ZINC OXIDE NANOPARTICLE PREPARATION, SPECTRAL ANALYSIS AND ANTIMICROBIAL PROPERTIES

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Abstract

With the continuous use of nano-metal oxides in the field of medicine, health care, and research, metal nanoparticles are developed enormously through various analytical methods which are affordable, research-oriented, and eco-friendly. In the current study, a simple and very useful approach has been followed for the preparation of Zinc oxide nanoparticles with the help of sol-gel method at 37^0C. The particles synthesized were analysed by X-ray diffraction, electron scanning microscopy, and UV-visible analysis. The temperature 37^0C, pH level (11.5-11.7) were maintained for the fabrication and the study of the nanoparticles. The λ_{max} value (360 nm) was found to be the most efficient value range in which ultraviolet B is being absorbed. The nanoparticles were then analysed for their antimicrobial activity against highly infectious microbes viz, *Bacillus subtilis, Carsonella ruddii, Pseudomonas syringae, Epulopiscium spp, Salmonella typhimurium, Escherichia coli, Myxococcus Xanthus, Bacillus anthracis.* The results of antimicrobial activity clearly show that the ZnO-NPs are good antibiotics against these mentioned contagious microbes. So these nanoparticles of zinc could be used as antimicrobial drugs in various forms for different treatments.

Keywords: Zinc Nanoparticles, XRD, SEM, TEM, Antimicrobial

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1. Introduction

Zinc oxide (ZnO) being a semiconductor with large energy gap of about 3.99 ev, in addition to great energy of binding 59.9 meV (at 298 K) (Zu P. et.al., 1997; Kim YS., 2005) . Zinc-oxide also had property with much more influence to make blue colored fluorescence and radiation absorption in UV range so is good for sunscreens, textile factories, catalysis, sensor materials, photodetector substances, and for solar energy absorption (Becheri A.et.al., 2008; Rao CNR. etal., 2004).

With a large number of application with different morphologies Zinc oxide nanoparticles have been developed in many forms like, nano-flowers, nano-rods, nano-belts, nano-tubes, nano-rings and nano-columns (Gao PX.et.al., 2007; Liu B.et.al., 2003) Various methods used for the preparation of ZnO nanoparticles so for, which includes sol-gel, the hydrothermal facile, solution and electric current heating, self-propagating high-temperature, nucleation spontaneous method, spray pyrolysis, gas-phase reaction and thermal evaporation, and so on (Zhao H. et.al., 2011; Bahnemann DW. et.al., 1987)

Also nowadays ZnO-NPs had become a hot topic for researchers as per their special type of properties in optics and chemical industry which have been done by simple change in a morphology of the constituents. Among the metal oxide family the ZnO-NPs are being utilized in many appliances with their use in electronics, communication, cosmetic materials, environmental analysis tools, biological and the pharmaceutical industry (C. Dagdeviren.et.al., 2013). More to gather, the zinc oxide particles have been found to have extra utilization in bio-analytical tools viz, biological sensing and labeling, gene and drug delivery systems and nanomedicine (J. W. Rasmussen. et.al., 2010) in addition to their antibacterial, antifungal, larvicidal and anti-diabetic activities (G. Applerot. et.al., 2009).

2. Materials and Methods

Zinc acetate dehydrate, cetyltrimethylammonium bromide (CTAB) and sodium hydroxide used was of analytical grade, from Merck. Nutrient agar, nutrient broth, dextrose agar, and were also of analytical grade from Hi Media (India). All other chemicals used were of (AR-grade).

2.1 Synthesis of ZnO nanoparticles. For the preparation of ZnO nanoparticles, 0.2 g of CTAB were mixed with 100 mL of double distilled water, in RD flask, to which 1% $Zn(CH_3COO)_2.2H_2O$, 10 mL solution was added. The complete solution was stirred on a mechanical stirrer at a stirring rate of 1500 rpm followed by the addition of NaOH solution in a drop-wise manner. Just after some time of agitation, a milky solution gets formed. The process was continued for about 30 minutes followed by cooling, centrifugation, and then dried at 25^0C in a desiccator, and finally washed with distilled water and then by methanol, and finally, the ZnO-NPs samples were stored for further use.

2.2. X-ray diffraction (XRD)

The crystal structure of Zinc nanoparticles were determined by X-ray diffractometer making use of a mono-chromatized X-ray beam using nickel-filtered Cu-Kα radiation in the 2θ range of 30°–70° in a step size of 0.01° with scanning rate of 0.02 steps/second.

