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Synthesis of ZnO nanorod for immunosensor application

Vu Y Doan, Pham Hung Vuong, Hoang Lan, Phuong Dinh Tam*

Advanced Institute for Science and Technology, Hanoi University of Science and Technology

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Abstract

This paper reported a facile method to synthesize ZnO nanorods for immunosensor application. The ZnO nanorods were synthesized by hydrothermal reaction. Synthesis time affecting on morphology of nanorods was also studied. The immobilization of anti-rotavirus onto ZnO nanorod-deposited sensor was performed via absorption method. The electrochemical responses of the immunosensor were studied by cyclic voltammetry (C-V) method with $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as redox probe. A linear decreased response in C-V for cell of rotavirus concentration was found in the range of 7.8×10^5 CFU/mL to 7.8×10^8 CFU/mL. The detection limit of the immunosensor was 7.8×10^5 CFU/mL. The results indicated application of ZnO nanorod sensor for label-free real-time detection of a wide dynamics range of biological species.

Keywords. ZnO, nanorod, immunosensor, nanowire, biosensor.

1. INTRODUCTION

One - dimensional nanostructures have been demonstrated as outstanding materials for biosensor applications because of their large surface - to - volume ratio as well as biocompatible. One such material has gained significant attention within a number of emerging materials is zinc oxide nanorod. Up to now, many works have been focused on fabrication of ZnO nanorod for biosensor applications. Choi et al. [1] reported a ZnO nanowire (NW) field effect transistor based on biosensor. ZnO nanowire synthesized by using pulsed laser deposition in a vacuum furnace. Then, it dispersed on Si wafer by spin coating method. A highly doped n-type Si wafer was used as a back gate electrode. Contact leads of Ti and Au were defined by using electron-beam lithography. N-type ZnO NWs field effect transistor (FET) used for the electronic detection of biotin-streptavidin binding system. The results indicated the electrical current changes of up to 22.5 nA when the binding of streptavidin with the concentrations of up to 250 nM. The ZnO NW biosensor could detect as low as 2.5 nM of the streptavidin with 7.5 nA of electrical current. A high performance glucose sensor based on ZnO NW was investigated by Pradhan et al. [2]. They demonstrated excellent electrocatalytic performance of biosensor with a high sensitivity of $19.5 \mu\text{A}/\text{mM}\cdot\text{cm}^2$ and a fast response time of < 5 s for the amperometric detection of glucose. In other study, an immunosensor based on the ZnO nanorod network for detection of H1N1 Swine Influenza

Virus (SIV) was developed by Jang et al. [3]. They used hydrothermal method to grow ZnO nanorod on a patterned Au electrode. The measurement results of the fabricated immunosensor showed that the reduction currents of immunosensor at 0.25 V logarithmically increased from 259.37 to 577.98 nA as the H1N1 SIV concentration changed from 1 pg/mL to 5 ng/mL, with a detection limit of 1 pg/mL. Park et al. [4] reported an electrochemical immunosensor based on a ZnO nanorods matrix electrode for detecting of Legionella pneumophila. ZnO nanorods was grown on Au working electrode using hydrothermal method at low temperature. A primary antibody was immobilized onto the ZnO nanorods by electrostatic interaction. The fabricated immunosensor could detect the L. pneumophila antigen concentrations from 1 to 5000 pg/mL. The detection limit is ~ 1 pg/mL. The using of ZnO nanorod as immobilization layers for interdigitated capacitive immunosensor was studied by P. Sanguino and co-worker [5]. In their study, ZnO layer was used as a sensitive layer coupled with interdigitated microelectrode in an affinity immunosensor format. The results show maximum response of the sensor was achieved in the 5–6 kHz frequency range. A ZnO nanorod-based on non-enzymatic optical glucose biosensor was studied by Sanagi et al. [6]. ZnO nanorod was synthesized by hydrothermal method. They showed the ZnO nanorods play the role of a catalyst similar to the oxidase used in the enzymatic glucose sensors. This makes to the photoluminescence (PL) intensity of the near-band edge emission of the ZnO nanorods

