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Hydrothermal Synthesis of ZnO Nano-Honeycomb Structures and their Activity against Pathogenic Bacteria

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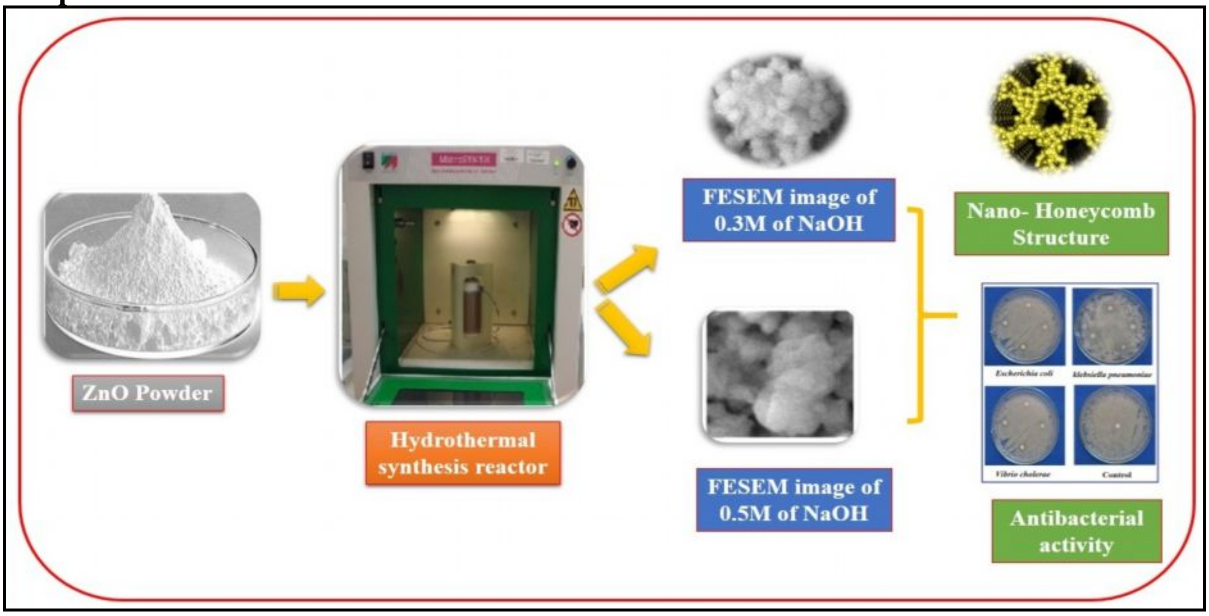
Abstract: Recently, honeycomb structure materials have attracted wide attention for both fundamental research and practical applications and have become an increasingly hot research topic. ZnO nano-honeycomb structures with different sizes and shapes have been successfully synthesized by simple hydrothermal method, using zinc nitrate and sodium hydroxide as the reactants. The synthesized zinc oxide nano-honeycomb structures were characterized by XRD, FTIR, UV and FESEM in order to confirm the phase purity and morphology of the sample. ZnO exhibit hexagonal wurtzite structure, which has been revealed by the XRD analysis. The growth mechanism of a ZnO honeycomb structure is attributed to the catalysis of metal nanoparticles. The antibacterial activity of ZnO nanoparticles were tested against Escherichia coli, klebsiella pneumoniae and Vibrio cholera organisms by agar diffusion method. Finally, the current study has clearly demonstrated that the ZnO NPs are responsible for significant higher antibacterial activities. These results suggest that ZnO nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems. Synthesized ZnO nanomaterials are excellent potential candidates for industrial applications.
Keywords: Hydrothermal, nano-honeycomb structure, FESEM, XRD, FTIR, UV.