The rays used for the experimentation have a power of 45 kV and 45 kA (Chen HC. et. al., 2012), and the crystallite size (D) of ZnO-NPs formed was calculated from highest intense peak (101) with the help of Debye–Scherrer equation:

$$D = k\lambda \beta \theta \cos \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad [1]$$

Where k = constant of proportionality with a value of 0.9; λ = X-ray wavelength, with a value of 1.54178 Å; β = full width at half maxima of the diffraction peak; θ is the Braggs' angle in degrees (Cullity BD. et.al., 1956).

2.3. UV-visible spectroscopy. The UV–Vis absorption measurements were done with Lambda 850 spectrophotometer (Perkin-Elmer) with an operational resolution of 1 nm. Distilled water was used as reference for correction purposes.

2.4. FT-IR spectroscopy. Infra-red analysis of the Zinc nano-particles synthesized were made using Fourier transform infrared (FT-IR) spectrophoto meter (Nicolet IS 10).
The spectra were recorded in spectroscopic grade KBr pellet (used at the ratio of 1:100) with resolution value of 4 cm^{-1} from 400 cm^{-1} to 4000 cm^{-1} frequency range in a diffuse reflectance mode. The identification and characterization of the functional groups and the structure chemically in ZnO-NPs was done by analysing various radiations at different frequencies. In total, the average of three readings of the sample was used for peak analysis.

2.5. Antimicrobial activity of ZnO-NPs

2.5.1. Test Organisms:

The synthesized ZnO-NPs were used for their antimicrobial activity against both Gram-positive

and Gram-negative bacteria. The test microbial strains include *Bacillus subtilis, Carsonella ruddii, Pseudomonas syringae, Epulopiscium spp, Salmonella typhimurium, Escherichia coli, Myxococcus Xanthus, Bacillus anthracis*. The synthesized **ZnO-NPs** were used with the concentration amount of 2 x10^{-5}M on the nutrient. The inoculation was done with 10% (v/v) actively growing inoculums and the cultures were incubated for about 24 hours on a rotary shaker with a speed of 150 rpm and maintained temperature of 37^0C**.**

The growth of the microbes was measured by incubation spectrophotometrically at 660 nm. The percentage growth was estimated with reference to the medium of reference but without any compound of inhibition.

2.6. Maintenance of Culture:
The culture used for bacteria was nutrient agar and then sub cultured accordingly.

3. Results and Discussion
Zinc oxide nanoparticles were synthesized in the form of nanotubes, nano rings, nano columns, etc (Haile SM. et.al., 1989) and have been prepared by various diverse techniques which include the sol-gel method (Gui Z. et.al., 2005), solution precipitation method [23] (Li F. et.al., 2008) spray pyrolysis (Li H. et.al., 2009) hydrothermal method (Khan MF.et.al., 2011), in a microwave-assisted technique (Chen HC. et.al., 2012). The technique used generally for the preparation of ZnO-NPs with little change in the temperature from low to ~1,000°C, pressure change from 1 atm to some Torr parameters with good techniques involved.

Reports are there related to the formation of flower-like ZnO nano rods by sono-chemical methods, but they are neither realistic nor cost-effective and therefore the present study provides paramount information for the generation of cheap and eco-friendly nanoparticles of ZnO at low temperature, at normal pH of the solution keeping in view the process to be very simple.

3.1. X-ray diffraction analysis
The X-ray diffraction studies that were obtained showed ZnO-NPs as crystalline with polycrystalline structure depicted in figure 1. The results of XRD show broad peaks at 2θ of 30°, 35°, 37° , 70°, which responds to planes of diffraction 101, 117, and 205 of ZnO crystals. The samples obtained show good results at different temperature values of 25°C and 75°C and the characteristic structures correspond to hexagonal wurtzite, having a lattice constant (c/a) of 1.7 where *a* and *c* are 3.25 Å and 5.21 Å, respectively. Also, the peaks (101), (117), and (205) clearly show structure wurtzite of ZnO (Rekha K.et.al., 2010) The XRD pattern shows the samples get obtained in a single phase. The peak with great intensity at (101) peak at 30° shows the growth of ZnO-NPs in an easy way for clear crystallization (Lin HF.et.al., 2005). No additional peaks corresponding to impurities have been detected in XRD spectra, making it clear that a pure form of ZnO-NPs are formed. With the help of the Debye–Scherrer equation, the average crystallite size of temperature-induced ZnO-NPs at 25°C and 75°C have been found to be 23.9 nm and 88.9 nm respectively. So the peak widths were found inversely proportional to crystallite sizes.

