

RESEARCH ARTICLE

Synthesis of *nano-ZnO* by chemical reduction method and their micro biocide activity against bacterial skin pathogens

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Manuscript details:	ABSTRACT
<p>Received: 07.03.2017 Accepted: 05.04.2017 Published : 30.06.2017</p> <p>Editor: Dr. Arvind Chavhan</p> <p>Cite this article as: Sharma Preeti and Nanoty Vijay (2017) Synthesis of <i>nano-ZnO</i> by chemical reduction method and their micro biocide activity against bacterial skin pathogens; <i>International J. of Life Sciences</i>, 5 (2): 233-240.</p> <p>Acknowledgement The authors would like to thank UGC; University Grants Commission, New Delhi, India for their financial support.</p> <p>Copyright: © 2017 Author (s), This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p>	<p>Zinc oxide nanoparticles (<i>nano-ZnO</i>) were prepared by chemical reduction method. Zinc nitrate was taken as the metal precursor and sodium hydroxide as a reducing agent. The formation of the <i>ZnO</i> nanoparticles (<i>nano-ZnO</i>) was monitored using UV-Vis absorption spectroscopy. The UV-Vis spectroscopy revealed the formation of <i>nano-ZnO</i> by exhibiting the typical surface plasmon absorption maxima at 360-380 nm. The average particle size of <i>ZnO</i> nanoparticles was analysed to be 48nm. We have used XRD, TEM, SEM and UV-Vis spectroscopy to characterize the nanoparticles obtained. The average size and morphology of <i>nano-ZnO</i> were determined by TEM. TEM photographs indicate that the nanopowders consist of well dispersed agglomerates of grains with a narrow size distribution (22.3 to 72.8 nm). The synthesized nanoparticles have been structurally characterized by XRD. The peaks in the XRD pattern are in good agreement and no peaks of other impurity crystalline phases were detected. Additionally, the antibacterial activity of the <i>ZnO</i> nanoparticles dispersion was measured by Kirby-Bauer method. The nanoparticles were found to be completely cytotoxic to most prevailing microorganisms in bacterial cutaneous infection like Methicillin Resistant <i>Staphylococcus aureus</i>, <i>Streptococcus pyogenes</i>, <i>Pseudomonas aeruginosa</i> and <i>Klebsiella</i>. The <i>ZnO</i> nanoparticles were found to exhibit antibacterial effects at low concentrations. These nanomaterials were shown to be an effective bactericide & may be suitable for the formulation of new types of bactericidal materials.</p> <p>Key words: Zinc oxide nanoparticles (<i>nano-ZnO</i>), chemicals reduction, XRD, TEM, SEM and UV-Vis spectroscopy, antimicrobial activity, Methicillin Resistant <i>Staphylococcus aureus</i>, <i>Streptococcus pyogenes</i>, <i>Pseudomonas aeruginosa</i> and <i>Klebsiella</i></p>
	<h2>INTRODUCTION</h2> <p>Nanoscale science and technology have emerged over the past decade as the forefront of science and technologies. The intersecting fields of study that create this domain of science and engineering perfectly typify the rapid, multidisciplinary advancement of contemporary science and technology. Inorganic materials such as metal and metal oxides have</p>

attracted lots of attention over the past decade due to their ability to withstand harsh process conditions (Fu L *et al.*, 2005). Of the inorganic materials, metal oxides such as TiO₂, ZnO, MgO and CaO are of particular interest as they are not only stable under harsh process conditions but also generally regarded as safe materials to human beings and animals (Stoimenov *et al.*, 2002).

Nanostructured materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical applications (Brigger *et al.*, 2002; Wu, X. *et al.*, 2003). Nanoparticles and nanostructure are becoming a part in human medical application, including imaging or the delivery of therapeutic drugs to cell, tissues and organs. Drug loaded nanoparticles interact organ and tissues and are taken up by cells. Nanomedical developments range from nanoparticles for molecular diagnostics, imaging and therapy to integrated nanosystems, which may perform complex repair actions at cellular level inside the body.

Hunter and Preedy (2011) reveal many reasons to consider NPs for medicine. Decreasing particle size results in increased surface interaction for both NPs and emulsions, which may result in increased solubility for both hydrophilic and hydrophobic drugs.