Graphical Abstract



Introduction

Nanostructures and Nanomaterials form the main research focus in material science due to their unique properties and extensive applications. During the last two decades, a great pile of inquiry has been performed on the synthesis of different nanostructures, which aids in the apprehension of the mesophysics phenomena of growing nanomaterial. Several nanostructures of different metal oxide semiconductors with potential applications have been explored. Among these, ZnO is considered to be a promising material for the nanoscale based device applications due to its wurtzite crystal structure, wide direct band gap of 3.37 eV and high exciton binding energy of 60 meV.

It has wide applications in different industries including photo detectors[1], sensors[2], solar cells, antibacterial for medical products[3-5] and cosmetics[6]. The powder is widely used as an additive in numerous materials and products including plastics, ceramics, glass, cement, rubber, lubricants, paints, pigments, food, batteries, ferrites, fire retardants, etc. ZnO is traced in earth's layer as a mineral zincite and a large amount of ZnO used commercially has been produced synthetically.

As a result of significant interest in relation to the specific properties of the one-dimensional (1-D) ZnO nanomaterials[7-9], current studies are centered mostly on the correlation of nano architecture morphology with deposition parameters and physical properties. On the other hand, achieving control over ZnO nanomaterial morphology is a challenging pursuit. Probably, ZnO nanostructures can be synthesized by various chemical or physical methods such as precipitation[10], sol-gel[11], chemical vapor deposition[12], spray pyrolysis[13], etc. Moreover, the specific properties of ZnO and its applications depend on its morphology, size and structure. Many researchers find new ways for the synthesization of nanomaterials. The hydrothermal method has received considerable attention due to its unique advantages. It is a low temperature (60-100^{0}C), high yield and more controllable process[14] than other methods.

ZnO nanoparticles have a broad spectrum of antibacterial activities. Zinc oxide (ZnO) is listed as "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration. Nano-sized particles of ZnO have more pronounced antimicrobial activities than large particles, since the small size (less than 100 nm) and high surface-to-volume ratio of nanoparticles allow for better interaction with bacteria. Recent studies have shown that these nanoparticles have selective toxicity to bacteria but exhibit minimal effects on human cells.

In this paper, we have synthesized ZnO nano-honeycomb structures by the simple hydrothermal method. The synthesized nanoparticles were characterized by XRD, FESEM, UV and FTIR spectrometer techniques[15]. We investigated antibacterial activity of ZnO nanoparticles prepared by a hydrothermal method against a gram-negative bacterium *Escherichia coli, klebsiella pneumoniae and Vibrio cholerae.*

Experimental details

Materials

All chemicals (zinc nitrate, sodium hydroxide, ethanol and acetone) were purchased from Merck Company. All chemical reagents were of analytical grade and used without any further purification.

Hydrothermal synthesis

In a typical process, 2.9747 grams of highly pure zinc nitrate ($Zn(NO_3)_2$) powder (99.9%) has been mixed with 50 ml of deionized water. After taking 0.3M of NaOH concentrated solution, 25 ml of deionized water was added.

Zinc nitrate solution was stirred with NaOH solution and added slowly with zinc nitrate. The mixed solution was stirred for 30 minutes with 420 rpm. The resultant solution has been processed for hydrothermal reaction at 150^{0}C. Finally, the white colored precipitate was obtained and then washed with deionized water and ethanol for several times. Then, the obtained precipitate was dried at 130^{0}C for 3 hours. The whole process was repeated by changing concentrations of NaOH from 0.3M to 0.5M and the variations were studied. Finally, the precipitate solution was filtered using 0.2 micrometer PTFE filter paper and washed in ethanol and acetone for three times. At last, the white color precipitate has been collected in a petri dish.

Then, the collected precipitate has been dried at 130^{0}C for 3 hours in Hot air oven. The obtained precipitate ground with an agate mortar.