linearly decreased with the increased concentration of H_2O_2 . Thus, glucose biosensor could test glucose concentration over the wide range of 0.5–30 mM, corresponding to 9–540 mg/dL. In other study, Marie et al. [7] reported an electrochemical biosensor based on ZnO nanorod for glucose detection. The hydrothermal sol–gel growth method was utilized to grow ZnO nanorods on indium tin oxide-coated glass substrates. The results indicated that the amperometric response time of sensor was 3 s, the limit of detection was 0.22 μM and the obtained sensitivity of the fabricated ZnO electrochemical sensor was 10.911 mA/mM \cdot cm 2 .

In this paper, we reported a facile method to synthesize ZnO nanorods for immunosensor application. ZnO nanorods were obtained through hydrothermal method. Antibodies were functionalized on ZnO nanorods by using absorption technique. Immunosensor response was tested by cyclic voltammetry method. The results indicated ZnO nanorod based sensor could be applied for label-free detection of a wide dynamics range of biological species.

2. EXPERIMENTAL

2.1. Chemical reagents

Zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$], ethanol (C_2H_5OH), zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) and methenamine ($(CH_2)_6N_4H_2O_2$), were purchased from Sinopharm Chemical Reagent co., Ltd, China. Anti-rotavirus was provided by Invitrogen Co. Phosphate-buffered saline (PBS 1 \times , pH 7.4), H_2SO_4 98%, KCr_2O_7 were purchased from Sigma-Aldrich. Potassium ferrocyanide and potassium ferricyanide were purchased from Beijing Chemical Reagent (China). All solutions were prepared with de-ionized (DI) water.

2.2. ZnO nanorods synthesis

The ZnO sol was prepared by dissolving 220 mg of [$Zn(CH_3COO)_2 \cdot 2H_2O$] in 10 ml C_2H_5OH by stirring at room temperature for 30 min, which served as the coating material. Then, the thin films were fabricated by spin coating method on substrate and were annealed at 400 $^\circ C$ for 30 min. Subsequently, the hydrothermal process was used for the growth of ZnO nanorod on the prepared ZnO seed layers. ZnO seed layers prepared sample was placed into a furnace. The reaction temperature was maintained at 150 $^\circ C$ for 3 h. The obtained product was directly precipitated on the silicon substrate, which was placed in the autoclave. Subsequently,

the nanomaterial could be dispersed in ethanol after the silicon substrate was removed. The products were dried in an oven for 12 h at 80 $^\circ C$ before antibody immobilization.

2.3. Immunosensor fabrication

In this work, the microelectrode was fabricated by sputtering 10 nm Cr and 200 nm Pt on a \sim 100 nm thick silicon dioxide (SiO_2) layer thermally grown on top of a silicon wafer. Then, the surface of the sensor was initially cleaned with KCr_2O_7 in 98 % H_2SO_4 , followed by cyclic voltammograms (swept potential from -1 V to +2 V; scan rate of 50 mV/s) in 0.5 M H_2SO_4 to activate the sensor surface. Then, 10 μL of ZnO nanorod were drop-coated on the sensor surface and were dried in a desiccator. Subsequently, 0.5 $\mu g/mL$ of anti-rotavirus was passed on the sensor surface. Finally, the immunosensor surface was rinsed with double-distilled water and dried in nitrogen flow. When not in use, the immunosensors were kept at 4 $^\circ C$ in the refrigerator. Principle of the immunosensor constructed by microelectrode was illustrated in Fig. 1.

