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HYDROTHERMAL MEDIATED SYNTHESIS OF ZnO NANORODS AND THEIR ANTIBACTERIAL PROPERTIES

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Abstract

Nanoceramics which possess antibacterial activity have recently received much attention as new inorganic antibacterial materials. Herein, we report the synthesis of nanostructured zinc oxide (ZnO) by surfactant assisted hydrothermal route using zinc acetate and hexamethylenetetramine (HMT). The as prepared ZnO nanoparticles were characterized by Fourier transform infrared spectroscopy (FT-IR), Ultraviolet- visible spectroscopy (UV-Vis), Photoluminescence spectroscopy, X-ray diffraction (XRD) and Field emission- scanning electron microscopy (FE-SEM). The XRD diffraction pattern corresponds to wurtzite structure of ZnO (JCPDS No.36:1451). The average crystallite size of the nanoparticles calculated from XRD, using Scherrer's equation, is approximately 10 nm. FE-SEM shows the as prepared ZnO are in the form of hexagonal nanorods. The antibacterial behavior of suspension of ZnO nanorods against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) showed an enhanced antibacterial activity as compared to the bulk ZnO.

Keywords *ZnO nanorods, hydrothermal, XRD, SEM, antimicrobial*

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Introduction

During last decade, nanomaterials are of considerable interest due to the functionalities unavailable to bulk materials. It is found that once the materials are prepared in the nanostructured forms, significant changes could occur to their physical, chemical and electrical properties [1]. Metal oxides such as TiO_2, ZnO, MgO and CaO are generally regarded as safe materials to human beings and animals, which not only exhibit strong antibacterial activity in small amounts even in absence of light but also stable under harsh process conditions [2]. ZnO is an important basic material due to its low cost, large band gap (3.31 eV), large exciton binding energy (60 MeV), luminescent properties and as biocompatible antimicrobial material. As compared to nano-silver, ZnO nanoparticles have the advantages of cost effectiveness, whiteness and UV blocking property. Moreover, ZnO nanoparticles are more stable when compared to organic UV-blocking agents [3]. Nano-ZnO particles are effective in inhibiting Gram-positive, Gram-negative bacteria and even spores that are high-temperature resistant and high-pressure resistant [1-3]. Factors, which influence the antibacterial activity of ZnO, are the concentrations of the metal oxides particles [4], the particle size of the metal oxide powder [5] and the specific surface area of the powder [6]. In this paper we report on the preparation, characterization of ZnO nanorods by surfactant assisted hydrothermal route and their antimicrobial activities against selected strains.

Experiment Details

Synthesis of ZnO nanoparticles. Zinc acetate, Zn $(CH_3COO)_2.2H_2O$ (99.5 %, GCS), HMT (99%, Merck), CTAB (Loba Cheme) were used as received without further purification. In a typical synthesis, 2.2 g of Zinc acetate was dissolved in 100 mL of distilled water along with 1 g of CTAB. To this, a solution of HMT (2.8 g in 50 mL of water) was added drop wise with constant stirring for 5 Hrs and the translucent contents formed were autoclaved in Teflon lined stainless steel bomb, placed in a thermo stated oven at 120°C for 8 Hrs. The white precipitate obtained was centrifuged, washed with water until neutral pH and finally with absolute ethanol and then dried in oven at 110°C to yield bright white powders of ZnO.

Characterization of ZnO nanoparticles. The as prepared nanoparticles were characterized by optical absorption using Perkin-Elmer (Model: Lambda 25) double beam scanning UV-Vis spectrophotometer in the range of 200-800 nm. FT-IR spectra were recorded as KBr pellets with a Perkin–Elmer FT-IR spectrophotometer (Model: Spectrum one) in the range of 4000–400 cm^{-1}. The crystal structure of ZnO nanoparticles were characterized by XRD (Philips PW, Philips Instruments, Eindhovan, The Netherlands) using Cu-Kα radiation (λ=1.54 A°)with a graphite monochromator. The applied voltage and current of XRD were set at 30 kV and 20 mA, respectively, with the scan speed of 3^{o}/min. The surface morphology and particle shape were studied by mounting the sample on double sided adhesive tape using FE-SEM (FE-SEM 1530, LEO Instrument, Cambridge, UK) at 10-15 kV. The room temperature photoluminescence (PL) spectrum of ZnO is recorded with fluorescence spectrometer (Model: FLS920) using Xe lamp as the excitation source upon excitation wavelength at 310 nm.