The recent development of effective and reproducible techniques has made it possible to synthesize stable aqueous dispersions of individual particles with sizes that can be accurately adjusted from a few nanometers to a few tens of nanometers. When nanoparticles are produced, the size of the inorganic core must be correctly adjusted to control its intrinsic properties. Synthesis is generally carried out in such a way as to favor particle nucleation rather than particle growth. Several articles report the synthesis of ZnO NPs via simple chemical route (Dwivedi *et al.*, 2014; Ban *et al.*, 2014). Inorganic NPs, including metal oxides, are promising materials for applications in medicine, such as cell imaging, bio sensing, drug/gene delivery, and cancer therapy. Zinc oxide (ZnO) NPs belonging to a group of metal oxides are characterized by their photocatalytic and photo-oxidizing ability against chemical and biological species. Among other metal nanoparticles, zinc oxide nanoparticles are very much important due to their utilization in gas sensors,

biosensors, cosmetics, drug-delivery systems, and so forth. ZnO NPs also have remarkable optical, physical, and antimicrobial properties (Sabir *et al.*, 2014)

ZnO NPs have a potential application as a bacteriostatic agent and may have future applications in the development of derivative agents to control the spread and infection of a variety of bacterial strains (Jones *et al.*, 2008). ZnO nanoparticles propose new opportunities including the improvement of the specific drug delivery and also manipulating cell membranes. No cytotoxic effects of ZnO nanoparticles were found in human glioma cells. ZnO nanoparticles are known to be one of the multifunctional inorganic nanoparticles with effective antibacterial activity (Kozgus *et al.*, 2015)

Skin infections caused by bacteria are the main obstacle in healing processes. Conventional antibacterial drugs face critical vital issues such as; more rapid delivery of the drug than intended which can result in bacterial resistance, dose related systemic toxicity, tissue irritation and finally delayed healing process that need to be tackled. Recently, studies have been focused on new drug delivery systems, overcoming resistance and finally localizing the molecules at the site of action in a proper dose. In this regard, nanotechnological approaches such as nanomedicine has to be developed to address accompanying problems mentioned above

In the present work on the preparation of *nano-ZnO* from aqueous solution of zinc nitrate, we employed as reductant sodium hydroxide and starch was employed as a stabilizer. These methods are referred to as soft chemistry, because they are generally carried out at room temperature. Metal salts are less expensive and less sensitive to humidity than metal alkoxides

MATERIALS AND METHODS

Materials

The objective of this study was to determine the antimicrobial susceptibility of *nano-ZnO* against *Methicillin Resistant Staphylococcus aureus (MRSA)*, *Streptococcus pyogenes* (gram positive), *Pseudomonas aeruginosa* and *Klebsiella* (gram negative) isolated from bacterial skin diseases. From March '15 to June '16, 205 consecutive, non-duplicate *Methicillin resistant*

Staphylococcus aureus, 232 *Streptococcus pyogenes*, 148 *Pseudomonas aeruginosa* and 86 *Klebsiella* isolates were recovered out of 743 samples from both admitted patients and those who attended the Out Patient Departments at various government and non-government hospitals in Akola (MS), India. Clinical specimens used for the study were pus and wound swabs. Information regarding patients age, sex, and type of specimen taken were also recorded. All the isolates were identified using colony morphology on Blood Agar, Mannitol Salt Agar, Nutrient Agar, Cetrimide Agar, Kings A and B medium, Gram stain characteristics, motility detection, positive reaction to oxidase, citrate utilization, etc. Chemicals of analytical grade like zinc nitrate ($Zn(NO_3)_2$), Sodium hydroxide (NaOH) and Starch were obtained and used without further purification. Double-distilled deionised water was used.

Preparation of nano-ZnO

The ZnO nanoparticles required for the present investigation were obtained by using zinc nitrate ($Zn(NO_3)_2$) and sodium hydroxide (NaOH) as precursors and soluble starch as stabilizing agent. 0.5% of soluble starch was dissolved in 500 ml of double distilled deionized water. Zinc nitrate, 14.874 g (0.1 M), was added in the above solution. Then the solution was kept under constant stirring using magnetic stirrer to completely dissolve the zinc nitrate.

After complete dissolution of zinc nitrate, 0.2 M of sodium hydroxide solution (20 ml was used in our study) was added under constant stirring, drop by drop touching the walls of the vessel. The reaction was allowed to proceed for 2 h after complete addition of sodium hydroxide.

