BVG Trust

J. Sci. Trans. Environ. Technov. 2013, 7(2) : 85-88

Scientific Transactions in Environment and Technovation

Influence of calcination temperature on the antibacterial efficiency of ZnO nanopowders synthesized using a simple soft chemical route

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Abstract

ZnO nanopowders were synthesized at four different calcination temperatures *viz*. 200, 300, 400 and 500 °C using an inexpensive soft chemical method. The effect of calcination temperature on the structural, antibacterial and surface morphological properties of ZnO nanopowders are studied and reported. The x-ray diffraction studies reveal that the degree of crystallinity of the nanopowder enhances gradually with the increase in the calcination temperature. The antibacterial studies through well diffusion test showed that the antibacterial efficiency of the ZnO nanopowder against the bacillus subtilis bacteria is very much enhanced when the calcination temperature is 400 °C. The scanning electron microscope studies agreed well with other results.

Keywords : nanopowder, ZnO, antibacterial studies, XRD, SEM

INTRODUCTION

Semiconductors of technological interest include the semiconducting oxides, like ZnO, SnO_2, NiO, MgO and CdO. ZnO, as one of the most important wide band gap semiconducting materials, has wide range of applications due to its non-toxicity, structure, electronic distribution and the type of conductivity. In addition, ZnO can form a large number of nanostructure configurations. Most importantly, zinc oxide is a green material that is bio-compatible, biodegradable and bio-safe for medical and environmental applications (Li *et al*., 2009).

In the biomedical field, ZnO nanopowders have been investigated for their strong antibacterial activity. In the recent years, nano structured zinc oxide as antimicrobial agent has received increasing attention, because it is stable under harsh processing conditions and it is generally regarded as safe to humans (GRAS) by the U.S. food and Drug Administration (Xie *et al*.,2011). The proposed mechanisms of antibacterial activity include generation of reactive oxygen species and damage to the cell membrane with subsequent interaction of the nanoparticle with the intracellular contents. Seil and Webster 2012 studied the bactericidal action of ZnO nanomaterials demonstrating their ability to induce membrane disruption of both gram-negative and gram-positive bacteria. Reddy *et al.* (2007) demonstrated that ZnO particles had minimal effects on primary human T-cell viability at toxic concentrations to both gram-negative and gram-positive bacteria.

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Nanopowders of ZnO have anti microbial and deodorizing qualities, and hence they are used for packaging purposes. This property along with its ability of neutralizing acids makes it ideal for use in antiseptic creams, healing creams etc. They are also an important component of toothpastes and dental prosthetics. Due to its ability to absorb ultraviolet light, ZnO is also used in sunscreens and sun blocks to prevent sunburns (Prasad and Jha, 2009)

Different techniques have been used to produce ZnO nanopowders like hydrothermal, polyol, soft chemical route, rapid microwave irradiation method, etc. Of these methods, soft chemical synthesis route has several advantages as it is facile, fascinated and inexpensive method which is suitable to prepare a large amount of sample with varied nanostructures (Saravana kumar *et al*., 2012). In this work, ZnO nanopowders have been synthesized using a soft chemical route and the effect of calcination temperature (200, 300…. 500 °C) on their physical and the antibacterial properties has been investigated and reported.

MATERIALS AND METHODS

Synthesis process

The ZnO nanopowders were synthesized using simple soft chemical method. Zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$) (0.2 M) was used as source precursor. The pH value was maintained at 7 by adding required amount of NaOH solution with the starting solution. The prepared solution was magnetically stirred for about 2 h at a temperature of 85 °C. After the completion of the stirring process, the resultant solution was cooled to room temperature and kept undisturbed for 1 h to get the required precipitate. Then,

it is filtered and washed separately with a mixture of ethanol and water kept in the ratio of 1:3. Finally, the product was calcined at four different temperature *viz.* 200, 300, 400 and 500 °C for 2 h.

Characterization of ZnO nanopowders

The crystalline structure of the synthesized powders was analyzed using x-ray powder diffraction (PANalytical-PW 340/60 X' pert PRO) technique using Cu-K_a radiation (l = 1.5406 Å). The surface images were observed using scanning electron microscope (SEM) (HITACHI S-3000H).

Evaluation of antibacterial activity

The antibacterial activity of the ZnO nanopowders was tested against *bacillus subtilis* bacteria, using the well diffusion method. Three wells each of 5 mm diameter were made in the agar plates with the help of sterile cork borer. The stock solution was prepared by dissolving 1 mg of nanopowder in 1 mL of water. Then the wells were inoculated with 100, 200 and 300 mL of dispersed stock solution of the product. All the plates were incubated at 37 °C for 24 h. After incubation, the plates were observed for the formation of clear inhibition zone around the well which indication the antibacterial activity of the samples. The zone of inhibition was noted by measuring the diameter of the inhibition zone around the well.

