The Improvement of the Band Gap Energy and Antibacterial Activities of CeO$_2$/ZnO Nanocomposites Prepared by High Energy Ball Milling

Sumetha Suwanboon*[a,d], Pongsaton Amornpitoksuk [b,d] and Phuwadol Bangrak [c,e]

[a] Department of Materials Science and Technology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.
[b] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.
[c] School of Science, Walailak University, Nakhon Si Thammarat 80161, Thailand.
[d] Center of Excellence in Nanotechnology for Energy (CENE), Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.
[e] The Research Unit of Natural Product Utilization, Walailuk University, Nakhon Si Thammarat 80161, Thailand.

*Author for correspondence; e-mail: sswanboon@yahoo.com; sumetha.s@psu.ac.th

Received: 22 April 2016
Accepted: 4 August 2016

ABSTRACT

CeO$_2$/ZnO nanocomposites were prepared by mechanical milling. The crystal structure, morphology and optical properties were characterized by X-ray diffractometry, scanning electron microscopy and UV-vis spectrophotometry, respectively. The antibacterial activity was tested by a broth microdilution method to obtain the minimum inhibitory concentrations (MIC). The crystallite size decreased from 29.78 to 20.45 nm whereas the micro-strain increased from 0.0814 to 0.1202 when the CeO$_2$ powders were loaded from 0 to 10 mol%. The widest optical band gap of 3.135 eV was obtained from 5 mol% CeO$_2$/ZnO nanocomposite. The antibacterial activity was improved as a function of the CeO$_2$ loading contents. The best antibacterial activity was obtained from 10 mol% CeO$_2$/ZnO nanocomposites that inhibited towards S. aureus and E. coli with MIC values of 0.78 mg/mL and 3.125 mg/mL, respectively.

Keywords: CeO$_2$/ZnO nanocomposite, mechanical milling, optical property, antibacterial activity

