Direct and indirect sonication affect differently the microstructure and the morphology of ZnO nanoparticles: Optical behavior and its antibacterial activity

Zahra Sharifalhoseini a, Mohammad H. Entezari a,b,⇑, Razieh Jalal c

a Sonochemical Research Center, Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, 91779, Mashhad, Iran
b Environmental Chemistry Research Center, Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, 91779, Mashhad, Iran
c Biochemical Research Center, Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, 91779, Mashhad, Iran

Article history:
Received 23 February 2015
Received in revised form 7 June 2015
Accepted 19 June 2015
Available online 19 June 2015

Keywords:
Green method
ZnO NPs
Sono-synthesis
Optical behavior

ABSTRACT

In the present study, the sono-synthesis of ZnO nanoparticles (NPs) was performed by simple, low-cost, and the environmentally friendly method. The synthesis of zinc oxide as an antibacterial agent was performed by an ultrasonic bath (low intensity) for the indirect sonication and a horn system (high intensity) for the direct sonication. The samples synthesized by these two kinds of sonication were compared with each other. Crystallographic structures and the morphologies of the resultant powders were determined by X-ray diffraction (XRD) and transmission electron microscopy (TEM). The XRD patterns showed that both ZnO samples were crystallized in their pure phase. The TEM images confirmed that the morphologies of the products were completely different from each other. Based on the obtained analysis, the probable growth mechanisms were proposed for crystallization of both samples. The antibacterial activity of the synthesized species was evaluated by the colony count method against Escherichia coli O157:H7. Moreover, the optical behavior of the samples was studied by UV–vis spectroscopy and the variation of the ZnO band gap was compared.

1. Introduction

Nanosized structures due to larger surface and higher efficiency than bulk materials have been widely used in many industries including materials science, optics, mechanics, plastics, medicine, and the textile industry [1]. ZnO is one of the most interest nanoparticle, which found as a safe and friendly material for human. This metal oxide has been developed in the dermatological applications as an antibacterial agent and UV absorber in creams, lotions, and ointments [2]. Furthermore, ZnO as a very important II–VI semiconductor with a wide band gap of 3.37 eV has been widely used in a broad range of the electronic, optical, and the piezoelectric applications [3–4].

Synthesis of the various ZnO nanostructures including nanotube [5], nanosheet [6], nanowire [7], and nanorod [8] has been reported. Recently, the successful efforts have been made to make the three-dimensional (3D) ZnO nanostructures due to their promising applications. For example, a scientific report confirmed that the ZnO hierarchical microstructures show high photocatalytic performance in comparison to the other nanoparticles, nanosheets and nanorods structures [9]. In addition, ZnO flower-like crystal, which used as the matrix enhances the enzyme immobilization in biosensor for the detector of H2O2 [10].

Various methods such as chemical vapor deposition [11], hydrothermal [12–13], microwave-assisted method [14], and electrodeposition [15] have been successfully performed for ZnO synthesis. In the past decades, the sonochemical method as the efficient and powerful technique was developed for the synthesis of the wide range of the materials with controlled properties [16–17]. For this purpose, generally high intensity sonicators equipped with a horn have been developed while the bath systems as the low power sonicators have been widely used for the cleaning applications and has been less used in the synthesis processes. In spite of this aspect, it should be mentioned that the use of this apparatus provides the conditions to reach the product with the different properties from those obtained under the high intensity irradiation or even under the conventional methods. Some
researchers have conducted the experiments on the progress of the reaction in the ultrasonic bath [18–19].

The current paper deals with fabrication of the ZnO nanocrystals with the flower-like feature under the low intensity ultrasonic irradiation. The phase structure, morphology and the biological activity of the product have been compared with the one sample prepared under the high acoustic intensity. Furthermore, with regard to the morphological studies, probable mechanisms have been proposed for the growth processes of two sono-synthesized samples.

2. Experimental

2.1. Materials

The reagents including zinc acetate dihydrate, sodium hydroxide, glycerol, and ammonium citrate were purchased from Merck and used without further purification.

2.2. Preparation of Zn(OH)$_2^2^-$

An aqueous zinc acetate dihydrate solution (1.25 mM) was prepared by dissolving 5.5 g of Zn(AC)$_2$·2H$_2$O, as a Zn$^{2+}$ source, in the 50 mL distilled water. Then, by the addition of 8 g of solid NaOH to this solution at 40°C, a transparent solution saturated with Zn(OH)$_2^2^-$ was formed.