Table 1: Structural parameters of ZnO nanopowders

Calcined Temperature °C	Lattice Constants(Å)		D (nm)	V(Å)^3
	a	c		
200	3.2356	5.1807	38	47.22
300	3.2378	5.1865	42	47.35
400	3.2409	5.1879	47	47.49
500	3.2435	5.1897	52	47.51

RESULTS AND DISCUSSION

Structural studies

The XRD patterns of ZnO nanopowders calcined at 200, 300, 400 and 500 C, respectively are presented in Fig. 1. The diffraction peaks located at 2q = 31.886°, 34.537°, 36.348°, 47.703°, 56.679°, 62.904°, 66.002°, 68.101°, 69.204° and 77.003°, correspond to the planes (100), (002), (101), (102), (110), (103), (200), (112), (201) and (202) of the hexagonal wurtzite structure of ZnO, respectively according to the JCPDS card no. 36-1451. From the Fig. 1, it is obvious that all the above mentioned peaks are present in all the four cases, irrespective of the calcination temperature. But, the intensity of the peaks increases gradually with the increase in the calcination temperature, indicating the gradual improvement in the crystalline quality of the samples. From the Fig. 1, it is seen that the preferential orientation is along the (101) plane. It is also observed that the predominancy of the (101) peak is not affected by the calcination temperature.

No diffraction peaks belonging to the impurities like $Zn(OH)_2$ are observed in the pattern, which indicates that all the samples have single phase of ZnO.

The broad and weak peaks along with amorphous nature observed in the case of lowest calcination temperature (200 °C) indicate that this temperature is insufficient for the formation of crystalline nanopowders. As the the calcination temperature increases from 300 to 500 °C, all the diffracted peaks become stronger and narrower. This is attributed to the proper periodic formation of atoms in the ZnO lattice at this temperature.

The average crystallite size is estimated using the well-known Scherrer's formula, (Jabena Begum and Ravichandran, 2013),

$$D = \frac{0.9\lambda}{\beta \cos\theta} \qquad (1)$$

where β is the broadening of the diffraction peak at half of its maximum intensity, λ is the wavelength of the X-ray used and θ is the Bragg's angle.

The crystallite sizes of the ZnO nanopowders calcined at temperatures of 200, 300, 400 and 500 °C are given in Table 1. The results show that the crystallite size increases gradually with the increase in the calcination temperature.

The lattice constants 'a' and 'c' and the volume of the unit cell are calculated using the formulae,

$$\frac{1}{d^2} = \frac{4}{3}\frac{(h^2 + hk + k^2)}{a^2} + \frac{l^2}{c^2} \qquad (2)$$

$$V = \frac{\sqrt{3}}{2} a^2 c \qquad (3)$$

The estimated lattice constant values and the volume of the unit cell are agreed well with the standard values as referred from the JCPDS file (JCPDS card no 36-1451).

As the sample prepared at the calcinations temperature 200 °C was found to exhibit poor crystalline quality, it was not considered for further investigations. Only three samples prepared at calcination temperatures like

300, 400 and 500 °C were characterized for their antibacterial and surface properties.

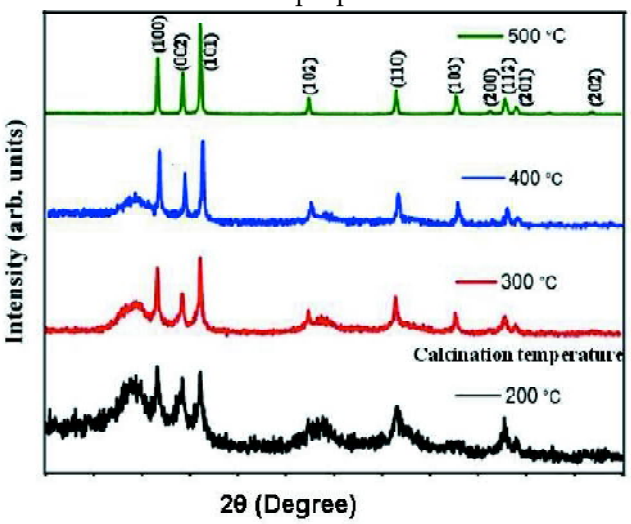

Fig. 1 XRD patterns of ZnO nanopowders calcined at different temperatures

Antibacterial activity

The antibacterial activity of zinc oxide nanoparticles calcined at 300, 400 and 500 °C, was investigated against *bacillus subtilis* using agar well diffusion technique and the corresponding inhibition zones are shown in Fig. 2. It is observed that the diameter of the inhibition zone is 14 mm when the calcination temperature was 300 °C and it increases to 19 mm for 400 °C. The zone again diminishes (16 mm) for 500 °C. The reason for these results may be explained as follows: (i) The poor antibacterial efficiency in the case of 300 °C may be attributed to the insufficient thermal energy required for the formation of crystalline ZnO, (ii) The good antibacterial efficiency observed at 400 °C is due to the generation of more number of reactive oxygen species (ROS) such as hydroxyl radicals (·OH), superoxides ($\cdot O_2^-$) and H_2O_2 caused by the presence of higher number of oxygen vacancies in the ZnO lattice, (iii) The degradation in the antibacterial efficiency beyond 400 °C is ascribed to the possible decrease in the concentration of oxygen vacancies which leads to the formation of near stoichiometric ZnO crystalline system which is confirmed from the XRD profile, and thus (iv) The antibacterial mechanism of ZnO nanopowders is related to the release of Zn^{2+} ions from the ZnO lattice and the generation of active species from the surface of ZnO (Amornpitoksuk *et al.*, 2011 and Snega *et al.*, 2013). The role of electron-hole pair for the generation of ROS can be expressed by the following equations (Ravichandran et al., 2013):