Characterization techniques

The crystalline structures of samples have been characterized by X-ray diffraction (XRD) on a D/Max 2005 Rigaku X-ray diffractometer that was operated at an acceleration voltage of 40 kV, using Cu-Kα1 radiation (λ= 1.5405Å). The size of the ZnO particles was estimated from the XRD graph. The morphology of samples was examined by scanning electron microscopy (FESEM, Phillips and Holland). The quality and composition of the samples were characterized by Fourier transform infrared (FTIR) spectroscopy (Shimadzo FTIR 1650 spectrophotometer, Japan) in the range of 400-4000cm^{-1}. The performance of FTIR measurements has been done with KBr pellets in the ratio of 1:100 containing the same amounts of ZnO samples to monitor the variation of defect contents in them qualitatively.

Results and discussion

XRD analysis

Fig. 1 shows the XRD pattern of ZnO nanoparticles, which have been synthesized by hydrothermal method with different concentrations (0.3 and 0.5M NaOH). The crystallite size (D) is calculated by using the Debye-Scherer's equation[16],

$D = k\lambda/\beta \cos\theta$

Where

β is the broadening of diffraction line measured at half of its maximum intensity (FWHM) in radians

λ is the wavelength of X-ray used ($\lambda = 1.5418 \times 10^{-10}$m)

K is a constant (taken as 0.94)

θ is the Bragg's angle

A definite line broadening of the diffraction peaks is an indication that the synthesized materials are in nanometer range. The average grain size of ZnO is determined using Scherrer's relation and it was found to be around 18 nm for 0.3M NaOH and 23 nm for 0.5M NaOH. All diffraction peaks are indexed according to the hexagonal phase of ZnO. No typical peaks of impurity phases except ZnO are found which exposed that good crystalline in nature of the samples. The broadining of the peaks in the XRD pattern can be atributed to the small particle size of the synthesized ZnO.

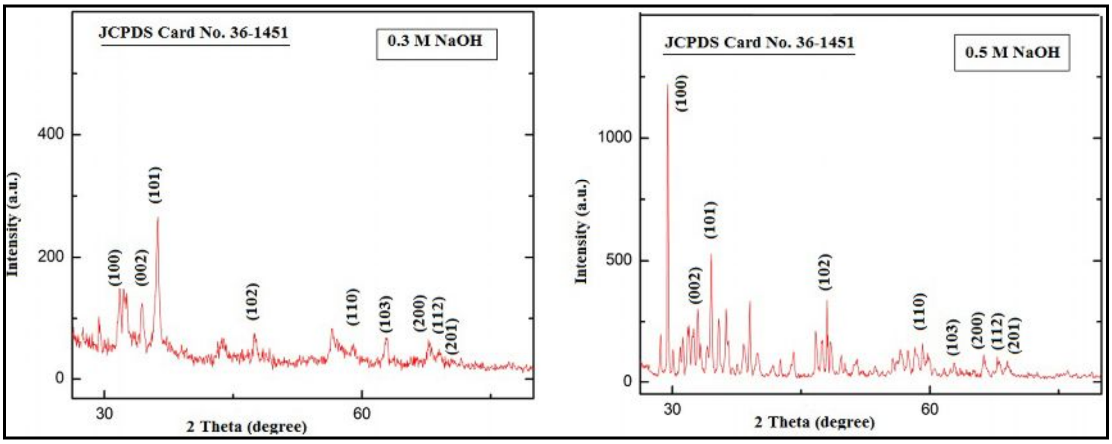


Fig. 1 XRD pattern of ZnO nanoparticles (0.3 and 0.5M NaOH)

FTIR analysis

Fig. 2 shows FTIR spectrum of ZnO nanoparticles with significant absorption peaks at 1388 and 441 cm^{-1} respectively. The absorption peak at 441 cm^{-1} attributes to Zn-O stretching vibration. The 0.3M of NaOH concentrated ZnO nanoparticles produce a peak at 1388 cm^{-1} and attributes to C-H stretching vibration. Similarly, the 0.5M of NaOH concentrated ZnO nanoparticles produce peaks at 1388 and 469 cm^{-1} and also attribute to the same. The merged spectrums also show merely the same peaks.