2.2.1. Direct method (method I)

The resulting solution (final volume = 55 mL) was dropped to the water-jacketed cell containing 100 mL of distilled water. The temperature of the water in the cell was adjusted at 65 °C. The ultrasonic irradiation was applied to the reaction medium by using an ultrasonic apparatus equipped with a horn (20 kHz, model XL 29 cm). The frequency (kHz) Intensity (W cm$^{-2}$) Temperature (°C) Reagents

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type of irradiation</th>
<th>Ultrasonic status</th>
<th>Ultrasonic parameters</th>
<th>Temperature (°C)</th>
<th>Reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Direct</td>
<td>Horn</td>
<td>20</td>
<td>65 ± 5</td>
<td>Zn(CH$_3$COO)$_2$·2H$_2$O NaOH</td>
</tr>
<tr>
<td>II</td>
<td>Indirect</td>
<td>Bath</td>
<td>40</td>
<td>65 ± 5</td>
<td>Zn(CH$_3$COO)$_2$·2H$_2$O NaOH</td>
</tr>
</tbody>
</table>

2.3. Preparation of nano fluids

The stability of the ZnO suspension during the antibacterial activity tests is an important factor. Therefore, ZnO nano fluids (ZnO NFs) were prepared with the dispersion of ZnO NPs in glycerol as the base fluid and ammonium citrate as dispersant. The weight ratio of dispersant to nanoparticles was kept at 1:1.

2.4. Antibacterial activity assay

2.4.1. Absorption method

To estimate the antibacterial action of ZnO NPs and the fluid components, including glycerol and ammonium citrate, absorption method was applied. For this evaluation, sample I was chosen. The ZnO nano fluid was prepared as a stock solution at a concentration of 5.6 mg mL$^{-1}$ and was diluted twofold serially with tryptic soy broth (TSB) prior to each experiment. Escherichia coli (E. coli) O157:H7 (NTCC: 12900) was grown overnight with aeration in TSB at 37°C. To prepare the logarithmic phase of bacteria in the beginning state, 10 μL of inoculum was transferred into an Erlenmeyer flask containing 10 mL TSB medium and was allowed to grow at 37°C for 3–4 h on a reciprocal shaker (120 rpm). A cell density was estimated by optical density measurement at 630 nm (OD$_{630}$). The final concentration of 10$^6$ colony forming units (CFU) mL$^{-1}$ was adjusted by dilution with TBS and then it was added into ZnO nano fluids with different concentrations (0.375, 0.750, and 1.500 mg mL$^{-1}$). The test samples were incubated at 37°C on a reciprocal shaker. For the absorption method which is based on the turbidity measurement, after 24 h, the growth of the cultures was monitored by measuring OD$_{630}$ using an ELISA microplate reader (Biotek. Elx800). To exclude the influence of the base fluid and dispersant agent on the results, fluids containing glycerol and ammonium citrate without ZnO NPs were also cultured under the same growth conditions. Bacterial suspension without ZnO NFs and culture medium (TSB) without microbial suspension were also considered as positive and negative controls, respectively. Composition of the fluids and ZnO NFs used in these experiments are given in Table 2.

2.4.2. Colony count method

The biological activity of the samples I and II were compared by colony count method. Concentration of 0.750 mg mL$^{-1}$ of ZnO nano fluids was chosen as the proper concentration based on the absorption method results. For this test, a bacterial culture with concentration of 10$^6$ CFU mL$^{-1}$ was added into TSB medium containing ZnO nano fluids with concentration of 0.750 mg mL$^{-1}$ and kept at 37°C for 24 h on a reciprocal shaker. One hundred microliter of the culture was then diluted serially in TSB and inoculated on tryptic soy agar (TSA) plates. After 24 h incubation at 37°C, the numbers of colonies were counted. To get access more accurate results all measurements were performed in triplicate.

2.5. UV–vis absorption

UV–vis spectroscopy was used for the comparison of the optical behavior of the sono-synthesized samples I and II. The UV–vis spectra were recorded by a Unico 2800 UV–vis spectrophotometer.
in the wavelength range of 200–800 nm. For these measurements, the ZnO powders were dispersed in the absolute ethanol in the ultrasonic bath for 5 min at the ambient temperature.

3. Results and discussion

3.1. XRD analysis

The crystallographic structures for the ZnO powders were determined by X-ray diffraction. Recording of XRD spectra was performed by using of X-ray diffractometer (D8 Advance) with Cu Kα radiation (\(\lambda = 0.154 \text{ nm}\)). Fig. 1(a) and (b) depict the XRD patterns for the prepared ZnO powders; (a) the sample was synthesized by ultrasonic horn, and (b) by ultrasonic bath.