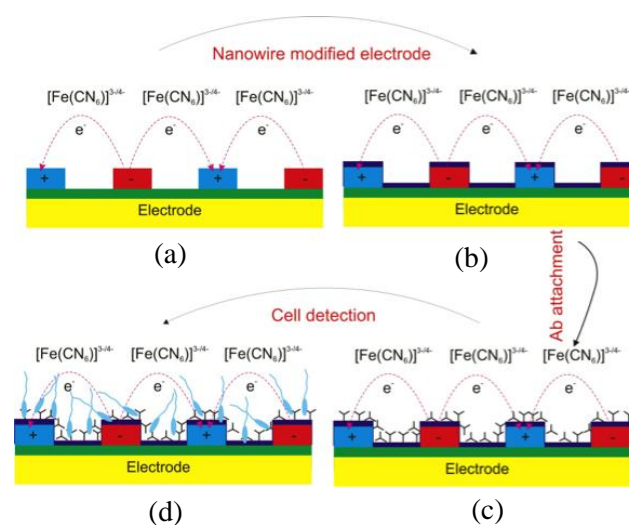


Figure 1: Schematic illustrates principle of the immunosensor constructed by microelectrode. (a) bare electrode, (b) ZnO nanorods modified electrode, (c) with antibody immobilization, (d) with cell binding

2.4. Bacterial binding measurement

IM6-impedance analyzer with IM6-THALES software was used to detect concentration of cell of *rotavirus*. In this work, anti-rotavirus modified sensor was immersed in a cells that was filled with 5 mL of 1 mM PBS solution (pH 7.3), containing defined

concentration of cells of *rotavirus* for 90 min at room temperature to form an antibody-antigen complex. Then, the immunosensor was rinsed thrice with buffer solution to remove the non-specifically adsorbed cells. The immunosensor responses were monitored using C-V method by dipping the modified sensor in 2 mL of 1 mM PBS solution containing 20 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as an indicator probe. The detected immunosensor was connected to the test and sense probe, and Pt electrode was connected to the counter electrode on the IM6 impedance analyzer. Ag/AgCl electrode was used as a reference electrode.

3. RESULTS AND DISCUSSION

3.1. ZnO nanorod characterizations

3.1.1. Fe-SEM & EDS

Fig. 2 shows field emission scanning electron microscope (FE-SEM) images of experimental samples grown at different times (3, 6, 9 and 12 h). Fig 2(a) displays the morphology of ZnO sample grown at 3h. It is evident that the sample mainly consists of ZnO nanorods and most of them assembly into rods like morphologies. The dimensions is typically about 1 μm in length and 50-100 nm in diameter. Fig. 2(b) shows the morphology of nanorods grown at 6h under the same conditions. These ZnO nanorods show diameter of 200 nm on average and length of 1-2 μm . When the synthesis process was performed at longer time (9h), longer nanorods were obtained (5-6 μm), as described in Fig. 2(c). Fig. 2(d) shows the morphology of nanorods grown at 12 h. In this case, the morphology of nanorods was changed with diameter 600 nm and long of 2-3 μm . Experimental results indicate that synthesis time plays an important role in the formation of the ZnO nanorods. In this experimental, optimal time for synthesis of ZnO nanorods was selected of 3 h.

The composition of the ZnO nanorods was investigated by energy dispersive spectroscopy (EDS). Fig. 3 shows EDS of ZnO nanorods synthesized at different time (3-12h). It can be seen that zinc and oxygen are the major components of the nanorods. Compositional analysis of the EDS indicated the atomic ratio of Zn/O in the ZnO nanorod is 53.3/46.7; 50.6/49.4; 50.5/49.5; and 55.2/44.8 for synthesis time to be 3; 6; 9; 12 h, respectively.