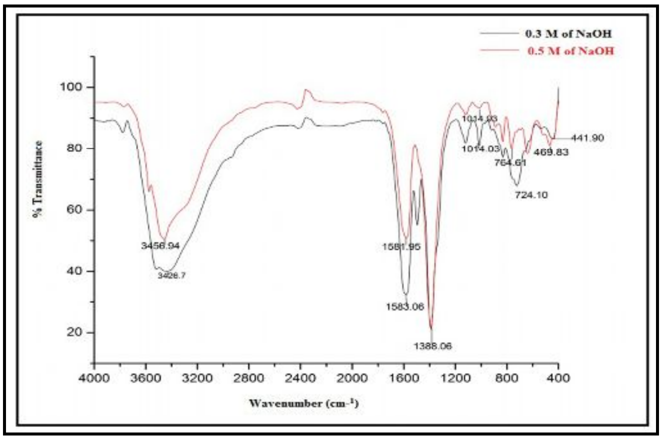


Fig. 2 FTIR spectrum of ZnO nanoparticles (0.3 and 0.5M NaOH)

UV-vis analysis

Fig. 3 depicts the UV- vis spectrum of ZnO nanoparticles (0.3M of NaOH and 0.5M of NaOH) and their broad absorption peaks are observed at 339.87 and 348.91nm respectively. UV-Visible absorption spectroscopy is broadly used to study the optical properties of nanoparticles. UV spectrum reveals that the ZnO nano particles have high absorption at low wavelength region and high transmission in high wavelength region. It may be due to free carrier absorption in the higher frequency region.

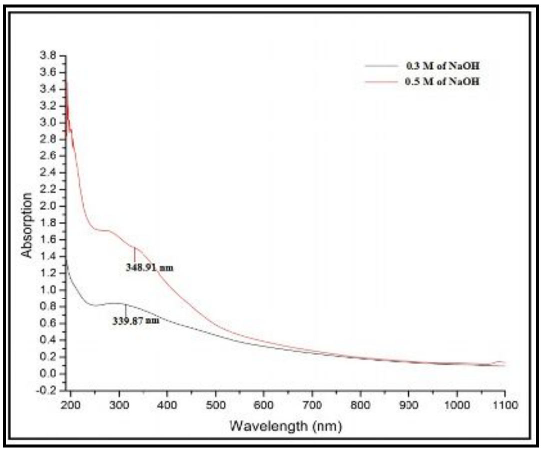


Fig. 3 UV-vis spectrum of ZnO nanoparticles (0.3 and 0.5M NaOH)