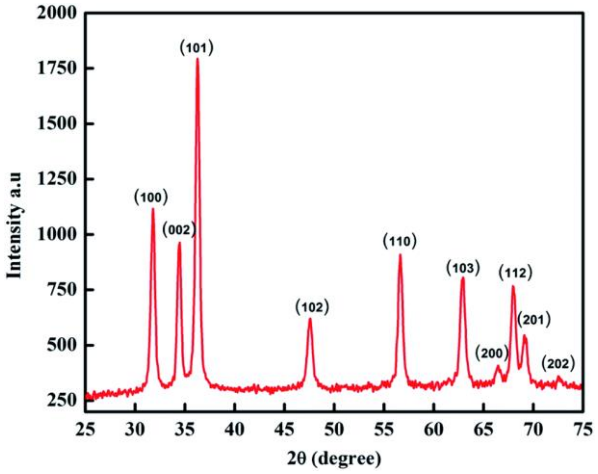

Fig 1: X-ray diffraction diagram of ZnO-NPs

3.2. Electron Microscopy. The SEM characterization of the ZnO nanoparticles showed flower-like structure for Zinc nanoparticles and the analysed structures appear small and sharper, obtained at higher stirring (Fig. 2). The SEM results completely agree with XRD results. The TEM images showed the diametric values between 3–50 nm for various samples prepared at high stirring conditions (Fig. 2). The sample thickness had been found inversely related to the speed of stirring. The aspect ratios (L/D; length by diameter) were found to be on average ~8.7 nm, ~10 nm, and ~19 nm, for ZnO-NPs which are prepared at 550, 1500, and 2500 rpm respectively. The increase in the aspect ratio was found to increase with the speed of agitation; making it clear to show an inversely proportional relation in relation to crystallite size. The increase in stirring conditions makes the proper mixing and dispersion with surfactants, making the growth in the length (c-axis), but a decline in the diameter.

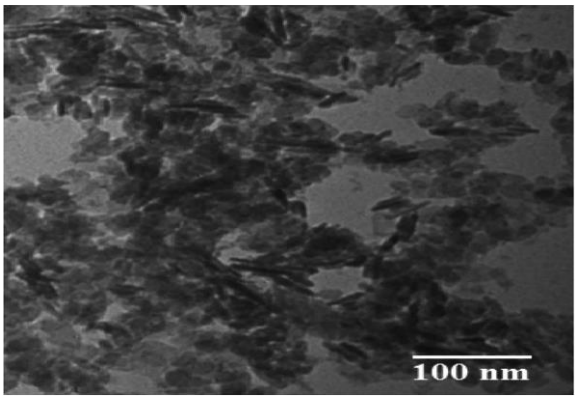

Figure 2: TEM images of ZnO-NPs

Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS)

ZnO-NPs morphology was analysed with help of SEM technique, and the morphological observations of ZnO-NPs and the results are shown in Fig 3. The respective figures clearly indicated a distinctive and abundant flower-shaped ZnO-NPs. The observed results clearly indicate that there are huge hexagonal arrays of ZnO-NPs leading to the formation of flower-shaped bundles. The results of the SEM pattern agree fully with the XRD results as per the size and shape. The compositional analysis of the ZnO-NPs particles clearly shows that Zn and O are present in the samples. The Zn and O phases, also show a few additional peaks, which are characteristically due to some foreign substance, which are shown in SEM imaging.