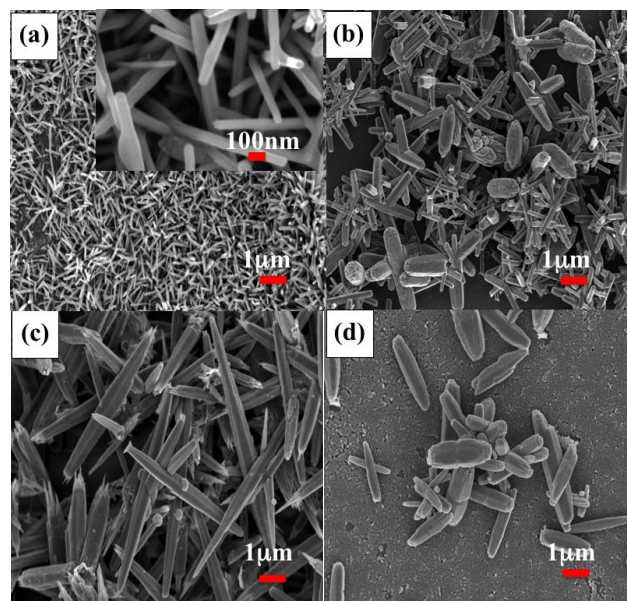


Figure 2: The FESEM images of ZnO nanorods grown from $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ 0.1 M at 150 °C with different time: (a) 3 h, (b) 6 h, (c) 9 h, (d) 12 h

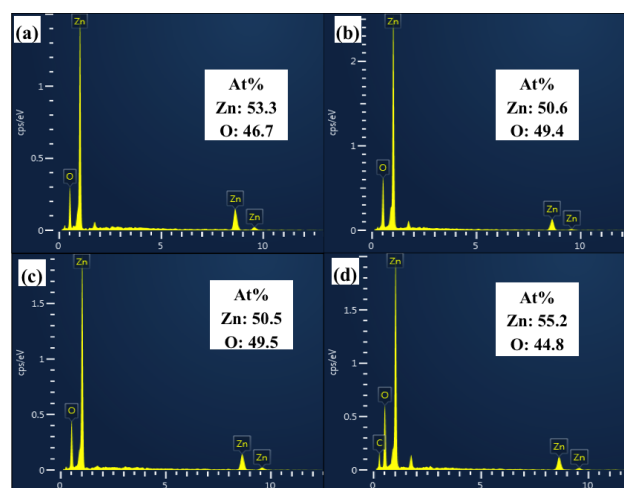


Figure 3: EDS spectrum of ZnO nanorods synthesized by the hydrothermal route at different time: (a) 3 h, (b) 6 h, (c) 9 h, (d) 12 h

3.1.2. X-ray diffraction spectroscopy

Fig. 4 illustrates the X-ray diffraction patterns of ZnO nanorod with different synthesis time. The results indicate that all of ZnO materials have a nanocrystal structure and they grow with hexagonal wurtzite type. No diffraction peak from other phases was observed in these samples. The main significant peaks for ZnO were found in accordance with values in the standard card (JCDPS number 05-0664 for ZnO).

The domain size of the crystal can be estimated from the full width at half maximum of the peaks by means of the Scherrer formula $D = \lambda / b \cos \theta$, where

λ is the wavelength of the incident beam (1.54 Å); b is the correct full width at half maximum of the peak in radians (2θ); θ is the diffraction angle, and K is Scherrer constant ($K = 1$). The average crystallite size calculated from (101) peaks was approximately 50 nm. ($2\theta = 36.67$).

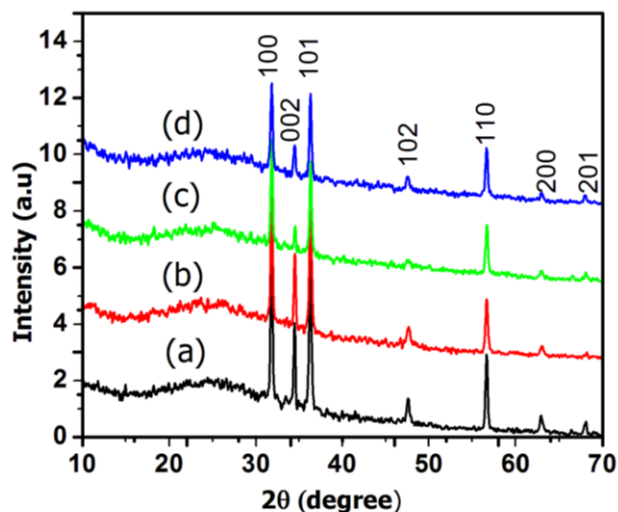


Figure 4: X-ray pattern of ZnO nanorods grown from $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ 0.1 M at 150 °C with different time: (a) 3 h, (b) 6 h, (c) 9 h, (d) 12 h