Characteristic diffraction peaks of ZnO including (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) were observed for two prepared samples. All the diffraction peaks can be well indexed to the hexagonal phase ZnO reported in JCPDS card (No. 36-1451, \(a = b = 3.249 \text{ Å} \) and \(c = 5.206 \text{ Å}\)). The presence of the high intensity peaks in Fig. 1(a) and (b) implied that the products were formed in the high crystalline structures. Moreover, these patterns confirm that the creation of the pure phases of ZnO occurred under the different sonication conditions because of no extra peaks were detected in the XRD analysis. The observation of the similar peaks with different intensity in two patterns is attributed to the various crystallographic structures. On the other word, the relative intensities of the diffraction peaks are implying that the preferred orientations occurred during the crystallization [20]. The (100)/(002) intensity ratio is reported 1.17 for the isotropic ZnO. This value is obtained from the XRD pattern collected for pic ZnO. This value is obtained from the XRD pattern collected for the ZnO powders [21]. As can be seen in Fig. 1, the intensity of the (001) peak with respect to the (002) peak of the samples I and II are different from each other. Based on our measurements, the values of this parameter are 1.61 and 0.94 for the samples I and II, respectively. Therefore, both of the samples show deviation from the isotropic structure. As an interesting result, these values of the (110)/(103) ratio were repeated in the obtained XRD patterns.

3.2. FTIR spectroscopy

The mid-IR spectra were recorded by using Fourier transform infrared spectrophotometer (FTIR, Bomem MB-154) in the range of 500–4000 cm\(^{-1}\). The mid-IR spectra are shown in Fig. 2(a) and (b). As can be seen from these figures, the characteristic band of ZnO could be detected in two samples at about 500–530 cm\(^{-1}\). This sharp peak is ascribed to the Zn–O bond [22]. Since this peak is located near the end of the spectra, far-IR technique was employed to get more accurate data on this stretching vibration mode. This technique by the scanning of the low wavenumber region provided a better sight of the spectrum and clarified the possible differences in the position of the ZnO peak for the samples I and II. The far-IR spectra were collected in the region 300–600 cm\(^{-1}\) using a Nexus 870 spectrometer (Thermo Nicolet) equipped with a solid substrate beam splitter. The far-IR spectra (Fig. 2(a)’ and (b)’) show clearly the differences in the peak position for the samples and confirm that the IR spectra are influenced by properties of a sample such as the morphology and the particle size. Based on the scientific reports, the pronounced peaks at 430 cm\(^{-1}\), 555 cm\(^{-1}\) and even at 670 cm\(^{-1}\) are assigned to the stretching vibrations of Zn–O bond [23]. Therefore, the presence of three peaks (at 400 cm\(^{-1}\), 506 cm\(^{-1}\) and 545 cm\(^{-1}\) and two peaks (at 425 cm\(^{-1}\) and 494 cm\(^{-1}\) observed in the far-IR spectra of the sample I and II respectively, could be assigned to the existence of the Zn–O bonds with different strengths. It appears that the differences in the crystalline structure of the samples I and II, which were confirmed by the XRD patterns resulted in the slight shift in the position of the characteristic peaks.

In FTIR method, absorption of some unwanted molecules such as \(\text{H}_2\text{O}\) and \(\text{CO}_2\) from the air on KBr pellet occurs during its preparation or in the recording of the spectrum. These molecules show the specific infrared bands. The peak at 3200–3600 cm\(^{-1}\) is attributed to the –OH bond and the peak at 1632 cm\(^{-1}\) is referred to bending

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of ZnO NPs (mg mL(^{-1}))</th>
<th>Concentration of Ammonium citrate (mg mL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluids(^a)</td>
<td>0</td>
<td>0.375</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.750</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.500</td>
</tr>
<tr>
<td>ZnO nanofluids(^a)</td>
<td>0.375</td>
<td>0.375</td>
</tr>
<tr>
<td></td>
<td>0.750</td>
<td>0.750</td>
</tr>
<tr>
<td></td>
<td>1.500</td>
<td>1.500</td>
</tr>
</tbody>
</table>

\(^a\) In all above suspensions glycerol was used as the base fluid.

