Antibacterial and physical properties of ZnO with pH-sensitive morphology

Chat Pholnak\textsuperscript{a}, Monthon Lertworapreecha\textsuperscript{b}, Chitnarong Sirisathitkul\textsuperscript{c} and Sumetha Suwanboond\textsuperscript{d}

\textsuperscript{a}Department of Physics, Faculty of Science, Thaksin University, Phatthalung, Thailand; \textsuperscript{b}Department of Biology, Faculty of Science, Thaksin University, Phatthalung, Thailand; \textsuperscript{c}Molecular Technology Research Unit, School of Science, Walailak University, Nakhon Si Thammarat, Thailand; \textsuperscript{d}Department of Materials Science and Technology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

\textbf{ABSTRACT}

The variation in morphology of zinc oxide (ZnO) with the pH of 0.05 M Zn(NO$_3$)$_2$ and 1.00 M NaOH aqueous solutions is demonstrated. Microstructured ZnO ranges from cuboid to spindle as the pH is reduced from 12 to 9. The ratio of visible-to-UV peak from photoluminescence spectra is also decreased, whereas the energy gap calculated from UV-Vis spectra remains comparable. Furthermore, nanospheroids and microellipsoid are, respectively, obtained when the molar concentration of NaOH is reduced to 0.20 and 0.10 M for the synthesis with the pH of 7. Due to their large surface-to-volume ratio, these ZnO nanospheroids exhibit a higher antibacterial activity than that of commercial ZnO in the disc diffusion method. According to this method as well as the minimal inhibitory concentration measurement, the effectiveness is substantial in the case of \textit{Staphylococcus aureus} and \textit{Escherichia coli}. By contrast, the inhibitions of \textit{Klebsiella pneumoniae}, \textit{Salmonella typhimurium} and \textit{Pseudomonas aeruginosa} are barely observed.

\textbf{ARTICLE HISTORY}

Received 9 March 2016
Accepted 17 July 2016

\textbf{KEYWORDS}

ZnO; pH; antibacterial activities; photoluminescence; electrical impedance

\section{1. Introduction}

Zinc oxide (ZnO) is a versatile material which has found a variety of applications. In addition to its electrical and optical characteristics for devices, ZnO nanostructures exhibit antifungal [1,2] and antibacterial properties.[2–12] A variety of Gram-positive and Gram-negative bacteria strains including \textit{Salmonella typhimurium}, \textit{Klebsiella pneumoniae}, [4] \textit{Staphylococcus aureus}, \textit{Escherichia coli},[5,6] \textit{Bacillus subtilis}, \textit{Aerobacter aerogenes},[7] \textit{Candida albicans} [8] and \textit{Streptococcus mutans} [9] have been under test. The antibacterial activity, dependent on both size [5,10,11] and shape [8,12] of ZnO, is further enhanced by either doping [13] or ultraviolet-visible (UV-Vis) lights.[2,14] This bacteriostatic agent could then be effectively used to control the bacterial infections under illuminations. Different mechanisms have been proposed to explain this antibacterial activity. ZnO nanoparticles theoretically exhibit strong cellular internalisation, high surface defect concentration, and generate large amount of hydroxyl radicals or reactive-oxygen species.
(ROS).[5,14] The surface-to-volume ratio and defects also regulate electrical and optical properties. The photon and electrical charge transfers are sensitive to the variations in size and shape of ZnO.