3.2. Antibody immobilization characterizations

3.2.1. FTIR spectroscopy

In this work, Fourier transforms infrared (FTIR) spectroscopy was used to verify the existence of ZnO nanorod and Anti-Rotavirus on the sensor's surface. Fig. 5 shows the FTIR spectra of (a) antibodies, (b) ZnO nanorod, and (c) antibodies/ZnO nanorod. The FTIR spectral features of antibodies sample displays in Fig. 5(a). The intense band observed around 1697 cm^{-1} is due to N-H bending of amine I form. The band at 1518 cm^{-1} is assigned to the N-H bending of amine II vibration. Fig. 5(b) shows the FTIR spectrum for ZnO nanorod. The peak at 1018 cm^{-1} is related to hydrogen bond of -OH groups of water molecules or surface -OH groups. The peak around 605 and 535 cm^{-1} is assigned to the Zn-O stretching band [8, 9]. When the antibody is absorbed on ZnO nanorod, it can be seen that the band related to N-H bending of amine I form and the N-H bending of amine II vibration of antibodies at 1697 cm^{-1} and 1518 cm^{-1} are shift to 1708 cm^{-1} and 1533 cm^{-1} , respectively, confirming the immobilization of antibodies (Fig. 5c). The presence of ZnO was also confirmed around 476 cm^{-1} [1]. Additionally, the peak at 1015 cm^{-1} is

related to the O-H stretching vibration of H_2O in sample.

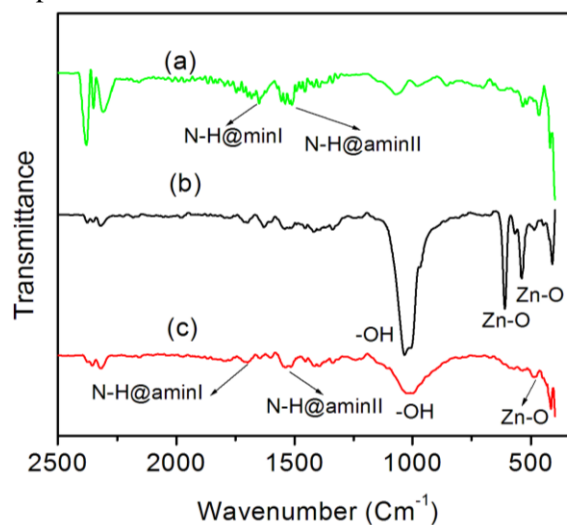


Figure 5: FTIR spectra of the FTIR spectra of (a) antibodies, (b) ZnO nanorod, (c) antibodies/ZnO nanorod

3.2.2. Fluorescence spectroscopy

The density of antibodies on a ZnO nanorod modified electrode was studied by using fluorescence microscopy as shown in Fig. 6. ZnO nanorod deposited electrode's surface was clearly black (Fig. 6 a) while the surface of the electrode with antibodies immobilized by using absorption clearly showed green fluorescence spots (Fig. 6b). This could confirm that antibodies were immobilized on the electrode's surface.