1. INTRODUCTION

Nanocrystalline materials are now being intensively investigated not only because of their fundamental scientific interest, but also for their many applications. At the present time, nanocrystalline inorganic metal oxides such as SnO$_2$ [1], TiO$_2$ [2], MgO [3], and ZnO [4] are being investigated worldwide to find ways to develop new procedures and products that take advantage of their unique properties. Among these oxides, nanocrystalline ZnO has received considerable interest and has been subjected to intensive investigations because ZnO has
many valuable and unique physical properties that allow for many potential applications such as for solar cells [5], as gas sensors [6], light emitting diodes [7], as a rubber additive [8], catalysts [4], pigments [9], cosmetics [10] and an antibacterial agent [4]. Another reason why ZnO has gained worldwide interest, is that the properties of ZnO can be modified and controlled by doping with small amounts of impurities such as Al [11], Mg [11], Ti [11], La [4], Ce [12], etc. Recently, cerium (Ce) has been one of the more interesting dopants and many research groups have studied the effects of Ce concentrations on enhancing some of the properties of ZnO. Some research groups had focused on the preparation of Ce-doped ZnO thin films produced by either chemical or physical methods. Tan et al. [12] prepared ZnO films doped with 2-20 at.% Ce by the sol-gel method and found that the photoluminescence of Ce-doped ZnO films exhibited a blue shift in the UV emission spectra and reduced the blue emission intensity compared with undoped ZnO film. Yousefi et al. [13,14] found that a CeO$_2$/ZnO nanocomposite film fabricated by the sol-gel method showed an increase of photocurrent density when doped with Ce and they also found that the average grain size increased as a function of the annealing temperature and CeO$_2$ phase was formed at 500°C. Anbia M. and Seyyed Ebrahim Moosaei F. [15] prepared a Ce-doped porous ZnO thin film by the sol-gel method for use as a humidity sensor and they reported that the Ce-doped ZnO sensor was highly sensitive to humidity and responded rapidly with good repeatability. Moreover, Chen et al. [16] reported that Ce-doped ZnO deposited by an electrodeposition method had a similar rod-like structure to the ZnO even though the Ce concentration was changed. The optical properties of the Ce-doped ZnO thin films changed when the concentration of Ce and the annealing temperature were changed. The as-deposited Ce-doped ZnO film prepared by the electrodeposition method showed different ratios of UV to visible emission whereas when annealed films were produced, they showed green and orange-red emissions. With as-prepared films by a physical method, Luo et al. [17] reported that Ce-doped ZnO prepared by DC magnetron sputtering exhibited a broad blue luminescence, but ZnO films showed a sharp UV luminescence. Other research groups have concentrated on the synthesis, characterization and properties of Ce-doped ZnO powders. Yayapao et al. [18] synthesized 0-3 mol% Ce-doped ZnO nanoneedles by a sonochemical method and they reported that Ce-doped ZnO nanoneedles exhibited an optical band gap of about 3.00 eV. Also the photocatalytic degradation of a methylene blue solution increased as a function of the Ce concentrations. Karunakaran et al. [19] prepared ZnO and 2 mol% Ce-doped ZnO nanoparticles by the sonochemical method, but they also calcined the samples at 300-900°C for 2 h. They found that the optical band gap decreased after doping with Ce. The Ce-doped ZnO catalyzed the oxidation of both cyanide and cyanate under UV-A irradiation. Moreover, the Ce-doped ZnO was a more effective inhibitor of E. coli than pure ZnO. Nowadays, hydrothermal methods have been used to produce Ce-doped ZnO nanoparticles. Dar et al. [20] synthesized Ce-doped ZnO nanorods and they found that the Ce-doped ZnO nanorods showed a highly reproducible sensitivity whereas Mahmoud et al. [21] reported that Ce-doped ZnO nanoplatelets synthesized at 150°C for 24 h exhibited a blue shift and had a weak UV emission compared with undoped ZnO. In addition, Rezaei M. and Habibi-Yangjeh A. [22] reported that the shape of Ce-doped ZnO changed, after being refluxed at 90°C for 3 h, from nanoplatelets to nanospheres when the mole fraction of Ce$^{4+}$ ions were increased and the ZnO that was doped with 0.05 mole fraction of Ce$^{4+}$ had the highest photocatalytic efficiency. However, use
of the sol-gel technique to control morphology is still fashionable. Muthu Kumaran S. and Gopalakrishnan R. [23] prepared Zn$_{1-x}$Ce$_x$O ($x = 0 - 0.1$) nanoparticles by the sol-gel method and they reported that the crystallite size of the samples decreased, but the micro-strain and optical band gap increased when the Ce concentration was increased. Moreover, Yang et al. [24] fabricated Ce-doped ZnO nanoparticles. They calcined Ce-doped ZnO at 500 °C for 6 h and showed that the UV emission peak shifted to a lower wavelength and the intensity of the visible peak decreased compared with pure ZnO. As far as this aspect is concerned, there have been no reports on the properties of both Ce-doped ZnO nanoparticles and CeO$_2$/ZnO nanocomposites prepared by high energy ball milling. Therefore, this study aimed to investigate the formation of CeO$_2$/ZnO nanocomposites by planetary ball milling and we have also investigated the structural, optical and antibacterial properties of CeO$_2$/ZnO nanocomposites.

2. MATERIALS AND METHODS
2.1 Preparation of CeO$_2$/ZnO Nanocomposites
The powders were weighed to obtain mixtures of ZnO (Fluka) with 3, 5, 7 and 10 mol% CeO$_2$ (Sigma-Aldrich). The milling was performed by a Pulversette 7 FRITSCH planetary ball mill in air. Both the vessel and balls used in this work were made from silicon nitride. The milling conditions were balls-to-powder weight ratio of 10:1, the total weight of mixtures was about 2 g, the milling speed was 400 rpm and the milling time was 20 h. The mixtures were milled for 10 min, alternating with a stop for 5 min in order to prevent overheating and rapid engine wear. The products were cooled naturally to room temperature and then collected for characterization using various techniques.

2.2 Characterization Methods
The crystalline structure and formation of CeO$_2$/ZnO nanocomposites were determined by X-ray diffraction (XRD) analysis using an X’Pert MPD diffractometer. The X-ray diffraction patterns were recorded from 2θ = 20° to 80° with a scanning rate of 0.2°/s. The morphology of the CeO$_2$/ZnO nanocomposites was observed by scanning electron microscope (SEM) with a QUANTA 400, FEI with an accelerating voltage of 20 kV. The diffuse reflectance spectra were recorded on a 2450 Shimadzu UV-vis spectrophotometer. The room temperature photoluminescence (PL) spectra were recorded by a luminescence spectrometer (LS/55, Perkin Elmer).