Antibacterial tests. *E. coli* MTCC 40 and *S. aureus* MTCC 87 from IMTECH, Chandigarh were grown aerobically in nutrient broth for 24 hours at 37°C. The qualitative assessments were carried by Agar Well diffusion assay [7] and the quantitative assessment by shake flask method (with slight modifications) respectively. The inoculum containing 5 x 10^5 cells was incubated along with 1% ZnO prepared by ultrasonic dispersion in water at 37°C for 5 hours under constant shaking. The percentage reduction of bacteria by the ZnO nanorods is reported as R, where R= 100*(B-A)/A. Here A is the number of bacteria recovered from the inoculated treated test specimen incubated over 5 h while B is the number of bacteria recovered from the treated test specimen immediately after inoculation (at '0' contact time) [8].

Results and Discussions

Different methods of preparation yield different particle sizes of ZnO, depending on the type of precursor [9 – 11], the pH [12, 13] and the temperature of the reacting solution [14 – 16]. The choice of method depends on the final application. In this study, we employed a hydrothermal precipitation method to prepare nanosized ZnO, because it allows control of size, morphology and crystallinity by tuning of the experimental variables [17]. As HMT decomposes into formaldehyde and ammonia with increasing temperature, OH^- species are released to the medium promoting the precipitation of the metal hydroxide and/or oxide [18]. The growth of ZnO is mediated through the formation of $Zn(OH)_4^{2-}$, which forms ionic pair with CAT^+ formed from the cationic surfactant, CTAB. Therefore the surfactant molecule is adsorbed on the crystal nuclei, thus acting as both growth director and also as surface protector from aggregation [19].

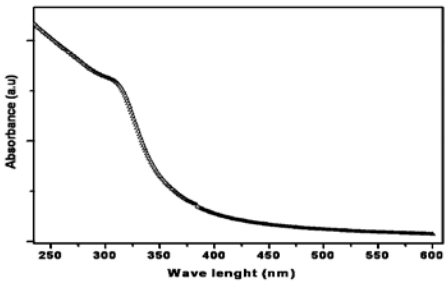


Figure 1: *UV-Vis spectrum*

Figure 1 shows that the excitonic absorption peak due to the ZnO nanoparticles is observed at 310 nm, which lies much below the band gap wavelength of 370 nm, reported for bulk ZnO [20].

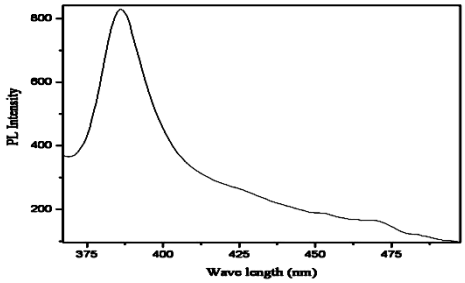


Figure 2: *PL spectrum*

Figure 2 shows the room temperature PL spectra of the nanocrystalline ZnO nanoparticles, mainly consisting of widened peaks from 365 to 490 due to UV emission, a strong UV emission broad band having emission maximum at ~ 385nm (3.55 eV) [21]. These UV emissions are attributed to near band edge emission of the wide band gap of ZnO due to the annihilation of excitons [22]. The blue or green luminescence is considered to be the result of radiative recombination of photo-generated holes with single ionized oxygen vacancies [23].

