Asian Journal of Chemistry; Vol. 27, No. 2 (2015), 639-642

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.17101

Preparation and Characterization of ZnO Nanoparticles and Their Effects on *Bombina orientalis*

EUN JI KIM[1], HAE SOO PARK[2,3], HOON CHUNG[1] and WEON BAE KO[2,3,*]

[1]Department of Animal Resource, Sahmyook University, Seoul 139-742, Republic of Korea
[2]Department of Convergence Science, Graduated School, Sahmyook University, Seoul 139-742, Republic of Korea
[3]Department of Chemistry, Sahmyook University, Seoul 139-742, Republic of Korea

*Corresponding author: Fax: +82 2979 5318; Tel: +82 23399 1700; E-mail: kowb@syu.ac.kr

Received: 22 January 2014; *Accepted*: 17 April 2014; *Published online*: 10 January 2015; AJC-16636

Nanosized zinc oxide was synthesized from zinc nitrate hexahydrate and sodium hydroxide in an aqueous-alcoholic solution under ultrasonic irradiation at room temperature. The crystallinity, size, morphology and optical property of ZnO nanoparticles were examined by X-ray diffraction, scanning electron microscopy, transmission electron microscopy and UV-visible spectrophotometry. *Bombina orientalis* were breeded in the laboratory at room temperature and exposed to ZnO nanoparticles at various concentrations. After hatching the tadpoles, the development, mortality and malformation of *Bombina orientalis* were investigated.

Keywords: Zinc oxide nanoparticles, *Bombina orientalis*, Development, Mortality, Malformation.

INTRODUCTION

Zinc oxide is a promising semiconductor material with a wide band gap and a high exciton binding energy of 3.37 eV and 60 meV, respectively[1,2]. Zinc oxide nanoparticles have attracted considerable interest in areas, such as ultraviolet lasers, light-emitting diodes, flat panel displays, photodetectors, gas sensors, catalysis, pH meters, nanogenerators and solar cells, on account of their unique physical and chemical properties[3-7]. In addition, ZnO nanoparticles are used in sunscreens, cosmetics, coatings, caulk and adhesives and have antimicrobial properties[8].

Over the recent decade, nanotechnology and the production of nanoparticles have developed rapidly. Regardless of the deficient data on their potential hazards, nanoparticles are being used increasingly in a variety of consumer products[9,10]. On the other hand, the use of ZnO nanoparticles increases the potential for their release to the environment. A recent study reported that the concentrations of ZnO nanoparticles were 10 ng/L in natural surface water and 430 ng/L in treated wastewater[11]. The environmental levels of ZnO nanoparticles are expected to increase continually because of their widespread applications[12].

Nanomaterial toxicity has been documented in the following aquatic organisms; Freshwater microalga (*Pseudokirchneriella subcapita*), *Daphnia magna* and zebrafish (*Danio rerio*)[13-15]. Exposure to ZnO nanoparticles at 1 mg/L increased the mortality significantly and decreased the hatchability of zebra fish[16]. Xenopus laevis showed increased mortality and negatively affected metamorphosis at 2 mg/L[17]. On the other hand, information on the ecotoxicological effects of ZnO nanoparticles is limited. This study examined the effects of ZnO nanoparticles on *B. orientalis* embryos and larvae.

This paper reports the preparation and characterization of ZnO nanoparticles under ultrasonic irradiation. This study examined the developmental effects of ZnO nanoparticles on *Bombina orientalis* growth and development. To accomplish this, *B. orientalis* were exposed to ZnO nanoparticles and divided into four groups (no exposure group, 0.1 g/L ZnO nanoparticles group, 0.01 g/L ZnO nanoparticles group and 0.001 g/L ZnO nanoparticles group). Tadpoles exposed to 0.1 g/L ZnO nanoparticles showed increased mortality and adversely affected metamorphosis of *B. orientalis*. The types of deformity were pectoral blisters, bent trunk and ventral dysplasia.

EXPERIMENTAL

$Zn(NO_3)_2{\cdot}6H_2O$, NaOH and ethanol were obtained from Samchun Chemicals. *B. orientalis* was collected from a mountain stream in Baekwoon (38°02′06″ N, 127°24′51″ E) in May 2011. Human chorionic gonadotropin (HCG) was purchased from Sigma-Aldrich.