2.3 Antibacterial Test
The minimum inhibitory concentrations (MIC) of CeO$_2$/ZnO nanocomposites were determined by a broth microdilution method [4]. Staphylococcus aureus (S. aureus) ATCC 25923 and Escherichia coli (E. coli) ATCC 25922 were used as representative microorganisms for Gram-positive and Gram-negative bacteria, respectively. In order to examine the antibacterial activity of the samples, 0, 3, 5, 7 and 10 mol% CeO$_2$/ZnO nanocomposites were suspended in sterile distilled water and sonicated for 20 min to yield a stock solution of 50 mg/mL and the samples were diluted using the twofold serial dilution method starting with 50 and diluting to 0.78 mg/mL. The bacterial suspensions were prepared in normal saline solution (NSS) with an optical density equivalent to the 0.5 McFarland standard, and diluted to 1:20 in NSS to obtain a final concentration of about 5×10$^5$ CFU/mL. Then triplicate 50 µL samples of each dilution were applied into a sterile 96-well microtiter plate. To each well was added 10 µL of 0.675% (w/v) reasurin solution as an indicator and 30 µL of 3.3 strength Mueller Hinton Broth (3.3×MHB). Finally, 10 µL of bacterial suspension (5×10$^5$ CFU/mL) was applied to achieve a concentration of 5×10$^3$ CFU/mL. The microtiter plates were prepared...
in triplicate. After incubation at 37 °C for 20 h, the lowest concentration at which no color change occurred was taken as the MIC value.

### 3. RESULTS AND DISCUSSION

#### 3.1 Crystal Structure and Morphological Study

The samples that were milled according to the milling conditions described in section 2.1 were characterized by the XRD technique and the XRD patterns of all samples are shown in Figure 1. It was evident that a CeO₂ phase appeared in a whole range of CeO₂ loading contents while the ZnO phase with a hexagonal structure were evaluated from the following relationship [25]:

\[
D = \frac{k\lambda}{\beta \cos \theta}
\]

where D is the crystallite size, k is a constant (k = 0.9), \( \lambda \) is the wavelength of the X-ray used (0.15406 nm), \( \beta \) is the full-width at half maximum in radian and \( \theta \) is the Bragg’s diffraction angle. Moreover, the lattice parameters: a and c for a hexagonal structure were evaluated from the following relationship [25]:

\[
\frac{1}{d_{hkl}^2} = \frac{4}{3} \left[ \frac{h^2 + \frac{1}{2}k^2}{a^2} + \frac{l^2}{c^2} \right]
\]

where \( d_{hkl} \) is the lattice spacing of the (hkl) plane and a as well as c are the lattice parameters. For lattice volume, it was calculated by the following relationship [23]:

\[
V = 0.866a^2c
\]

where V is the lattice volume, a and c are the lattice parameters.

In addition, the dependence of the micro-strain on the CeO₂ loading content was also investigated. The micro-strain was calculated by the equation below [23]:

\[
\varepsilon = \frac{\beta \cos \theta}{4}
\]

### Table 1

<table>
<thead>
<tr>
<th>CeO₂ (mol%)</th>
<th>D (nm)</th>
<th>lattice parameter (nm)</th>
<th>V (nm³)</th>
<th>( \varepsilon )</th>
<th>E₉ (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>29.78</td>
<td>0.3249</td>
<td>0.5210</td>
<td>0.04766</td>
<td>0.0814</td>
</tr>
<tr>
<td>3</td>
<td>26.36</td>
<td>0.3251</td>
<td>0.5207</td>
<td>0.04769</td>
<td>0.0917</td>
</tr>
<tr>
<td>5</td>
<td>26.29</td>
<td>0.3252</td>
<td>0.5212</td>
<td>0.04777</td>
<td>0.0918</td>
</tr>
<tr>
<td>7</td>
<td>24.22</td>
<td>0.3249</td>
<td>0.5207</td>
<td>0.04763</td>
<td>0.0943</td>
</tr>
<tr>
<td>10</td>
<td>20.45</td>
<td>0.3247</td>
<td>0.5207</td>
<td>0.04757</td>
<td>0.1202</td>
</tr>
</tbody>
</table>