After the completion of reaction, the solution was allowed to settle for overnight and the supernatant solution was then discarded carefully. The remaining solution was centrifuged at 10,000 $\times g$ for 10 min and the supernatant was discarded. Thus obtained nanoparticles were washed three times using deionized distilled water. Washing was carried out to remove the byproducts and the excessive starch that were bound with the nanoparticles. After washing, the nanoparticles were dried at 80°C for overnight. During drying, complete conversion of $Zn(OH)_2$ into ZnO takes place. The white powder of ZnO nanoparticles was thus obtained and stored. The progress of the above chemical reaction was monitored with thin layer

chromatography (TLC) because of its simplicity and speed.

The stability of nanoparticles was examined by exposing them to ambient conditions for one month. To carry out all characterization methods and interaction of the silver nanoparticles with bacteria, the silver nanoparticle powder in the freeze-drying cuvette was re-suspended in deionised water; the suspension was homogenized with an ultrasonic machine.

Characterization of silver nanoparticles

1. Characterization using UV-Visible Spectroscopy:

Characterization of silver nanoparticles was done using UV-Visible Spectrophotometer by using model Single Beam UV-Visible Spectrophotometer with software (BI/CI/SP/SB-S-03) of BioEra make. The UV-Vis spectroscopy reveals the formation of nanoparticles by exhibiting the typical Surface Plasmon Absorption maxima from the UV-Vis spectrum.

2. Characterization using Transmission Electron Microscopy (TEM):

The studies of size, morphology and composition of the synthesised silver nanoparticles were performed by means of magnified TEM image. The TEM measurements were carried out from Sophisticated Analytical Instrumentation Facility (SAIF), All India Institute of Medical Sciences, New Delhi.

3. Characterization using Scanning Electron Microscopy (SEM):

The SEM was used to show very detailed 3D images of nanoparticles at much high magnifications with great clarity. The SEM measurements were carried out from Sophisticated Analytical Instrumentation Facility (SAIF), All India Institute of Medical Sciences, New Delhi.

4. Characterization using X-ray Diffraction (XRD):

The structure and composition of the synthesized ZnO nanoparticles were determined by the powder X-ray diffractometer technique. X-ray diffraction (XRD) was based on constructive interference of monochromatic X-rays and crystalline samples. The diffraction pattern of the synthesized NPs were measured at the range of $2^\circ < 2\theta < 70^\circ$. The XRD of prepared nano-ZnO were carried out from Nanobeach, New Delhi with model XPERT-PRO X-ray diffractometer operated at 45kV and a current of 40 mA with Cu K radiation in a θ - 2θ configuration.

A) Organism preparation or Standardization of inoculums

To standardize the inoculums density for a susceptibility test, bacterial isolates were grown overnight in nutrient broth at 37°C and McFarland standards were used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria were within a given range, that is, same number of colony forming units were present per ml of solution. The standard can be compared visually to bacterial suspension.

B) In-vitro bacterial susceptibility to nano-ZnO:

The agar well diffusion method was exclusively employed to assess the therapeutic application of prepared silver nanoparticles as recommended by NCCLS, National Committee of Clinical Laboratory Standards, (NCCLS, 2000 & 2003). *MRSA*, *S. pyogenes* (gram positive) and *P. aeruginosa*, *Klebsiella* (gram negative) isolated from bacterial skin infections were tested against zinc oxide nanoparticle by this method. Mueller-Hinton Agar containing 2ml of McFarland Turbidity Standardized bacterial inoculums were poured in a Petri dish and then solidified. Wells of diameter 8mm were made onto each bacteria-inoculated agar plate. Six different concentrations (1:10 diluted) of zinc oxide nanoparticles like 100, 90, 80, 70,

60, 50, 40, 30, 20, 10 $\mu\text{g ml}^{-1}$ of the diluted nano-ZnO sol were loaded on marked wells with the help of micropipette. Evaluation of the bactericidal effect of drugs were based on analysis of diameter of inhibition zone around the sample-loaded wells after incubating at 37°C for 24 hours in agar well diffusion tests. The measurements were carefully noted in tabulated form and minimum inhibition concentration (MIC) was recorded.

RESULTS

The isolates were obtained from 743 human skin wound swabs of indoor and outdoor, 384 (51.69%) male and 359 (48.31%) female patients visiting or admitted to Government (400 samples) and non-government (343 samples) hospitals. **Table 1** shows the distribution of 743 samples of human skin wound collected from 5 different wards. The age groups were categorized in to five; 0-15, 16-30, 31-45, 46-60, 61 and above. The result showed that the occurrence of *MRSA* (205), *Klebsiella* (86) and *S. pyogenes* (232), *P. aeruginosa* (148) was higher in 31-45 age groups and in 16-30 age groups respectively than in infants and elderly age group.