Fig. 1. XRD patterns for the prepared ZnO powders; (a) the sample was synthesized by ultrasonic horn, and (b) by ultrasonic bath.
mode of H$_2$O. Presence of CO$_2$ is characterized by a weak peak at 2400 cm$^{-1}$, which is due to the asymmetric stretch –CO bond.

3.3. TEM images

Fig. 3(a) and (b) show the morphology of the samples I and II, respectively. The images showed that the direct sonication produces the flower-like with the sharp part at the end of the branches (needle form) while under indirect sonication, particles crystallized in a star appearance with the leaf like parts. From the morphological studies, some points could be concluded as follows:

- The presence of the small particles and the ordered crystals with the large size, which could be detected in TEM images implying that the Ostwald ripening process, happened during the particle growth. According to this phenomenon, small crystals tend to dissolve and deposit on the surface of the larger

![Fig. 2. FT-IR analysis for the prepared samples; (a & b) point to mid-IR, and (a' & b') are attributed to the far-IR spectra of the samples I and II, respectively.](image)

![Fig. 3. TEM images of ZnO prepared under the different conditions; (a) flower-like of the sample I synthesized under high intensity, and (b) star-like of sample II fabricated under low intensity irradiation.](image)
particles. Indeed, the formation of the larger crystals by reduc-
tion of the external surface leads to approach the lower energy
levels. Therefore, this process happens spontaneously.

- The irradiation under direct and indirect ways promoted the
crystallization in two different paths. In addition to the inequality
of the frequency of these two systems, the different types of
the ultrasonic wave propagation through the reaction medium,
created the different structures.

- The comparison of the crystallographic structures of the sam-
ples I and II with the conventional sample (prepared without
the ultrasound and the TEM images are shown in the
Supplementary data, Fig. 1S) confirmed that the primary nucle-
ation was deeply affected by the presence of the ultrasonic irra-
diation. Applying of ultrasonic energy led to make the cluster
form of ZnO while in the conventional procedure the isolated
nanorod particles were formed. This phenomenon was assigned
to the cavitation. As it is known, the strong streams, including
shockwaves and microjets produced during the cavitation pro-
cess agitate the medium in the effective way and increase the
possibility of the particle joining. Based on our results the
growth mechanisms were proposed for both samples.

3.3.1. Growth mechanisms

For crystallization of the flower-like structure (sample I), two
possibilities could be considered:

i. Joining of the nuclei and generation of the symmetrical form
in the primary steps of the growth process.

ii. Agglomeration of the grown particles and formation of the
ordered structure in the next steps of the crystallization.

As it is known, many examples could be found in the nature
with the symmetric patterns in the macro and even in the micro
levels. It has been scientifically proven that the existence of these
patterns is due to the possessing of more stability in symmetric
configurations.

Concerning to the tendency of having the symmetry and obser-
vation of the flower-like structure with the equal length branches,
the first path seems more possible to happen. By this assumption,
the probable mechanism for crystallization of the flower-like struc-
ture could be illustrated as follows:

At first, the hexagonal prisms were considered as the primary
nuclei of ZnO. As it is known, a hexagonal prism includes two
hexagonal “basal” faces and six rectangular “prism” faces. This
structure has two polar planes consist of (001) face
(zinc-terminated plane) and (001) face (oxygen-terminated
plane). The (001) face, which belongs to the symmetry group
(C15) has higher symmetry and growth occurs along it (c-axis) [21].

It could be assumed that some primary nuclei (such as six or
eight nuclei) were joined. The extension along the c-axis of each
nucleus and the formation of the pyramid after this step resulted
in the final structure that is similar to the shape of the sample I.
By the comparison of the morphology of the conventional sample
(synthesized under the static state) and the sample I (produced
under the high intensity irradiation), it can be concluded that the
aggregation or fusion of the ZnO nuclei in the sample I was due
to the transferring of the acoustic energy to the reaction medium.
The implosive collapse of the bubble generates localized hot spots
through adiabatic compression or shock waves. Fusion of these
nuclei could be illustrated by the high temperature (~5000 K)
and high pressure (~20 MPa) produced in the hot spots. Fig. 4 rep-
resents a possible growth path for this state in a schematic view.

Under the low intensity irradiation, crystallization progress
occurred in another path. The final structure of the sample II
may be shaped by the particle coalescence. The alignment of the
particles created the flake-like pieces. These blocks were oriented
and formed the low-energy configuration in the star-like (Fig. 5).
As it is well known, the combination of the small particles and
their agglomeration during and even at the end of the crystalliza-
tion is due to the inherent tendency for reduction of the fee energy
of the particles. The oriented attachment process occurred under
continuous sonication by the ultrasonic bath is in agreement with
the results reported in the literature [19].