FESEM with EDAX analysis

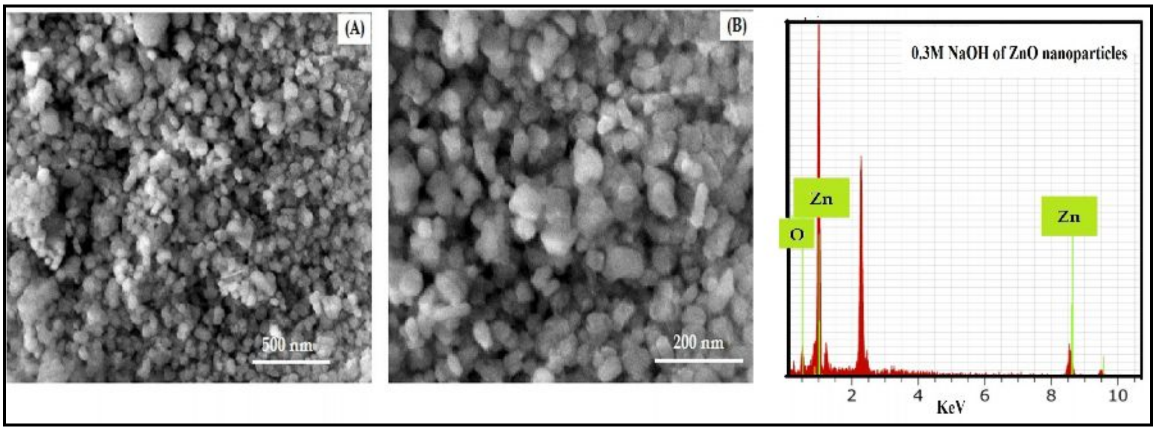


Fig. 4 FESEM with EDAX image of 0.3M NaOH of ZnO nanoparticles

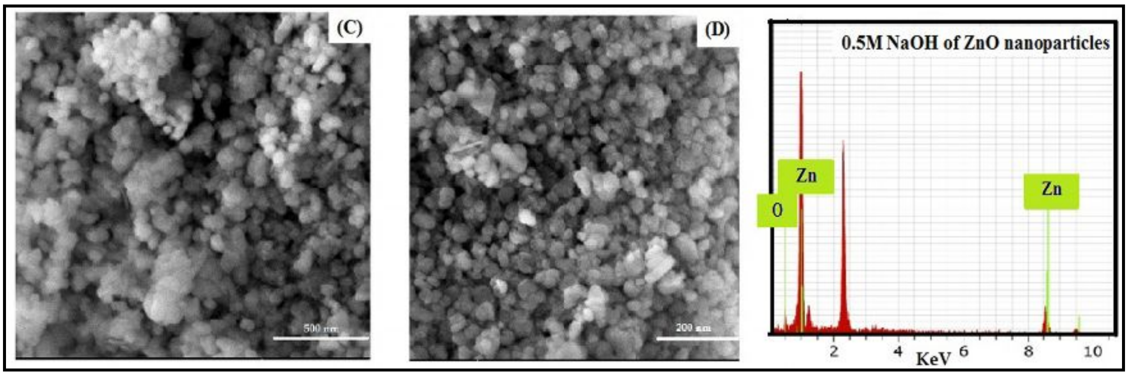


Fig. 5 FESEM with EDAX image of 0.5M NaOH of ZnO nanoparticles

The FESEM micrograph of the ZnO by hydrothermal method is shown in Fig. 4 and Fig. 5. The nano crystallite is wurtzite in nature and belongs to the hexagonal base. The FESEM image confirms the nano-honeycomb structure of ZnO. The nanoparticles were subjected to EDAX analysis (Fig. 4 & 5) which shows the general composition of ZnO studied, thus clearly confirms the presence of Zn and O. The detailed elemental compositions are presented in Table 1.

Table 1 Elemental composition of ZnO NPs.

S. No.	Sample	Elements	Atomic (%)	Weight (%)
1	0.3M NaOH of ZnO	Zn O	72.35 27.65	56.20 43.80
2	0.5M NaOH of ZnO	Zn O	71.43 28.57	57.24 42.76

Antibacterial activity of ZnO nanoparticles

ZnO nanoparticles were tested for its antibacterial activity against the gram-negative bacterial pathogens, *Escherichia coli, klebsiella pneumoniae and Vibrio cholerae* by Kirby-Bauer disk-diffusion method[17,18] and shown in Fig. 6 & 7. Zone of inhibition values determined for the treated 0.3M NaOH of ZnO and 0.5M NaOH of ZnO nanoparticles were shown in Table 2. Both treated fabric and ZnO nanoparticles pronounced significant growth inhibitory effect against both bacteria due to their large surface area by their nanosize.

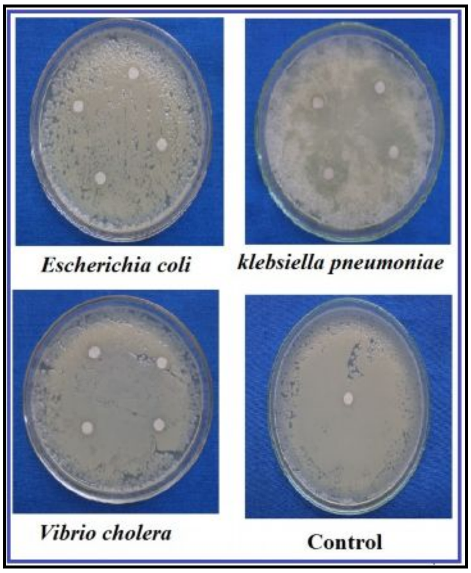


Fig. 6 Antibacterial activity of 0.3M NaOH of ZnO nanoparticles treated against *Escherichia coli, klebsiella pneumoniae and Vibrio cholerae*