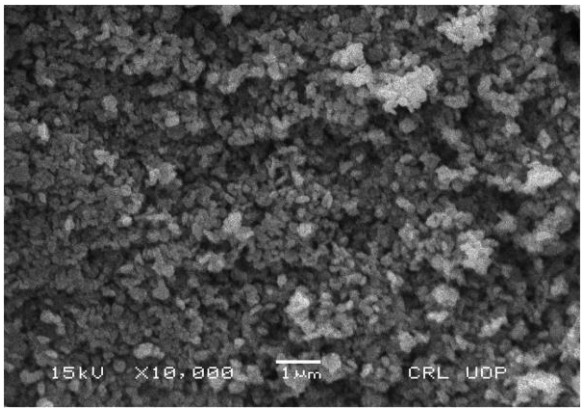

Figure 3: SEM images of ZnO-NPs

3.3. UV-visible absorption spectroscopy

The optical study of ZnO-NPs nanoparticles was carried out by a double-beam UV-Visible spectrometer at 25^0C and the obtained results are shown in figure 4. The samples were analysed for the study of reference just after their preparation of a few minutes and the results obtained are in full agreement with the results of (Guan XH.et.al., 2007). The peaks of the analysed samples were found in the VU-B region (280-320 nm) and the differences are observable in the peaks with notable intensity with a wavelength of maxima (λmax). The obtained results of the study of reference are in agreement with the previously reported results in various studies making it clear that the peak shifts are dependent on the crystallite size, the nature of the solvent, the temperature of the system, and the methods of preparation (Hale PS. et. al., 2005). The ZnO-NPs at 60^0C showed lowest λ_{max} at 510 nm which corresponds to maximum absorbance, however, the nanoparticles show highest λ_{max} at 360 nm which corresponds to the least absorbance, and the temperature of the system was adjusted at 30^0C. The highest λ_{max} values obtained for the ZnO-NPs that are prepared at 60^0C show that the most effective absorbance of radiation comes under UV-B. The non-appearance of other peaks in the spectrum directs confirms the purity of the sample.

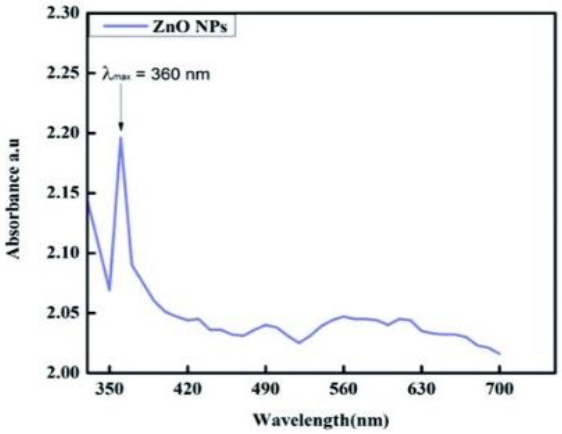

Figure 4: UV-Visible spectra of ZnO-NPs

3.4. Antimicrobial Activity

The metal nanoparticles synthesized are the most important particles with great importance as anti-pathogenic agents are considered an emerging field of research. The ZnO-NPs synthesized at near-25^0C temperatures have been characterized and tested as anti-bactericidal agents. Also, it had been found that different concentrations of the ZnO-NPs exhibit a characterized antibacterial activity against both Gram-positive and Gram-negative bacteria. The antimicrobial activity as observed seems to be concentration-dependent, as with every increase in concentration the activity against the microbe increases. The highest antimicrobial activity by the ZnO-NPs was shown against the Epulopiscium spp and the least activity was observed against the Salmonella typhimurium. The rest of the microbial strains were found to have an effect of the nanoparticles in between Epulopiscium spp and Salmonella typhimurium. The obtained data so far may suggest that the antimicrobial activity of ZnO-NPs could be dependent on the size and morphology of the nanoparticles.

Table 1. Antimicrobial Effect of ZnO-nano particles

Bacterial Species	**ZnO-NPs Particle Concentration (% w/v)** Zone of Inhibition (mm)			
	25%	50%	75%	100%
Bacillus subtilis	0	5	7	10
Carsonella ruddii	0.9	6	10	12
Pseudomonas syringae	2	9	11	13
Epulopiscium spp	5	10	16	24
Salmonella typhimurium	0	0	2	5
Escherichia coli	4	7	14	23
Myxococcus Xanthus	0	4	7	11
Bacillus anthracis	3	8	14	20

The anti-microbial activity of the ZnO-NPs could be suggested as the particles seem to be more diffusible in the growth medium, which allows their greater interaction with microbial cells. The same type of antimicrobial results with other ZnO-NPs synthesized by different workers (Wahab R. et. al., 2010). Among these studies, it had been mentioned that the nano-particles could interact with the thiol group of the important enzymes and thereby inactivate their activity. Although the exact site of interaction between the ZnO-NPs and the microbial wall is clearly understood, the results show that Gram-positive bacteria are susceptible to get affected by the ZnO-NPs, due to different types of their cell wall bio-molecules viz, proteins and the bio saccharides (Xie Y. et. al., 2011).

4. Conclusion

The synthesis and characterization of ZnO-NPs were done by a simple sol-gel method at room temperature. The SEM results showed the existence morphology of the ZnO-NPs as needle-like with bunches of colonies. The crystallite size of ZnO-NPs on an average was observed as 23.7 nm at 25°C. The growth order of the ZnO-NPs showed that the growth rate is maximum at normal conditions of temperature and pressure. The absorbance of light in a significant manner in the UV-B range by ZnO-NPs demonstrated clearly that the particles could be used in sunscreens. The ZnO-NPs synthesized also show characterized and significant antimicrobial activity, making them better antibiotics against bacterial and other microbial infections. So in general, it can be mentioned that the synthesized ZnO-NPs, with other formulation interactions, could be used as better antimicrobial agents in many types of ointments and lotions.

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