The crystallite size and morphology of the synthesized ZnO nanoparticles were analyzed by transmission electron microscopy (TEM, JEOL Ltd, JEM-2010) at an acceleration voltage of 200 kV. The surface of the synthesized ZnO nanoparticles was examined by scanning electron microscopy (SEM, JEOL Ltd, JSM-6510) at an acceleration voltage of 0.5 to 30 kV. The structure of the synthesized ZnO nanoparticles was characterized by X-ray diffraction (XRD, Bruker, D8 Advance). UV-visible spectroscopy of the samples was performed using an UV-visible spectrophotometer (Shimazu, UV-1601 PC). Ultrasonic irradiation of the sample was carried out in continuous mode using an ultrasonic generator UGI1200 (Hanil Ultrasonic Co., Ltd.) with a frequency 20 KHz and nominal power of 750 W. The ultrasonic generator was a horn type system with a 13 mm horn tip.

Preparation of ZnO Nanoparticles under Ultrasonic Irradiation: 1 M $Zn(NO_3)_2 \cdot 6H_2O$ and 10 M NaOH were dissolved separately in 6 mL of distilled water. Ethanol (15 mL) was then added to each solution. The two solutions were mixed in a 100 mL Erlenmeyer flask and stirred vigorously for 45 min. This mixture solution was reacted under ultrasonic irradiation for 45 min at room temperature. After the reaction was complete, the solution was removed and the white precipitate was washed 5 times with ethanol.

Breeding: *B. orientalis* was breeded in the laboratory. Two mating pairs of *B. orientalis* adults were breeded. To induce reproduction, the males and females were injected with 250 IU and 750 IU of HCG in the dorsal lymph sac, respectively.

Embryo collection began approximately 48 h after the HCG injection. Ten days prior to starting the experiments, a 100 L outdoor tube was filled with water. Each test tank was filled with 3 L water and supplied air at room temperature. The water temperature in the tank ranged from 11 and 20 °C throughout the experiment and the photoperiod was not controlled. Tadpoles were fed a rabbit food. The test was begun the day following eggs collection. The embryos were grouped into control, 0.001, 0.01 and 0.1 mg/L based on exposed concentration of ZnO nanoparticles. Each concentration was tested twice in with 10 eggs in each replica.

Zinc oxide nanoparticles injection continued after hatching tadpoles. After 3 days hatching, the tadpoles were measured from the snout-vent length (SVL). The mortality and malformation were recorded. The mortality was determined from the lack of movement or response from external stimuli. To analyze data from the experiments that four groups, we used one-way ANOVA and Bonfferoni test. All statistical analysis were performed using SPSS Ver. 20.0 (SPSS, Chicago, IL, U.S.A).

RESULTS AND DISCUSSION

The synthesized ZnO nanoparticles were dispersed in distilled water to examine the peak value of ZnO nanoparticles. Fig. 1 shows the optical properties of ZnO nanoparticles synthesized using the sonochemical method for 45 min at λ_{max} = 360 nm. The wavelength of the peak of the UV-visible spectrum of the synthesized ZnO nanoparticles was determined by the nature of the electronic transition from the top of the valence band to the bottom of the conduction band as well as by the particle size distribution and band gap distribution[18,19]. In addition, the shape of the absorption edge depends on the distribution of the particle band gap and particle size. This means that the absorption edge was narrow, which confirmed the formation of monodispersed ZnO nanoparticles by ultrasonic irradiation[20].

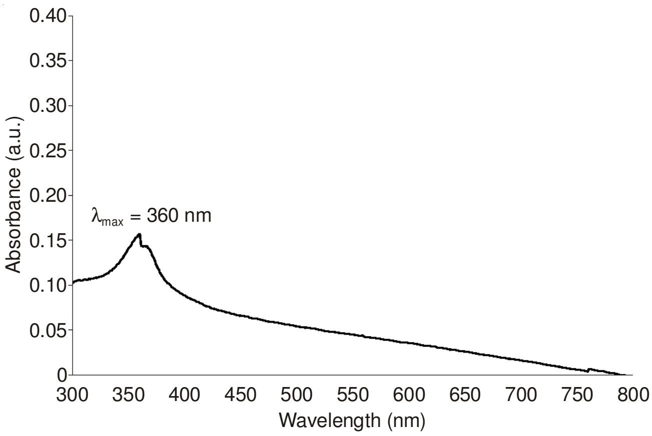

Fig. 1. UV-visible spectrum of the synthesized ZnO nanoparticles

Fig. 2 shows the XRD patterns of the ZnO nanoparticles synthesized by the sonochemical method. The crystal structure of the synthesized ZnO nanoparticles was characterized by XRD using CuK_α (λ = 1.5406 Å) radiation. The characteristic peaks of the synthesized ZnO nanoparticles were observed at 31.82°, 34.44°, 36.27°, 47.55°, 56.61°, 62.85°, 66.30°, 67.93° 69.10°, 72.42° and 76.88° 2θ, which were assigned to the indices [(100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (002)]. The synthesized ZnO nanoparticles were highly crystalline and had a hexagonal type structure[3]. The mean crystallite size was calculated using Scherrer's formula:

$D = \frac{0.9\ \lambda}{\beta \cos\theta}$, where D is the crystallite size, λ is the wavelength of X-rays (k = 1.5406 Å), β is the full width at half maximum (FWHM) of the diffraction peak and θ is the Bragg diffraction angle of the XRD peak. The mean crystallite size was 32.32 nm[21].