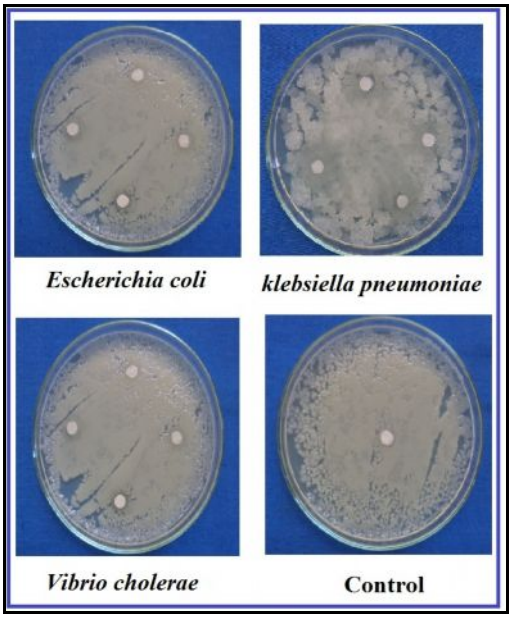


Fig. 7 Antibacterial activity of 0.5M NaOH of ZnO nanoparticles treated against *Escherichia coli, klebsiella pneumoniae and Vibrio cholerae*

Table 2 Zone of inhibition (mm) values against test organisms.

Samples	**Bacterial Pathogens Zone of inhibition (mm)**		
	Escherichia coli	***klebsiella pneumoniae***	***Vibrio cholerae***
control	0	0	0
0.3M of NaOH ZnO NP's	16.2	20.3	17.6
0.5M of NaOH ZnO NP's	16.3	21.4	18.3

Bacteria have a small pore in cell membrane. Reactive oxygen species (ROS) are produced from the ZnO-nanoparticles actively penetrates the cell membrane using pores of the cell. The outflow of proteins, minerals and some matters from the cell for the reason that ROS penetrate the cell wall. The cell membrane is spoiled, so the bacteria are killed of cell growth[19,20]. A plausible mechanism of ZnO inactivation of bacteria involves the direct interaction between ZnO nanoparticles and cell surfaces, which affects the permeability of membranes where nanoparticles enter and induce oxidative stress in bacterial cells, subsequently resulting in the inhibition of cell growth and eventually in cell death. Consequently, the present result evidently revealed that the synthesized ZnO nanoparticles are destroyed or inhibited of cell growth of *Escherichia coli, klebsiella pneumoniae and Vibrio cholerae*.

Conclusion

The composition and quality of the nanoparticles were analyzed by FTIR and EDAX studies. The XRD confirms the crystal structure of the sample. The surface morphology of ZnO was studied by FESEM technique which shows that the size of nanoparticles is strongly dependent on NaOH concentration. The grain size of the particles is calculated from the Scherrer's formula by using XRD result. The study shows that 0.3M of NaOH results in average grain size around 18 nm and 0.5M of NaOH results in average grain size around 23 nm. When the concentration of NaOH increases, the size of nanoparticles also increases. ZnO nanoparticles exhibited remarkable antibacterial activity against the pathogenic bacteria. Their superior antibacterial activity and environmental friendly preparation give them potential applicability in bioengineering and other fields. Finally, the work suggests a novel hydrothermal approach for the synthesis of ZnO nanoparticles without any templates. Further, hydrothermal approach is hazardous-free and cost effective. This method helps to produce nanoparticles at large scale in Industries.

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