Figure 1. The XRD patterns of milled ZnO nanoparticles and CeO₂/ZnO nanocomposites with different CeO₂ loading contents.
where $\varepsilon$ is the micro-strain, $\beta$ is the full-width at half maximum in radian and $\theta$ is the Bragg’s diffraction angle. The calculated crystallite size, lattice parameters, lattice volume and micro-strain as a function of CeO$_2$ loading contents are shown in Table 1. From Table 1, it was clearly seen that the lattice parameters and lattice volumes tended to increase when the CeO$_2$ loading content was increased from 0 to 5 mol%. This might be due to some Ce$^{4+}$ ions or defects could incorporation into ZnO lattice. With further increase of the CeO$_2$ loading contents, the excess CeO$_2$ phase could adsorb onto the surface of the ZnO. In this case, the crystal lattice was suppressed and led to a contraction of the lattice parameter and lattice volume [4]. As a matter of fact, the Ce$^{4+}$ ion (ionic radius of 92 pm) is bigger than that of Zn$^{2+}$ ion (ionic radius of 74 pm). Therefore, the substitution of Zn$^{2+}$ ion by Ce$^{4+}$ ion is difficult and the separate CeO$_2$ phase was the preferred form as confirmed by the XRD result.

The crystallite size of the ZnO slightly decreased when the CeO$_2$ was loaded from 0 to 5 mol% and the crystallite size significantly decreased when the CeO$_2$ was further loaded to 10 mol%. This might be due to the formation of a complex of Ce$^{4+}$ ions with the surface oxygen of ZnO [12]. In this study, the micro-strain increased when the CeO$_2$ loading content was increased. This is because the many lattice imperfections produced and affected a peak broadening that has also occurred in the case of Ti-doped ZnO nanoparticles [26].

The secondary electron images of the starting materials and the milled CeO$_2$/ZnO nanocomposites at different CeO$_2$ loading contents are presented in Figure 2.

The ZnO and CeO$_2$ powders have an irregular shape and a large particle size. After milling, the CeO$_2$/ZnO nanocomposites became shaped in a spherical structure and the particles agglomerated as clusters to reduce the total surface energy of the system [26].

Figure 2. SEM images of (a) commercial ZnO and (b) commercial CeO$_2$ powders as well as CeO$_2$/ZnO nanocomposites with different loading contents (c) 0 mol%, (d) 3 mol%, (e) 5 mol%, (f) 7 mol% and (g) 10 mol%.
3.2 Optical Study

We know that the optical properties of the ZnO powders depended upon many parameters such as crystallite size, particle size, particle shape, defects, lattice strain and lattice volume. In this study, the optical absorption behavior was employed to study the effect of the CeO$_2$ loading content on the optical band gap energy of the CeO$_2$/ZnO nanocomposites. Since, the interband transition in ZnO is the allowed direct transition; the optical band gap energy of the CeO$_2$/ZnO nanocomposites can be expressed by [4]:

\[(\alpha E)^2 = B(E - E_g)^{1/2}\] (5)

where $\alpha$ is the absorption coefficient, $E$ is the photon energy, $B$ is a constant and $E_g$ is the optical band gap energy.

The $E_g$ values of the CeO$_2$/ZnO nanocomposites were determined from the intercept of $(\alpha E)^2$ versus $E$ curves as shown in Figure 3 and the extrapolated values are given in Table 1. Noticeably, the crystallite size decreased when the CeO$_2$ loading content increased, but the $E_g$ values slightly increased when the CeO$_2$ loading content increased from 0 to 5 mol% only and then when the CeO$_2$ loading content was higher than 5 mol%, the $E_g$ value suddenly decreased. Therefore, other parameters affected the change of the $E_g$ value of the CeO$_2$/ZnO nanocomposites.

Figure 3. Plots of $(\alpha E)^2$ versus $E$ of the ZnO nanoparticles and the CeO$_2$/ZnO nanocomposites with different loading contents for evaluating the $E_g$ value.
nanocomposites. Considering the correlation between the $E_g$ value and lattice volume, it was evident that the $E_g$ values significantly changed when the lattice volume changed. The $E_g$ value increased when the lattice volume increased. This might be due to the fact that the $E_g$ is directly proportional to the interatomic distance as occurred in the case of Cr-, Mn- and Fe-doped ZnO nanostructures [27].