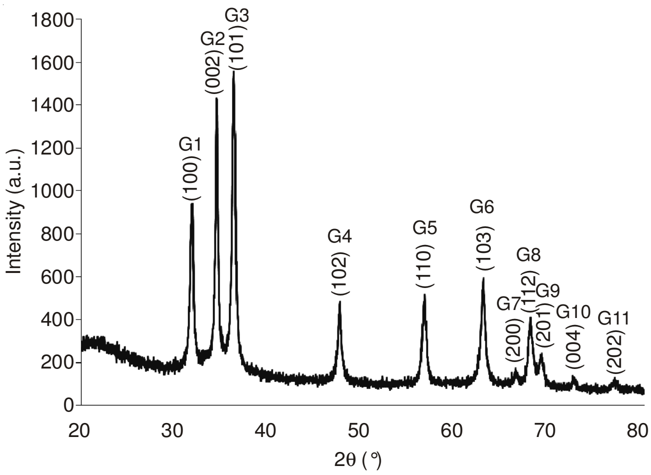

Fig. 2. XRD pattern of the synthesized ZnO nanoparticles

Fig. 3 shows a TEM image of the ZnO nanoparticles synthesized using the sonochemical method. The shape of the synthesized ZnO nanoparticles showed a rod, triangle and spherical-like shape. The mean size of the synthesized ZnO nanoparticles was 40-70 nm.

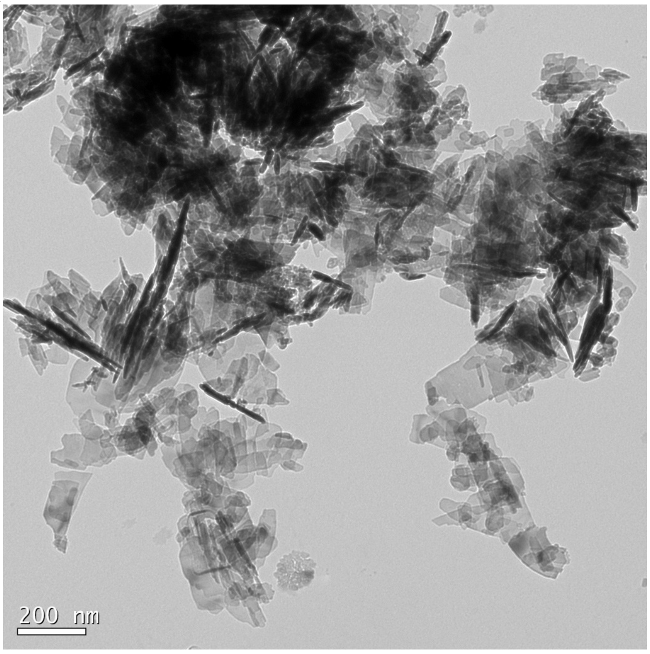

Fig. 3. TEM image of the synthesized ZnO nanoparticles

Fig. 4 shows a SEM image of the ZnO nanoparticles synthesized using the sonochemical method. The synthesized ZnO nanoparticles were finely agglomerated. The surface of the synthesized ZnO nanoparticles showed a snow-flower-like shape with overlapping needles.