A considerable advantage of ZnO is also derived from its facile synthesis. Various morphologies have reportedly obtained from reaction in aqueous solutions of Zn(NO₃)₂ and NaOH involving the ions.

\[
\text{Zn(NO}_3\text{)}_2 \leftrightarrow \text{Zn}^{2+} + 2\text{NO}_3^{-} \tag{1}
\]

\[
\text{NaOH} \leftrightarrow \text{Na}^+ + \text{OH}^- \tag{2}
\]

The reaction, like one between Zn(NO₃)₂ and hexamethylenetetramine, can be assisted with ultrasonic irradiation. Under ultrasonic irradiation, the dehydration reaction takes place as follows:

\[
\text{Zn}^{2+} + 4\text{OH}^- \rightarrow [\text{Zn(OH)}]_4^{2-} \tag{3}
\]

\[
2[\text{Zn(OH)}]_4^{2-} \rightarrow 2\text{ZnO} + 4\text{H}_2 + 3\text{O}_2 \tag{4}
\]

The effects of sonication condition as well as other synthesis parameters have been reported. Single phase ZnO is obtained from the solution growth using Zn(NO₃)₂ around 0.05 M. Either higher or lower concentration may lead to a mixture with other zinc compounds.[15] In addition to reagent concentrations, the ultrasonic irradiation time has influenced the growth mechanism and optical properties of ZnO nanoparticles.[16] The sonication power from standard ultrasonic baths is commonly sufficient for the synthesis. High sonication powers and temperatures tend to cause agglomerations into microclusters.[17]

Since the concentration of Zn(OH)₄²⁻ is increased with increasing pH of aqueous solutions, the pH is expected to play an important role in the ultrasonic-assisted solution growth of ZnO. According to Xiao et al.,[18] the pH condition and zinc salt counter ions are key parameters in the formation of rod-like and sheet-like ZnO synthesised from various Zn compounds and NaOH by the sonochemical method. In this study, the morphology of ZnO was investigated by varying the pH in the ultrasonic-assisted synthesis. The antibacterial activity of ZnO is then tested on E. coli, S. aureus, K. pneumoniae, S. typhimurium and P. aeruginosa which are common causes of skin inflammation and digestive disorders. Electrical and optical properties are also measured for the possible enhanced functionality.

2. Experimental

ZnO was synthesised from the reaction in 0.05 M Zn(NO₃)₂ and 1.00 M NaOH aqueous solutions under ultrasonic irradiation. The solutions were prepared by dissolving the Zn(NO₃)₂ and NaOH in deionised water and slowly mixed in a beaker under stirring at 50 °C. The beaker was then transferred to a 150 W ultrasonic bath and sonicated at 46 kHz for 30 min. The particles were collected, repeatedly washed, and finally dried at 105 °C for 18 h. Four samples (C1–C4) were prepared to compare the effect of pH from
9 to 12 on the morphology of ZnO. Two other samples were also synthesised at the pH of 7 by decreasing the concentration NaOH to 0.20 M (Sample B0) and 0.10 M (Sample C0). The synthesis conditions are listed in Table 1. The pH was adjusted by adding NaOH while keeping the ratio between Zn(NO₃)₂ and NaOH constant for each sample. The change in pH was monitored up to 30 min. It was later found that the pH tended to change by less than 0.3. The value recorded at the beginning is then used as the reference to each sample.

The dried ZnO were characterised by X-ray diffractometry (XRD; Phillips X’pert MPD) using Cu-Kα radiation and scanning electron microscopy (SEM; FEI Quanta 400) using accelerating voltage of 25 kV. The UV-vis spectrophotometry (Shimadzu UV-2450) from 200—800 nm and photoluminescence (PL) spectrophotometry (PerkinElmer LS/55) were used in optical characterisations. To measure electrical properties of Sample B0, 1.6 g of dried ZnO powder was milled in an agate mortar with a slow addition of 25 ml polyethylene glycol (PEG-2000). The powder was then pressed, under 4—5 tons for 40 s, into a pellet of 15 mm in a diameter and 2.5 mm in thickness. After that, ZnO pellets were sintered in a tube furnace at 900 °C with a heating rate of 300 °C/h for 4 h and then slowly cooled to room temperature. To obtain parallel electrodes, gold contacts were pasted on both sides of each pellet. The impedance spectroscopy data were collected by using a Hioki 3522-50 LCR Hi-Tester in the frequency range 1 Hz to 1 MHz under laboratory ambient conditions.