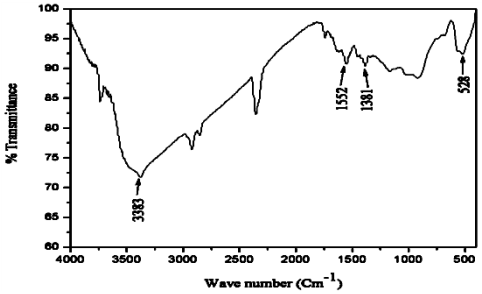


Figure 3: *FTIR spectrum*

Figure 3 FT-IR shows main absorption bands due to O-H stretching of hydroxyl group at 3383 cm^{-1}, asymmetric and symmetric C=O stretching of zinc acetate at 1552 and 1381 $cm^{-1}$and Zn-O stretching of ZnO at 528 cm^{-1} [24].

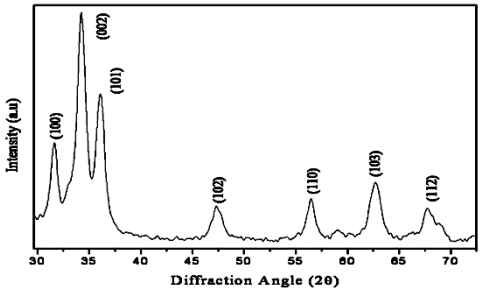
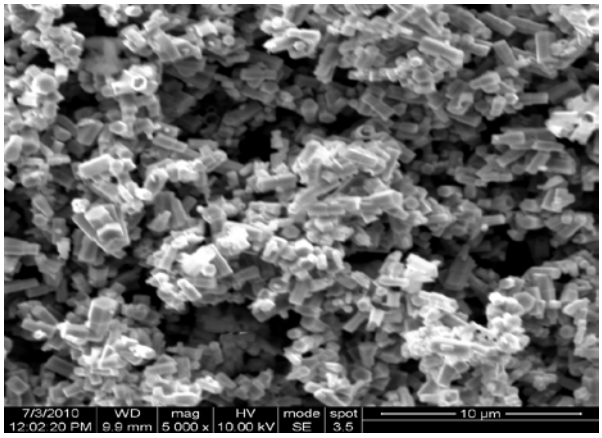
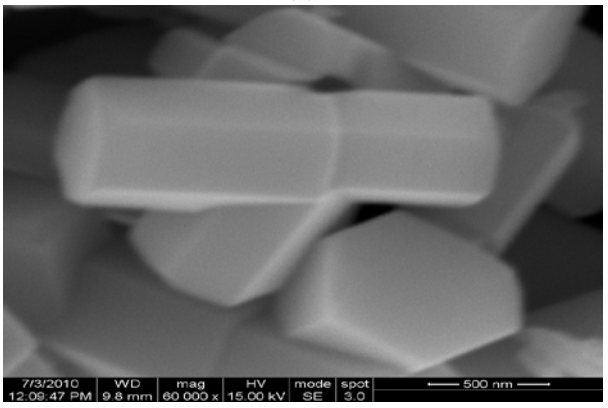


Figure 4. *XRD spectrum*

The XRD pattern of ZnO is shown in Figure 4 and peaks were broadened due to decrease in the particle size. All the peaks match very well with standard ZnO of wurtzite structure (JCPDS No.36:1451). Additionally, higher intensity and narrower spectral width of ZnO (0 0 2) peak, compared to other observed ZnO peaks in the spectrum affirms the growth in c-axis direction [25]. The crystallite sizes were estimated from the line broadening by using Debey-Scherrer's formula: $D = (0.9\ \lambda) / (\beta \cos \theta)$. Here, D is the coherent length, λ is the wavelength of X-ray radiation, β is the full-width at half-maximum (FWHM) of the peak and θ is the angle of diffraction [21]. The average crystallite size of the nano ZnO calculated is in the range of 10 nm ± 2 nm.