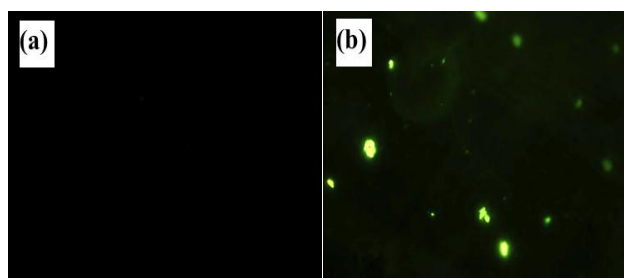


Figure 6: Fluorescence images of antibody immobilized electrode: (a) ZnO nanorod modified electrode, (b) anti-rotavirus/ZnO nanorod modified electrode

3.3. Cyclic voltammetry characterization of immunosensor

Cyclic voltammetry studies on the immunosensor detection of bacterial cells were performed in PBS solution containing 20 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at a scan rate of $100\text{ mV}\cdot\text{s}^{-1}$ versus a

Ag/AgCl reference electrode.

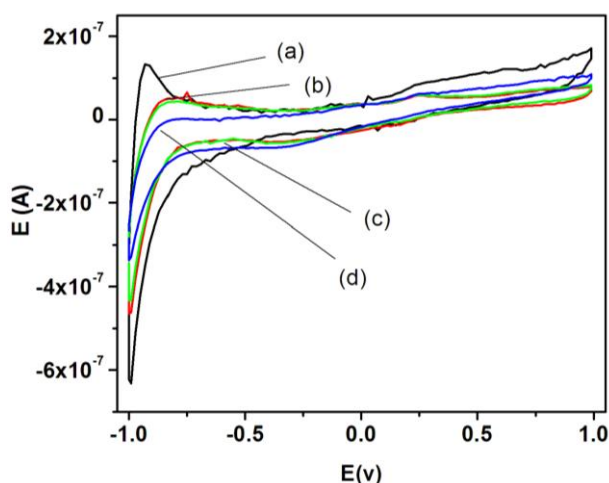


Figure 7: Cyclic voltammetry of microelectrode in the presence of 20 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at a scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$ versus a Ag/AgCl reference electrode.

(a) bare electrode, (b) ZnO nanorod deposited electrode, (c) after antibody ($0.5 \mu\text{g}/\text{mL}$ of Anti-rotavirus) immobilization and (d) after rotavirus cell interaction

Figure 7 presents the cyclic voltammetry characterizations of the immunosensors prepared by antibody immobilization through absorption. We can observe that when the bare sensor was immersed in an electrolyte solution containing the redox probe, the reduction process of the redox probe occurred, and electrons were transferred between the two electrodes through the redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$. The electron transfer was not blocked by any monolayer on the sensor's surface. The peak current was determined to be 132 nA. When ZnO nanowire modified the surface of sensor, a thin film was formed that could hinder electron transfer of hexacyanoferrates into the conductive sensor surface. The peak current obviously decreased to 51.5 nA. On anti-rotavirus/ZnO nanowire-modified sensor surface, a thinner film was formed, and the electron transfer of hexacyanoferrates was continuously inhibited. A slight decrease in the peak current (33.7 nA) was found. Thus, the current change showed that antibodies were attached to ZnO nanowire-modified sensor surface. The antibody immobilization was continuously confirmed by interaction tests between antibodies and rotavirus cells. When the rotavirus cells interacted with the anti-rotavirus modified electrode's surface, a decrease in the peak current of 2.08 nA was observed as shown in Fig. 7(d). The variation in the cyclic voltammetry value induced by binding with cells of *Rotavirus* at different concentrations was

also studied. When the concentration of cells increased from $7.8 \times 10^5 \text{ CFU}/\text{mL}$ to $7.8 \times 10^8 \text{ CFU}/\text{mL}$, the current decreased linearly (data not shown). The detection limit of the immunosensor was $7.8 \times 10^5 \text{ CFU}/\text{mL}$.