$$ZnO + h\nu \rightarrow h^+ + e^- \quad (4)$$

$$h^+ + H_2O \rightarrow OH^\bullet + H^+ \quad (5)$$

$$O_2 + e^- \rightarrow O_2^{\bullet -} \quad (6)$$

$$O_2^{\bullet -} + H^+ \rightarrow HO_2^\bullet \quad (7)$$

$$H^+ + HO_2^\bullet + e^- \rightarrow H_2O_2 \quad (8)$$

$$H_2O_2 + O_2^{\bullet -} \rightarrow OH^\bullet + O_2 + OH^- \quad (9)$$

After finding the optimized calcination temperature (400 °C) for the antibacterial studies, the well diffusion test experiment was repeated for different amount of stock solution *viz* 100, 200 and 300 µL. The results observed for this study are shown in Fig 3. The Fig.3 depicts that the antibacterial efficiency is remarkable when the amount of the stock solution is 300 µL (values

Fig. 2 Inhibition zone of ZnO nanopowders calcined at different temperatures

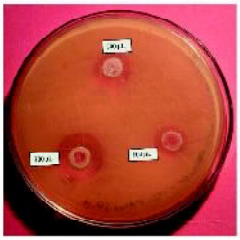

Fig. 3 Inhibition zones for different amount of ZnO nanopowders calcined at 400 °C

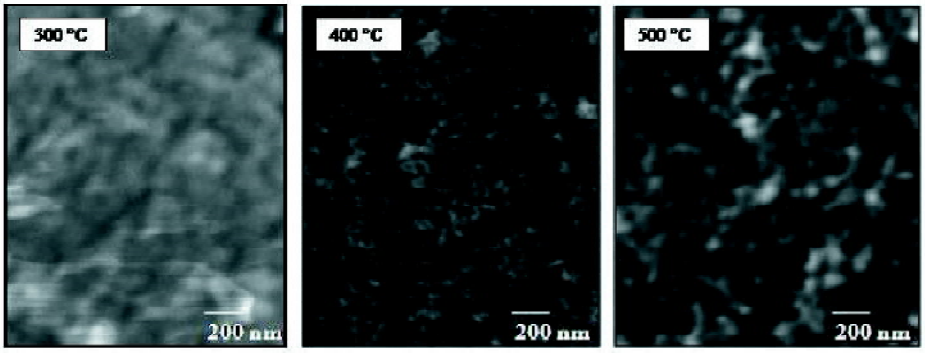

Fig. 4 SEM images of ZnO nanopowders with different calcination temperature

are given in Table. 2). From these results, it is found that greater the amount of the stock solution the higher the antibacterial efficiency. This may be due to the increase in the liberation of Zn^{2+} ions from the nanopowder which could lead to the rupturing of the cell wall and leakage of cell contents which is the main reason for the enhancement in the antibacterial efficiency (Vasanthi *et al*., 2013).

Table 2: Diameter of zone of inhibition of ZnO nanopowders (calcined at 400 °C) against bacillus subtilis bacteria

Amount of stork solution (µL)	Zone of inhibition (mm)
100	15
200	19
300	22

Scanning electron microscope studies

The surface imaging of the ZnO nanopowders calcined at 300, 400 and 500 °C are shown in Fig. 4. From the Fig. 4, it can be seen that the size of the grains is altered due to the increase in the calcination temperature. At low temperature (300 °C), the grains seem to be not well defined. As the calcination temperature increases to 400 °C, the grain size becomes smaller and uniform in size and appears homogeneous. The surface looks more compact and smooth with good packing density. The surface to volume ratio is found to be larger in this case which may be one of the reasons for the good antibacterial efficiency at this temperature. The nanopowders have larger grains with well defined grain boundaries at 500 °C.

CONCLUSION

Good quality ZnO nanopowders were successfully synthesized using a soft chemical route and the effect of calcination temperature on their antibacterial and certain other properties were studied. It is found that at the calcination temperature of 400 °C, the antibacterial efficiency is higher compared with the other values. The XRD and scanning electron microscope studies supplement the results.

Acknowledgements

Financial support from the University Grants Commission of India through the Major Research Project (F. No. 40-28/2011(SR)) is gratefully acknowledged.

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