The antibacterial activity was tested with five bacteria strains, namely *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (ATCC 700603) and *S. typhimurium* (isolated strain), cultured for 15—20 h. They were adjusted by saline (0.85%) in a densitometer (Biosan) to obtain 0.5 McFarland, equivalent to 1 × 10⁸—2 × 10⁸ CFU/ml of bacteria. In the Minimal Inhibitory Concentration (MIC) measurement in a 96 well-microplate, the bacteria were diluted with Mueller—Hinton broth (Himedia) in a ratio of 1:100. ZnO nanoparticles were dispersed in a 3 M NaOH solution to obtain the concentration of 8192 μg/ml. The bacteria were then added to obtain 5 × 10⁴ CFU/ml and incubated at 37 °C for 24 h. In addition to the MIC, the antibacterial activity of the ZnO nanoparticles was compared to commercial ZnO by the disc diffusion method. The ZnO suspensions in deionised water were prepared with the concentration of 40 mg/ml. They were sonicated in ultrasonic bath at room temperature and then kept in a refrigerator overnight before their use. The ZnO suspension was dropped on Petri dishes containing bacteria-streak Mueller—Hinton agar). The dishes were then incubated at 37 °C for 24 h before measuring the clear zone diameter.

| Sample | Zn(NO₃)₂ (M) | NaOH (M) | Starting pH of the solution | Morphology |
|--------|-------------|----------|-----------------------------|------------|
| B0     | 0.10        | 0.20     | 7                           | Nanospheroid |
| C0     | 0.05        | 0.10     | 7                           | Microellipsoid |
| C1     | 0.05        | 1.00     | 9                           | Spindle-like |
| C2     | 0.05        | 1.00     | 10                          | Star-like |
| C3     | 0.05        | 1.00     | 11                          | Flower-like |
| C4     | 0.05        | 1.00     | 12                          | Cuboid |
3. Results and discussion

3.1. Morphology and phase

All samples exhibit characteristic XRD peaks of ZnO as exemplified in Figure 1. The XRD peaks of Samples C1–C4 at 36.2°, 31.7°, 34.3°, 47.5°, 56.5°, 59.6°, 62.8°, 66.3°, 67.8° and 69.0°, respectively, corresponding to (101), (100), (002), (102), (110), (103), (200), (112), (201) and (004) diffraction planes, are matched by those of wurtzite ZnO with a space group of P63mc (JCPDF No.01-079-2205). No impurity phase is detected. With the Zn(NO₃)₂ to NaOH molar ratio of 1:2, the variations in pH from 9 to 12 do not lead to any change in crystalline ZnO phase.

By contrast, the morphology of ZnO is very sensitive to the variation in the starting pH. As revealed by SEM images in Figures 2(b,c), the shape of ZnO evolves from microellipsoid to symmetric spindle-like structure when the pH is raised from 7 to 9 at a fixed concentration of Zn(NO₃)₂ and NaOH. The ends of ellipsoid become sharper and smaller particles are incorporated into the larger spindle-like structures. A further increase in pH to 10 promotes the linkage between these microspindles resulting in the star-like structures. The formation mechanism of spindle- and star-like structures was described in a previous report.[19] The agglomeration continues at high pH beyond 10 and aggregates from microplates are increased at the expense of spindle- and star-like morphology. At the highest pH used, bulky cuboids with the dimension over 1 μm are obtained. According to the previous suggestion on the growth mechanism of ZnO polyhedron,[16] zinc

![Figure 1](image_url)

**Figure 1.** XRD patterns of Samples (a) C1, (b) C2, (c) C3 and (d) C4.
hydroxide (Zn(OH)$_4^{2-}$) in the mixed solutions is increased with increasing pH value. In a strong alkali medium, the growth units of Zn(OH)$_4^{2-}$ and Zn(OH)$_{4-x}$(ONa)$_x(\mathrm{OH})^{2-}$ complex are formed by the dehydration reaction between shielding growth units. During the crystallisation, the activation of OH$^-$ ligands introduced on the different interfaces of Zn$_x$O$_y$(OH)$_{z-1}$[(ONa)$_m(\mathrm{OH})^{2-}$ results in the growth of various faces of polyhedron. Interestingly, the size of ZnO can be reduced not only by the pH reduction but also the increase in molar concentrations of Zn(NO$_3$)$_2$ and NaOH in the reaction. Figure 2(a) reveals spheroidal ZnO nanoparticles in Sample B0 as a result of the double concentration in the synthesis compared to that for Sample C0. Due to its enhanced surface-to-volume ratio, this ZnO nanospheroid is selected for further investigation for electrical and antibacterial applications.