3.4. UV–vis analysis

The room temperature UV–vis absorption spectra (Fig. 6) show
an exciton band at 370 nm for both samples. These absorption

Fig. 4. Schematic view of the possible mechanism for the formation of the flower-like structure (sample I). The hexagonal prism was considered as a simple model describing
of the hexagonal wurtzite ZnO.
peaks present a blue shift compared to the bulk exciton absorption of ZnO (380 nm). This shift is due to the size effect of the nanostructures [22]. The sample I shows marginally higher UV absorption in comparison to the sample II. Moreover, absorption in the visible range of wavelength could be observed for this sample. This optical behavior could be attributed to the existence of the defect energy levels in this structure [24].

3.5. Biological activity

Evaluation of antibacterial activity was performed for the fluids and ZnO NFs with three concentrations of 0.375, 0.750, and 1.500 mg mL\(^{-1}\). The growth of the treated groups was compared to the positive control and the percentage of growth inhibition in comparison with the positive control was determined using Eq. (1):

\[
\text{Growth inhibitory percentage} = 100 \times \frac{OD_{630 \text{ for the test sample}}}{OD_{630 \text{ for the positive sample}}} (1)
\]

The results of these measurements are represented graphically in Fig. 7.

As can be seen from the graph, ZnO NFs in low concentration (0.375 mg mL\(^{-1}\)) shows the low antibacterial activity (about 16%) while in high concentration (1.5 mg mL\(^{-1}\)), in addition to the ZnO NFs, the fluid shows strong antibacterial action alone. This behavior is assigned to the presence of the high concentration of ammonium citrate, which has biological activity toward \(E. \ coli\) [25]. In the medium concentration (0.750 mg mL\(^{-1}\)), the values of the growth inhibitory percentage were 75% and 20% for the ZnO NFs and fluid respectively. Therefore, this concentration was chosen as a proper value for the colony count method.

Comparison of the antimicrobial activity of the samples I and II was carried out by using of colony count method and the recovery of \(E. \ coli\) on the TSA plate is shown in Fig. 8.

The colonies were counted and the results shown as a graph (Fig. 9). The values of the colonies grown on the plate are about 18% and 22% respect to the positive control (set as %) for the samples I and II, respectively (this value for the conventional sample was evaluated 21%). The reported data for each specimen is an average of three measurements.
Many researchers have been conducted on the evaluation of the antibacterial activity of ZnO nanoparticles against a broad spectrum of microorganisms. In addition, different groups have worked to find the mechanism of the biocidal activity of this antibacterial agent. For instance, the results of a study carried out into the role of size scale of ZnO nano- and micro-particles on toxicity to bacteria and osteoblast cancer cells indicated that the ZnO nanoparticles show antibacterial activity apparently greater than microparticles [26]. On the other hand, another study claimed that the ranges of differently sized ZnO powders (10^1–10^4 nm) exhibited the similar antibacterial activity and this precluded discerning the effect of size on toxicity [27]. In fact, the effect of some factors, including testing methods and physicochemical properties of the particles such as size, crystal structure, defects, surface energy, surface charge, surface morphology, surface area, solubility, and even agglomeration state on the antibacterial activity of the species is the reason behind these observations [28]. In the case of its mechanism, the biological activity of ZnO is mostly attributed to the generation of the reactive oxygen species (ROS) and the existence of H_2O_2 in the presence of ZnO has been affirmed [29]. However, the exact mechanism of this generation has not yet been clarified because of zinc oxide shows this property even under dark conditions. Release of zinc ion, membrane dysfunction, and nanoparticle internalization are other paths considered for the biological action of this metal oxide [30].

4. Conclusion

In summary, sono-synthesis of ZnO NPs was carried out via high-efficiency and green approach under direct and indirect sonication. Characterization of the resulting samples showed that the pure phases of ZnO NPs were synthesized with the high crystalline structures. The low and high intensity irradiation led to make the particles with different morphologies. The optical property of the samples were investigated. The preparation of the 3D structure of ZnO NPs with high purity and good antibacterial activity confirmed that the bath sonicator could be more attended as a useful vessel for the synthesis processes of the materials.

Acknowledgements

The support of Ferdowsi University of Mashhad (Research and Technology) for this work (3/21049, 08/03/2012) is appreciated.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ultsonch.2015.06.016.

References