Table 1: Correlation of skin pathogens isolated from skin disease patients from varied wards

Bacteria	Burn (N=23)		Dermatology (N=443)		ICU (N=92)		Maternity (N=43)		Medicine (N=142)	
	n	%	n	%	n	%	n	%	n	%
<i>MRSA</i>	6	26.1	111	25.1	25	27.2	16	37.2	47	33.1
<i>S.pyogenes</i>	4	17.4	145	32.7	33	35.9	18	41.9	32	22.5
<i>P.aeruginosa</i>	12	52.2	78	17.6	23	25	9	20.9	26	18.3
<i>Klebsiella</i>	4	17.3	48	10.8	11	12	7	16.3	16	11.3

N=Valid cases for each hospital ward, n= Number of isolates, %= Isolation rate

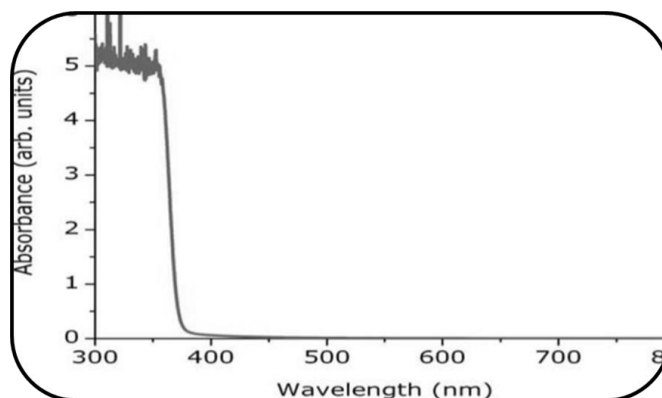


Figure 1: UV-Visible Absorption Spectra of Zinc oxide Nanoparticles/ *nano-ZnO*

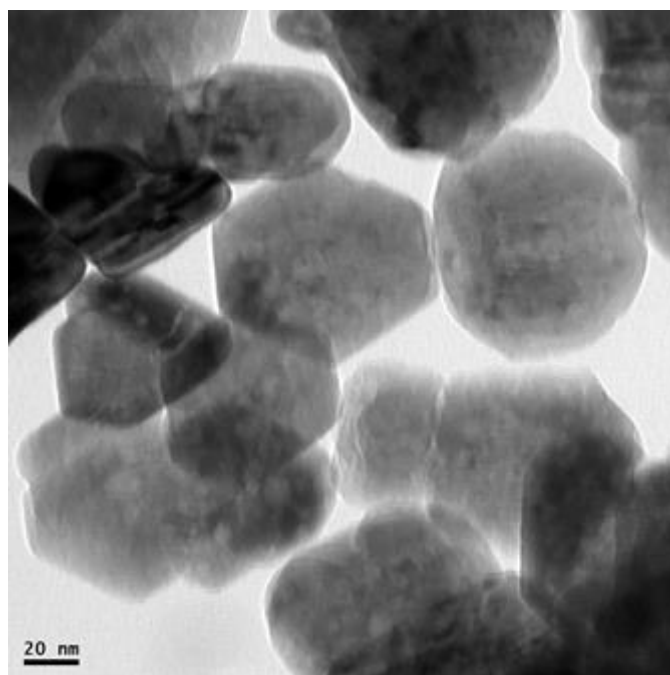


Figure 2: TEM image of Zinc oxide Nanoparticles/ *nano-ZnO* displays the XRD pattern of ZnO nanoparticles. On applying Debye-Scherer equation on different peaks of XRD graph of *nano-ZnO*, it was perceived that particles have different sizes of *nano-ZnO*. The average size of the *nano-ZnO* was analysed to be 48 nm).