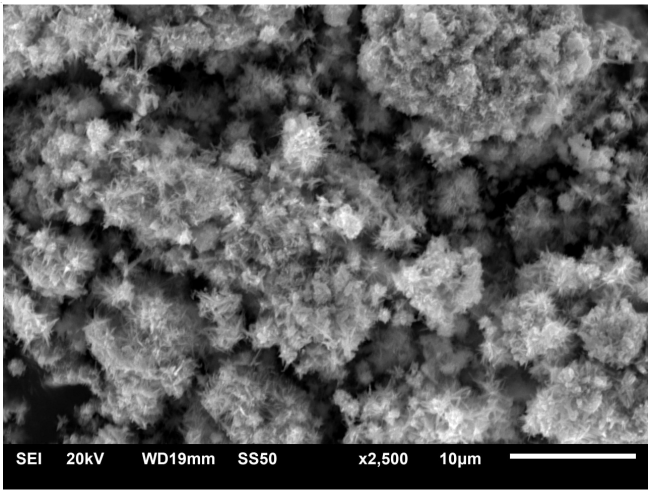

Fig. 4. SEM image of the synthesized ZnO nanoparticles

Mortality: The control concentration (0 mg/L) exposed tadpoles showed a mortality rate of 20 %. A significant increase in mortality was observed at 0.01 mg/L ZnO compared to the other treatments and decreased at 0.001 mg/L concentration Table-1. Previous studies have demonstrated that ZnO nanoparticles increase mortality in zebrafish, crustaceans and frog[16,17,22]. Exposure of *Danio rerio* to nano ZnO induced a 96 h LC_{50} of 1.793 mg/L[16]. *Daphnia magna* experienced a 48 h LC_{50} of 3.2 mg/L ZnO while *Thamnocephalus platyrus* had a 24 h LC_{50} of 0.18 mg/L ZnO[22]. *Xenopus laevis* experienced a 0.799 mg/L Zn nanomaterials[17]. *B. orientalis* in this study did not exhibit a dose-response relationship for mortality. Highest mortality was not observed at the highest tested concentration, but observed at the second highest tested concentration (0.01 mg/L ZnO). We considered that further studies were needed for these results (Table-2).

TABLE-1
AVERAGE CRYSTALLITE SIZE OF THE SYNTHESIZED ZnO NANOPARTICLES BY SCHERRER'S FORMULA

Peak	2 (θ)	FWHM	D (nm)
G1	31.82	0.4529	36.48
G2	34.44	0.3545	46.92
G3	36.27	0.3939	42.45
G4	47.55	0.5317	32.65
G5	56.61	0.6302	28.64
G6	62.85	0.6105	30.50
G7	66.30	0.3741	50.73
G8	67.93	0.7484	25.60
G9	69.10	0.9453	20.41
G10	72.42	1.0241	19.23
G11	76.88	0.9259	21.91
Average			32.32

TABLE-2
MORTALITY AND MALFORMATION OF *B. orientalis* INDUCED BY EXPOSURE OF ZnO NANOPARTICLES

ZnO (mg/L)	Mortality (%)	Malformation (no.)
0(control)	20	0
0.001	10	1
0.01	40	3
0.1	30	4

Snout-vent length: The snout-vent length of the control (0 mg/L) tadpoles was 11.08 ± 0.47 mm (mean ± SD). The longest snout-vent length (11.95 ± 0.62 mm (mean ± SD)) was observed at 0.001 mg/L ZnO. The 0.01 and 0.1 mg/L concentration resulted in a snout-vent length of 10.74 ± 0.77 and 10.47 ± 0.47 mm, respectively (Fig. 5). *X. laevis* tadpoles exposed to 0.067 mg/L ZnO appear to be larger than controls, while tadpoles exposed to 0.513 and 0.799 mg/L ZnO appear to be smaller than controls[17]. This results is similar to results of our research. *B. orientalis* in this study did not exhibit a dose-response relationship for snout-vent length.

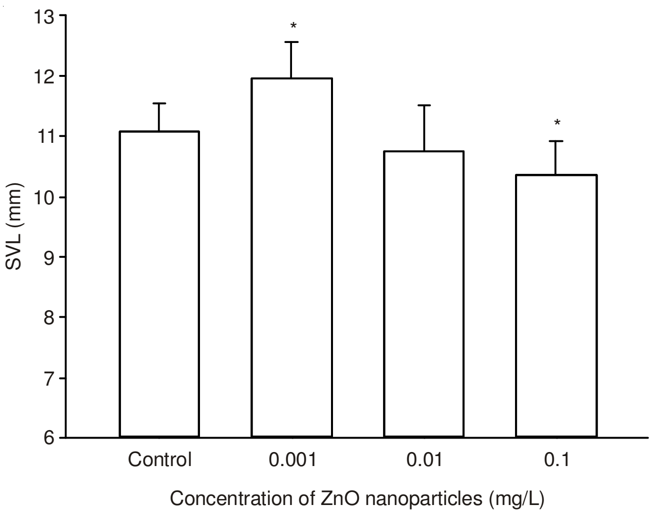

Fig. 5. Snout-vent length of *Bombina orientalis* induced by exposure to ZnO nanoparticles; *: $p \leq 0.05$ ANOVA and Bonfferoni

Malformation: Four types of malformation were observed in 8 individuals; 3 types were pectoral blisters, bent trunk and ventral dysplasia (Fig. 6). Malformation of ZnO exposed amphibians was not reported. So, results of this study have significance (Table-2).