### 3.3 Bactericidal Study

In addition to the study of the antibacterial activity of ZnO nanoparticles and CeO$_2$/ZnO nanocomposites, the antibacterial activity against *S. aureus* and *E. coli* was investigated and the antibacterial results are shown in Table 2. It was found that the CeO$_2$/ZnO nanocomposites showed excellent antibacterial activities. Moreover, the CeO$_2$/ZnO nanocomposites inhibited *S. aureus* at lower concentrations than for *E. coli*. This could be due to the *S. aureus* and *E. coli* having different cell wall structures, cell physiology, metabolism or degree of contact [28]. As we know, the cell wall of *S. aureus* and *E. coli* is composed of surface proteins that they used for adhesion and colonization and components such as polysaccharides and teichoic acid for protection against host defenses and environmental conditions. Therefore, the CeO$_2$/ZnO nanocomposites can attach and inhibit on contact the bacteria cell [29]. Recently, many possible antibacterial mechanisms were proposed for metal oxide powders such as the release of Zn$^{2+}$ ions, the penetration of metal oxide powder into the cells and the production of $\cdot O_2^-$ reactive species from the surface of metal oxide powders [30, 31], but the actual mechanism is still not clearly present. In this study, these three mechanisms might inhibit towards the bacterial cells. As a matter of fact, the smaller particle size showed the larger specific surface area, so the Zn$^{2+}$ ions and $\cdot O_2^-$ reactive species could more generate and then these species could affect the antibacterial activity increasingly when the particle size of ZnO powder decreased. As we know, the samples that had higher oxygen vacancies can produce more $\cdot O_2^-$ reactive species, thus the PL spectra were measured as shown in Figure 4. The PL spectra showed the broad band emission from interstitial atoms and oxygen vacancies centered around 650 nm [32]. It was observed that the highest and lowest PL emission intensities were obtained from the 10 mol% CeO$_2$ loading content and pure ZnO powders, respectively. Therefore, the $\cdot O_2^-$ reactive species could more produce from the samples loaded with higher CeO$_2$ contents and this resulted in the better antibacterial activities. Moreover, the bacterial activity increased as a function of CeO$_2$ loading contents because of a reduction of particle size of ZnO powders. The smaller particle size could penetrate increasingly into the bacteria cell and this resulted in enhancement of antibacterial activity. In this study, pure CeO$_2$ did not inhibit both *S. aureus* and *E. coli*.

### Table 2. The antibacterial properties of ZnO nanoparticles and CeO$_2$/ZnO nanocomposites.

<table>
<thead>
<tr>
<th>CeO$_2$ (mol%)</th>
<th>Minimum Inhibitory Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S. aureus$</td>
</tr>
<tr>
<td>0</td>
<td>6.25</td>
</tr>
<tr>
<td>3</td>
<td>3.125</td>
</tr>
<tr>
<td>5</td>
<td>1.5625</td>
</tr>
<tr>
<td>7</td>
<td>1.5625</td>
</tr>
<tr>
<td>10</td>
<td>0.78</td>
</tr>
<tr>
<td>Pure CeO$_2$</td>
<td>0</td>
</tr>
</tbody>
</table>

### 4. Conclusions

The CeO$_2$/ZnO nanocomposites with different CeO$_2$ loading contents were prepared by using the high energy ball milling method. The milling conditions were a milling speed of 400 rpm, a milling time of 20 h, and a ball-to-powder weight ratio of 10:1. The XRD results always showed a principle phase of ZnO with
a hexagonal wurtzite structure. The crystallite size of the samples decreased because of the formation of the CeO$_2$ phase. The microstrain increased significantly as a function of crystallite size and CeO$_2$ loading content. The $E_g$ value depended upon the lattice volume due to the $E_g$ value being directed proportional to the interatomic distance. The widest $E_g$ value was obtained when the lattice volume was the largest. The samples inhibited the growth of both $S$. aureus and $E$. coli. In this study, the samples were more active against $S$. aureus than $E$. coli due to the differences in their cell wall structures.

ACKNOWLEDGEMENT

The authors would like to thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education and the authors would like to acknowledge Dr. Brian Hodgson for assistance with the English.

REFERENCES