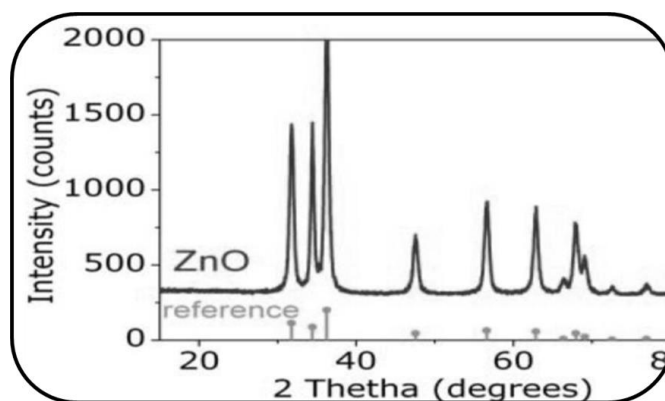


Figure 3: X-ray diffraction pattern of Zinc oxide Nanoparticles/ *nano-ZnO*

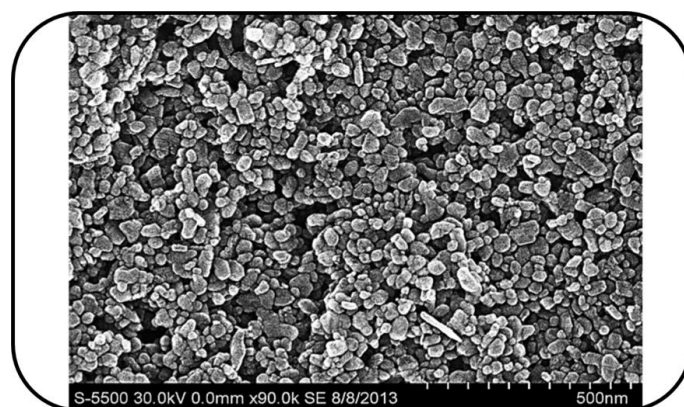


Figure 4: SEM image of Zinc oxide Nanoparticles/ *nano-ZnO*

The surface plasmon resonance peak in absorption spectra of ZnO nanoparticles is shown by an absorption maximum at 300-400 nm. The optical absorption spectra of *nano-ZnO* shift to longer wavelengths with increasing particle size. The surface peaks were found to vary with size and concentration of metallic nanoparticles. The Figure 1 shows the spectrum of *nano-ZnO*. As the distribution of shapes in the sample is broad, it is clear that the characteristic absorption of these nanoparticles arises from the contribution of different shapes and sizes, which agrees with the TEM observations. The prepared ZnO nanoparticle presents a peak at 360-380 nm. These results indicate that the nanoparticles preparations of this metal are mainly composed of small spherical and hexagonal *nano-ZnO*.

High resolution Transmission Electron Microscopy (TEM) reveals that the size of *nano-ZnO* lies in the range of 22.3 to 72.8 nm. The average particle size of *nano-ZnO* was analysed to be 48 nm. The TEM image also indicates that most of the particles exist separately. However, some TEM images of *nano-ZnO* indicate that few of them exist as aggregated form. Additionally, high resolution Transmission Electron Microscopy (TEM) demonstrated that the *nano-ZnO* is

composed of several morphologies. As the distribution of shapes in the sample is broad, it is clear that the characteristic absorption of these nanoparticles arises from the contribution of different shapes and sizes. The TEM image of *nano-ZnO* is shown in Figure 2.

High resolution Scanning Electron Microscopy (SEM) reveals the surface morphology and the structure determination of *nano-ZnO*. They are single, ocular crystallites although the tendency of agglomerate formation appears to be high. The SEM image of *nano-ZnO* shows that most of the *nano-ZnO* are spherical in shape and few of them are hexagonal or cubic including multi-twinned nanoparticles with different fold symmetries. Figure 4 epitomizes the SEM image of *nano-ZnO*.

The obtained results of zone of inhibition of bacterial study for ZnO nanoparticles have been shown in Table 2. The bacterial skin pathogens *MRSA*, *S. pyogenes* (gram positive) and *P. aeruginosa*, *Klebsiella* (gram negative) all are significantly susceptible to antibacterial action of *nano-ZnO*. On the basis of outcomes obtained, it was interpreted that *nano-ZnO* shows prevailing bactericidal properties even at lower concentration ($10 \mu\text{g ml}^{-1}$).