### 3.2. Optical properties

UV-Vis spectra of ZnO indicate the strong UV absorption up to 400 nm but negligible effect in the visible range. As compared in Figure 3, the absorption is gradually increased with increasing pH from 9 to 12 in the solution growth. This enhancement can be attributed to the large planar surface of cuboids previous shown in the morphological evolution of ZnO with the pH. The variation in pH from 9 to 12 does not affect the energy gap and the values evaluated from the slopes of the plots in Figure 4 remain around 3.2 eV for all samples with different morphologies.

The room temperature PL spectra in Figure 5 reveals four major bands around 318, 390, 410 and 650 nm, respectively, assigned to UV-B, UV-A, violet and red emissions. The UV emission centred at 318 nm is known as the near band edge emission and the UV peak around 390 nm is originated from the recombination of free excitons under low excitation intensities.[20–22] The UV emissions of ZnO are mostly attributed to the near
Figure 3. Room temperature UV-vis spectra of Samples C1—C4.

Figure 4. Slope of plots from UV-Vis spectra to determine energy band of Samples; (a) C1/pH9/spindle, (b) C2/pH10/star, (c) C3/pH11/flower and (d) C4/pH12/cuboid.
band edge emission from the recombination of free excitonic emissions. The narrow violet peak at 412 nm is attributed to the transition from the shallow defect level to the valence band. A broad visible band ranging from 500 to 800 nm presented in all sono-synthesised products is associated with the deep level emission from the intrinsic defects including Zn-interstitial (Zn\(_i\)), O-interstitial (O\(_i\)), Zn-vacancy (V\(_{Zn}\)), O-vacancy (V\(_O\)) and extrinsic impurities.\(^\text{[23]}\) The intensity of this visible peak is highly sensitive to environment which suggests a high density of interstitial atoms and vacancies. Such intensity is also slightly increased with further increase in concentrations because of deep defect levels well below the conduction band. The high intensity of these visible peaks compared to that of the UV absorption is testament of a high density of defects in ZnO crystals. It is fitting that the visible-to-UV peak ratio of spindle- and star-like structures is much higher than those of aggregated samples.

### 3.3. Electrical properties

Electrical transport properties of ZnO nanospheroids can be deduced from electrical impedance spectra. The plot between the real and imaginary parts in Figure 6 is approximately parabolic in the frequency up to 0.35 MHz. According to the grain boundary effect,\(^\text{[24]}\) the electrical conduction through the grain boundaries has a time constant at low temperatures similar to the grain interior of a bulk sample measured in the high frequency range. The arc of impedance spectrum can be modelled as an equivalent circuit shown in an inset of Figure 6. The circuit consists of a parallel \(R_g\) and \(C_g\) elements connected in series with \(R_s\). The \(R_g\) and \(C_g\) are, respectively, the resistance and the capacitance due to the grain boundary of ZnO nanospheroids whereas the \(R_s\) represents the contact resistance. The mean relaxation time of the grain boundary process is \(\tau_g = R_g C_g\). An enhancement of electrical conductivity due to the grain boundary contribution is higher than that of the grain interior. In the case of high surface-to-volume ratio of ZnO...
Nanospheroids, a large number of charge carriers are accumulated at the grain boundary region.