Fig. 6. Various malformations in tadpoles of *B. orienralis*; (a) Bent trunk, (b) Ventral dysplasia, (c) Pectoral blister, (d) Normal

Zinc oxide nanoparticles adversely affected growth, mortality and malformation incidence of several aquatic species[13,16,17,22]. This was also observed in the ZnO nanoparticles-exposed embryos and tadpoles. On the other hand, exposure to lower concentrations (0.001 mg/L) increased the tadpole snout-vent length and decreased mortality. Therefore, the release of ZnO nanoparticles, *albeit* at low concentrations, might have beneficial effects on some aquatic species.

Conclusion

Zinc oxide nanoparticles were synthesized under ultrasonic irradiation. The ZnO nanoparticles exhibited a rod, triangle and spherical-like shape with a mean size of 40-70 nm. The ZnO nanoparticles showed an absorption wavelength of 360 nm. Zinc oxide nanoparticles have an adverse effect on the growth of *Bombina orientalis*. On the other hand, exposure to lower concentrations of ZnO nanoparticles increased the growth of *Bombina orientalis* and decreased their mortality. Nevertheless, ZnO nanoparticles released into aquatic ecosystems at high concentrations might have detrimental effects on aquatic organisms, such as amphibians.

ACKNOWLEDGEMENTS

This study was supported by Sahmyook University research funding in Korea.

REFERENCES

1. K. Siraj, K. Javaid, J.D. Pedarnig, M.A. Bodea and S. Naseem, *J. Alloys Comp*., **563**, 280 (2013).
2. A.K. Srivastava, *Mater. Lett*., **62**, 4296 (2008).
3. A.S. Lanje, S.J. Sharma, R.S. Ningthoujam, J.-S. Ahn and R.B. Pode, *Adv. Powder Technol*., **24**, 331 (2013).
4. V.R. Shinde, T.P. Gujar, C.D. Lokhande, R.S. Mane and S.-H. Han, *Mater. Sci. Eng. B-Adv. Funct. Solid-State Mater*., **137**, 119 (2007).
5. V.R. Shinde, T.P. Gujar and C.D. Lokhande, *Sol. Energy Mater. Sol. Cells*, **91**, 1055 (2007).
6. Y. Li, J. Gong and Y. Deng, *Sens. Actuators A*, **158**, 176 (2010).
7. Z.L. Wang and J. Song, *Science*, **312**, 242 (2006).
8. D. Ali, S. Alarifi, S. Kumar, M. Ahamed and M.A. Siddiqui, *Aquat. Toxicol*., **124-125**, 83 (2012).
9. A. Nel, T. Xia, L. Mädler and N. Li, *Science*, **311**, 622 (2006).
10. B. Nowack and T.D. Bucheli, *Environ. Pollut*., **150**, 5 (2007).
11. F. Gottschalk, T. Sonderer, R.W. Scholz and B. Nowack, *Environ. Sci. Technol*., **43**, 9216 (2009).
12. C.G. Daughton and T.A. Ternes, *Environ. Health Perspect*., **107**, 907 (1999).
13. N.M. Franklin, N.J. Rogers, S.C. Apte, G.E. Batley, G.E. Gadd and P.S. Casey, *Environ. Sci. Technol*., **41**, 8484 (2007).
14. S.B. Lovern, J.R. Strickler and R. Klaper, *Environ. Sci. Technol*., **41**, 4465 (2007).
15. S.B. Lovern and R. Klaper, *Environ. Toxicol. Chem*., **25**, 1132 (2006).
16. X. Zhu, L. Zhu, Z. Duan, R. Qi, Y. Li and Y. Lang, *J. Environ. Sci. Health Part A*, **43**, 278 (2008).
17. S. Nations, M. Long, M. Wages, J. Canas, J.D. Maul, C. Theodorakis and G.P. Cobb, *Ecotoxicol. Environ. Saf*., **74**, 203 (2011).
18. L. Wang and M. Muhammed, *J. Mater. Chem*., **9**, 2871 (1999).
19. N.S. Pesika, K.J. Stebe and P.C. Searson, *J. Phys. Chem. B*, **107**, 10412 (2003).
20. S.K. Hong, J.H. Lee, J.M. Kim, M.H. Kwon and W.B. Ko, *J. Nanosci. Nanotechnol*., **11**, 593 (2011).
21. Y. Zhang, B. Lin, Z. Fu, C. Liu and W. Han, *Opt. Mater*., **28**, 1192 (2006).
22. M. Heinlaan, A. Ivask, I. Blinova, H.C. Dubourguier and A. Kahru, *Chemosphere*, **71**, 1308 (2008).