Table 2: Inhibition rate of ZnO nanoparticles against bacterial skin pathogens

Test concentration of ZnO NPs ($\mu\text{g ml}^{-1}$)	<i>MRSA</i>	<i>S. pyogenes</i>	<i>P. aeruginosa</i>	<i>Klebsiella</i>
	Zone of inhibition in mm			
100 $\mu\text{g ml}^{-1}$	23	24	26	22

90 $\mu\text{g ml}^{-1}$	21	22	24	21
80 $\mu\text{g ml}^{-1}$	19	20	23	21
70 $\mu\text{g ml}^{-1}$	18	18	20	19
60 $\mu\text{g ml}^{-1}$	17	15	18	17
50 $\mu\text{g ml}^{-1}$	15	13	17	15
40 $\mu\text{g ml}^{-1}$	12	11	15	13
30 $\mu\text{g ml}^{-1}$	12	10	13	12
20 $\mu\text{g ml}^{-1}$	11	10	12	10
10 $\mu\text{g ml}^{-1}$	9	9	11	10

DISCUSSION

The metal nanoparticles of *nano-ZnO* prepared by simple laboratory wet chemical reduction methods were noticed and found to exhibit considerably prominent and powerful antibacterial activity against all pathogenic bacteria causing primary and secondary bacterial skin diseases. They also demonstrate low cytotoxicity, chemical stability and thermal resistance as compared to conventional and organic antibacterial agents (antibiotics) prescribed in the study zone. The lowest concentration of the ZnO nanoparticles that inhibited bacterial growth was determined as the MIC for that particular bacterium. The MICs of *nano-ZnO* was observed to be 10 $\mu\text{g ml}^{-1}$.

The considerable amounts of evidences are now available to support the antibacterial potentialities of metal nanoparticles. Many studies have shown that some nanoparticles made of metal oxides, such as ZnO nanoparticle, have selective toxicity to bacteria and only exhibit minimal effect on human cells, which recommend their prospective uses in agricultural and food industries (Brayner *et al.*, 2006; Reddy *et al.*, 2007). The antimicrobial activity of zinc oxide nanoparticles have been studied against the food related bacteria *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens* (Jiang *et al.*, 2009).

The metallic nanoparticles are promising bactericides as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Gong P *et al.* 2007). The *nano-ZnO* prepared by simple

laboratory wet chemical reduction methods in this research also shows promising micro biocide properties.

Synthesis and characterization of nanoscaled materials in terms of novel physicochemical properties is of great interest in the formulation of bactericidal materials. The growth on agar plates is a more ready means of distinguishing antimicrobial properties of zinc oxide nanoparticles of different shapes than liquid growth experiments. In our study, complete inhibition of bacterial growth was observed on agar plates supplemented with nanoparticles (Table 2). It is noteworthy that inhibition depends on the concentration of the zinc oxide nanoparticles as well as on the initial bacterial number.

The development of new resistant strains of bacteria to current antibiotics has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides. Because of this, our studies and research was fabricated to look for an alternative form antibacterial medication.

CONCLUSION

The three key steps have been developed for this nanoscience and nanotechnology based study: the first is synthesis of material (*nano-ZnO*) preparation and second is property characterization and third is scrutiny/ analysis of antibacterial potentialities of prepared *nano-ZnO*.

Preparation of nanomaterials was advanced by simple wet chemical reduction techniques. The chemical techniques applied for synthesis of ZnO nanoparticles produced nanocrystals with well-defined structures

and morphology. The technique described in this article is facile as compared to other preparative techniques such as sonochemical method, sol-gel technique, electrochemical method, etc.

The challenging task of characterization comprised of structural analysis as main category. In our research work the structure analysis of synthesized NPs was carried out using microscopic techniques and spectroscopic techniques. UV-Visible (UV-Vis) spectroscopy was used to assure the complete reduction of the precursor compound. X-ray diffraction (XRD) method was used to demonstrate which elements were present in sample, to obtain information that the particles were amorphous or not. Imaging methods such as transmission electron microscope (TEM) and scanning electron microscope (SEM) were used to establish particle morphology and to provide a quantitative description of the particle size distribution.

The nanoparticles show antibacterial activity against all four bacterial strains *MRSA*, *S. pyogenes* (gram positive) and *P. aeruginosa*, *Klebsiella* (gram negative). Bacterial cell size usually ranges in micron range. These cells have cellular membranes which contain pores in nanometer range. The nanoparticles which were synthesised have a size less than that of the pore size in the bacteria and thus they have a unique property of crossing the cell membrane without any hindrance. It can be hypothesised that these nanoparticles form stable complexes with vital enzymes inside cells which hamper cellular functioning resulting in their death.

Conflicts of interest: The authors stated that no conflicts of interest.

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