In Figure 7, the imaginary part of the impedance ($Z''$) of ZnO nanospheroids with frequency from 1 Hz to 1 MHz exhibits single peak characteristics which mark the transition in electrical transport from a low frequency to a high frequency range.\cite{24,25} The asymmetric broadening of impedance peak is attributed to the electrical relaxation phenomena.
in ZnO. This peak frequency gives an estimate conduction relaxation time according to the relation

$$\omega \tau = 1$$  \[24\]

Additionally, the hopping of charge carriers between defect sites also contributes to the conduction mechanism in ZnO nanospheriods at room temperature.

### 3.4. Antibacterial properties

Nanospherooids from Sample B0 were selected for the antibacterial test because of large contact areas between ZnO and micro-organism cells.[10] Moreover, the reduction in size enhances the photocatalytic activity of ZnO and eventually leads to hydrogen peroxide detriment to bacteria. The MIC measurement confirms that the control sample without ZnO does not affect the bacteria growth. The MIC of 64 μg/ml for *S. aureus* and 125 μg/ml for *E. coli* indicates the effectiveness on both bacteria strains. The antibacterial properties are reduced with increasing MIC as 512, 1024 and 1024 μg/ml, respectively, for *K. pneumoniae*, *P. aeruginasa* and *S. typhimurium*. In Figure 8, the disk diffusion method emphasises the effective inhibition of *S. aureus* and *E. coli* by ZnO nanospheroids in creating a clear zone with a diameter of 25 and 20 mm. This antibacterial activity is stronger than that of commercial ZnO which leads to the smaller clear zones of 21 and 16 mm in diameter. However, the clear zone is not apparent in the case of three other bacteria strains. The distinct effectiveness for both *S. aureus* and *E. coli* are in good agreement with the previous reports.[5,6,26,27]

### 4. Conclusions

A morphological evolution of ZnO is shown as a function of pH of aqueous solutions of NaOH and Zn(NO$_3$)$_2$. Owing to the large surface-to-volume ratio, the nanosized ZnO
particles are selected for antibacterial tests. The synthesised ZnO nanoparticles effectively inhibit both Gram-positive \((S.\text{ aureus})\) and Gram-negative \((E.\text{ coli})\) pathogenic bacteria. The main bacterial inhibitory mechanisms are the generation of hydrogen peroxide and the electrostatic binding of ZnO nanoparticles on the bacterial surface causing a breaking of cell membrane and then a cell death. The visible emission due to a high defect density in ZnO should lead to a good luminescence, charge transport as well as antibacterial activity.

Acknowledgments
This work was supported by Thaksin University under Grant number R03-6/2557. The authors would like to acknowledge Dr Supab Choopan and the staff of Applied Physics Research Laboratory, Department of Physics and Materials Science, Faculty of Science, Chiang Mai University for the access to the LCR meter.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
Thaksin University [grant number R03-6/2557].