(a)



(b)

Figure 5: *FE-SEM images of ZnO hexagonal nanorods a) Lower magnification and b) Higher magnification*

Figure 5 shows the FE-SEM micrograph of as prepared ZnO nanoparticles are in the shapes of hexagonal nanorods. The growth rate of ZnO is known to be the fastest along the c-axis [26]. The dimension of ZnO nanorods prepared through hydrothermal routes on

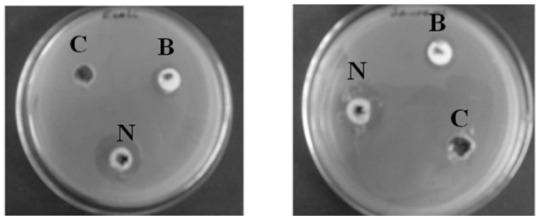
glass substrates depends on the pH of the reaction medium [27-28]. In our method ZnO nanorods of lengths ranging from 1.25 µm to 1.75 µm and the widths from 0.5 µm to 0.75 µm with aspect ratios 2.3 to 2.5 were observed by FE-SEM. These nanorods might be composed of individual 10 nm sized nanocrystalline ZnO as calculated from line broadening of XRD. In a recent study based on solution-based molecular route, Mikhail *et al* have reported ZnO nanorods of 5-6 µm, which composed of sub-5 nm ZnO nanocrystals [29].

Zone of inhibition of bulk and nano ZnO against Gram-positive as well as Gram-negative bacteria are shown in Table 1. The antibacterial activity of the nanoparticles for *S. aureus* was more intensive (12 mm) than that observed for *E.coli* (10 mm), whereas bulk ZnO exhibited 5 mm and 4 mm inhibition zones for *S. aureus* and *E. coli* respectively.

Table 1: *Growth inhibition zones of control, bulk and nano ZnO*

SAMPLES	ZONE OF INHIBITION in mm	
	S. aureus	*E. coli*
Control – Phosphate Buffer Saline	-	-
Bulk ZnO – 10mg/ml	5± 0.2	4 ± 0.2
Nano ZnO – 10 mg/ml	12 ± 0.2	10 ± 0.2

The results of the qualitative and quantitative antibacterial assessments are shown in Figure 6 and Table 2.



a) **b)**

Figure 6: *The qualitativr diffusion assay of ZnO Nanorods activity against a) E. coli and b) S. aureus, where C - Control (Phosphate buffer saline); B- Bulk ZnO and C - Nano ZnO*

The bactericidal efficacies of ZnO nanoparticles, after an incubation time of 5 h shows that the Nano-ZnO are more effective than the bulk ZnO as reported earlier [30]. This can be explained on the basis of the enhanced reactive oxygen species released on the surface of nano ZnO, which cause fatal damage to microorganisms [31]. In contact with water, ZnO catalyse the conversion of dissolved oxygen molecules to super oxide radical anions ($\bullet O^{-2}$), which in turn react with H^+ to generate ($HO^2\bullet$) radicals, and converted to hydrogen peroxide anions (HO^2-).

Table 2: *Percentage reduction of S. aureus and E.coli with control, bulk ZnO and nano ZnO*

SAMPLES	% REDUCTION	
	S.aureus	*E. coli*
Control – Phosphate Buffer Saline	NR	NR
Bulk ZnO – 1%	94.4	92.1
Nano ZnO – 1%	99.1	97.2

Mean of 3 values; NR-No Reduction

They react with hydrogen ions to produce molecules of H_2O_2. The generated H_2O_2 can penetrate the cell membrane and kill the bacteria [32]. The higher surface to volume ratio of nanoparticles results in the generation of larger number of active oxygen species as compared to bulk ZnO. The results have also demonstrated that the ZnO nanofluids have better antibacterial activity against *S. aureus* than *E. coli*. The results may attributed to the fact that Gram-negative bacteria have an unique outer membrane, a thinner layer of peptidoglycan, and a periplasmic space between the cell wall and the membrane, exhibiting a protective effect against the ZnO nanofluids, which are more active against the Gram-positive strain.

Conclusions

The work presented here reports the preliminary studies on the antibacterial activities of the ZnO nanofluids. The sizes of hydrothermally produced ZnO in presence of CTAB and HMT were in the range of 10 nm. The studies show that ZnO nanofluids (1% suspension in water) exhibit bactericidal activity of more than 90% on 5 hrs interaction time, against both Gram- positive and Gram- negative organism, as a result of damage in the bacterial wall due to reactive oxygen species generated by ZnO nanorods.

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