4. CONCLUSION

In summary, ZnO nanorods were synthesized by the facile hydrothermal method. The characteristic of the ZnO nanorod based biosensor was tested by cyclic voltammetry technique. Results show that the optimal hydrothermal time for synthesis of ZnO nanorod was 3 h. A linearly decreased response in C-V for cell of rotavirus concentration was found in the range of $7.8 \times 10^5 \text{ CFU}/\text{mL}$ to $7.8 \times 10^8 \text{ CFU}/\text{mL}$. The detection limit of the immunosensor was $7.8 \times 10^5 \text{ CFU}/\text{mL}$. These results showed potential application of ZnO nanorod biosensor in biomedicine and environmental field. These are initial results to use ZnO nanorod for biosensor fabrication. In the next time, the results of the parameters affecting on immunosensor response will be investigated, as follows: pH value, immunoreaction time, incubation temperature, and anti-rotavirus concentration.

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REFERENCES

1. Ahmi Choi, Kyoungwon Kim, Hyo-II Jung, Sang Yeol Lee. *ZnO nanowire biosensors for detection of biomolecular interactions in enhancement mode*, Sensors and Actuators B, **148**, 577-582 (2010).
2. Debabrata Pradhan, Farnaz Niroui, K. T. Leung, High-Performance. *Flexible Enzymatic Glucose Biosensor Based on ZnO Nanowires Supported on a Gold-Coated Polyester Substrate*, ACS Appl. Mater. Interfaces, **2**, 2409-2412 (2010).
3. Yunseok Jang, Jungil Park, Youngmi Kim Pak, James Jungho Pak. *Immunosensor Based on the ZnO Nanorod Networks for the Detection of H1N1 Swine Influenza Virus*, Journal of Nanoscience and Nanotechnology, **12**, 5173-5177 (2012).
4. Jungil Park, Xueqiu You, Yunseok Jang, Yoonkyung Nam, Min Ja Kim, Nam Ki Min, James Jungho Pak. *ZnO nanorod matrix based electrochemical immunosensors for sensitivity enhanced detection of Legionella pneumophila*, Sensors and Actuators B, **200**, 173-180 (2014).
5. P. Sanguino, Tiago Monteiro, S. R. Bhattacharyy, C. J. Diasb, Rui Igrej, Ricardo Franco. *ZnO nanorods*

- as immobilization layers for interdigitated capacitive immunosensors*, Sensors and Actuators B, **204**, 211-217 (2014).
6. Sachindra Nath Sarangi, Shinji Nozaki, Surendra Nath Sahu. *ZnO Nanorod-Based Non-Enzymatic Optical Glucose Biosensor*, Journal of Biomedical Nanotechnology, **11**, 988-996 (2015).
 7. Mohammed Marie, Sanghamitra Mandal, Omar Manasreh. *An Electrochemical Glucose Sensor Based on Zinc Oxide Nanorods*, Sensors, **15**, 18714-18723 (2015).
 8. Mutasim I. Khalil, Maha M. Al-Qunaibit, Aisha M. Al-zahem, Joselito P. Labis. *Synthesis and characterization of ZnO nanoparticles by thermal decomposition of a curcumin zinc complex*, Arabian Journal of Chemistry (2013), <http://dx.doi.org/10.1016/j.arabjc.2013.10.025>.
 9. Samira Bagheri, Chandrappa K. G., Sharifah Bee Abd Hamid. *Facile synthesis of nano-sized ZnO by direct precipitation method*, Der Pharma Chemica, **5(3)**, 265-270 (2013).
 10. C. Amutha, A. Dhanalakshmi, B. Lawrence, K. Kulathuraan, V. Ramadas, B. Natarajan. *Influence of Concentration on Structural and Optical Characteristics of Nanocrystalline ZnO Thin Films Synthesized by Sol-Gel Dip Coating Method*, Progress in Nanotechnology and Nanomaterials Jan. **3**, 13-18 (2014).

Corresponding author: **Phuong Dinh Tam**

Advanced Institute for Science and Technology
Hanoi University of Science and Technology
No.1, Dai Co Viet, Hai Ba Trung Hanoi
E-mail: phuongdinhtam@gmail.com.