Reference
[1] Sanoop PK, Mahesh KV, Nampoothiri KM, et al. Multifunctional ZnO-biopolymer nanocomposite coatings for health-care polymer forms and fabrics. J Appl Polym Sci. 2012;126: E233–E244.
[2] Sharma D, Rajput J, Kaith BS, et al. Synthesis of ZnO nanoparticles and study of their antibacterial and antifungal properties. Thin Solid Films. 2010;591:1224–1229.
[3] Sirelkhatim A, Mahmud S, Seeni A, et al. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Lett. 2015;7:219–242.
[4] Wahab R, Mishra A, Yun S-I, et al. Fabrication, growth mechanism and antibacterial activity of ZnO micro-spheres prepared via solution process. Biomass Bioenergy. 2012;39:227–236.
[5] Applerot G, Perkas N, Amarian G, et al. Coating of glass with ZnO via ultrasonic irradiation and a study of its antibacterial properties. Appl Surf Sci. 2009;256:S3–S8.
[6] Azam A, Ahmed AS, Oves M, et al. Antimicrobial activity of metal oxide nanoparticles against gram-positive and gram-negative bacteria: a comparative study. Int J Nanomed. 2012;7:6003–6009.
[7] Jain A, Bhargava R, Poddar P. Probing interaction of Gram-positive and Gram-negative bacterial cells with ZnO nanorods. Mater Sci Eng. 2013;33:1247–1253.
[8] Phaechamud T, Mahadlek J, Aroonrerk N, et al. Antimicrobial activities of ZnO-doxycycline hyclate thermosensitive gel. ScienceAsia. 2012;38:64–74.
[9] Eshed M, Lellouche J, Matalon S, et al. Sonochemical coatings of ZnO and CuO nanoparticles inhibit \(Streptococcus\ mutans\) biofilm formation on teeth model. Langmuir. 2012;28:12288–12295.
[10] Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir. 2011;27: 4020–4028.
[11] Yamamoto O. Influence of particle size on the antibacterial activity of zinc oxide. Int J Inorg Mater. 2001;3:643–646.
[12] Talebian N, Amininezhad SM, Doudi M. Controllable synthesis of ZnO nanoparticles and their morphology-dependent antibacterial and optical properties. J Photochem Photobiol B. 2013;120:66–73.
[13] Sharma N, Jandaik S, Kumar S, et al. Synthesis, characterisation and antimicrobial activity of manganese- and iron-doped zinc oxide nanoparticles. J Exp Nanosci. 2016;11:54–71.
[14] Leung YH, Chan CMN, Ng AMC, et al. Antibacterial activity of ZnO nanoparticles with a modified surface under ambient illumination. Nanotechnology. 2012;23, article ID 475703.
[15] Pholnak C, Sirisathitkul C, Suwanboon S, et al. Effects of precursor concentration and reaction time on sonochemically synthesized ZnO nanoparticles. Mater Res. 2014;17:405–411.
[16] Yadav RS, Mishra P, Pandey AC. Growth mechanism and optical property of ZnO nanoparticles synthesized by sonochemical method. Ultrason Sonochem. 2008;15:863–868.
[17] Pholnak C, Suwanboon S, Sirisathitkul C. Evolution and temperature dependence of ZnO formation by high power sonication. J Mater Sci. 2013;24:5014–5022.
[18] Xiao Q, Huang SP, Zhang J, et al. Sonochemical synthesis of ZnO nanosheet. J Alloys Compd. 2008;459:118–122.
[19] Yang JL, Huang WZ, Cheng YL, et al. Morphology-controlled synthesis of gadolinium fluoride nanocrystals via ultrasonic and salt assisted method. CrystEngComm. 2012;14:899–907.
[20] Wu QH. ZnO nanostructures prepared using a vapour transport method. J Exp Nanosci. 2015;10:161–166.
[21] Mishra SK, Srivastava RK, Prakash SG. Photoluminescence and photoconductivity studies of ZnO nanoparticles prepared by solid state reaction method. J Mater Sci. 2013;24:125–134.
[22] Rai P, Kim SG, Yu YT. Microwave assisted synthesis of flower-like ZnO and effect of annealing atmosphere on its photoluminescence property. J Mater Sci. 2012;23:344–348.
[23] Yang J, Li X, Lang J, et al. Effect of mineralizing agent on the morphologies and photoluminescence properties of Eu3+-doped ZnO nanomaterials. J Alloys Compd. 2011;509:10025–10031.
[24] Samuel MS, Koshy J, Chandran A, et al. Electrical charge transport and dielectric response in ZnO nanotubes. Curr Appl Phys. 2011;11:1094–1099.
[25] Chaari M, Matoussi A. Electrical conduction and dielectric studies of ZnO pellets. Physica B. 2012;407:3441–3447.
[26] Ekthammathat N, Thongtem S, Thongtem T, et al. Characterization and antibacterial activity of nanostructured ZnO thin films synthesized through a hydrothermal method. Powder Technol. 2014;254:199–205.
[27] Siddique S, Shah ZH, Shahid S, et al. Preparation, characterization and antibacterial activity of ZnO nanoparticles on broad spectrum of microorganisms. Acta Chim Slov. 2013;60:660–665.