THE SCATTERING PATTERN STUDY OF ZINC OXIDE NANOROD ON SILICA GLASS SUBSTRATE FOR PROTEIN IMMOBILIZATION PLATFORM

[1]THANIT APIWATTANADEJ, [2]PORNPIMOL SRITONGKHAM, [3]WALEED S. MOHAMMED

[1,2]Biosensor laboratory, Department of Biomedical engineering The Faculty of Engineering, Mahidol University, Salaya, Nakhon Pathom 73170, Thailand,
[3]Center of Research in Optoelectronics, Communication and Control Systems, School of Engineering, Bangkok University, Pathum Thani 12120, Thailand
E-mail: [1]thanit_ap@yahoo.com, [2]pornpimol.srt@mahidol.ac.th, [3]waleed.m@bu.ac.th

Abstract- In this work, the light scatterings from Zinc oxide (ZnO) nanorods on silica glass substrate for protein immobilization are reported. ZnO nanorods were grown using hydrothermal process. The effects of the growth time and the effects of Dimercaptosuccinic acid (DMSA) concentration on the scattering pattern of ZnO nanorods were experimentally measured using a nephelometer. According to the results, the growth time increased the length of nanorod and reduced the number of nanorod per unit area. The optimum results for 12 hours growth was 6.35 nW (9.76 %RSD) for the scattering power and the scattering peak angle was 54.22 degree (0.03 %RSD). DMSA solution was used to functionalize carboxylic group on the ZnO surface. Hence, in the presence of 0.1 mM DMSA, the maximum scattering power of ZnO nanorods was reduced up to 1.5%. These outcomes were resulted from the dissolving of ZnO nanorod in acidic solution. ZnO nanorods on silica glass substrates demonstrated a potential to be conjugated with other proteins, which could be applied for biosensor fabrications.

Index Terms- Light scattering, Optical biosensors, Hydrothermal process, Zinc oxide nanorod

I. INTRODUCTION

Zinc oxide (ZnO) is a well-known material in term of electrical and optical applications since 1960s [1]. The Wurtzite crystal structure of ZnO is an asymmetry structure, which has unique properties such as, strong piezoelectric and pyroelectric effects. The wide band gap (3.37 eV) of the ZnO material absorbs the ultraviolet spectrum. The high exciton binding energy (60 meV) allows excitonic emissions at room temperature. A large surface to volume ratio of the ZnO nanostructure makes it a suitable material for sensing applications. A humidity sensor [2] and an ammonia gas sensor [3-5] are examples of ZnO in sensor applications. One of the important properties of the ZnO nanostructure is a high isoelectric point (pI = 9.5) [6], which allows ZnO to be immobilized with acidic proteins via electrostatic interaction [6]. There are several applications of biomolecule interfacing on a ZnO surface, including: biofunctional on flexible substrates [7], DNA hybridization on ZnO nanorods [8], enzyme-based biosensors [6, 9] and antibody-based biosensors[10]. In our previous work [5], the ZnO nanorods were successfully synthesized on an optical fiber employing a hydrothermal process. In general, the hydrothermal synthesizing method is a simple technique that does not require a high temperature and a vacuum system, which makes it inexpensive. Also, during the process, the morphology of ZnO nanorods can be controlled [1, 5, 11]. Hence, the effects of hydrothermal growth conditions on light coupling with optical fibers were experimentally investigated. This study opened an opportunity to fabricate a biosensing platform, which is sensitive to alterations of light scattering patterns. In order to fabricate a biosensor, the protein immobilization on the ZnO nanorods must be thoroughly studied and optimized. There is evidence showing that the ZnO nanorods are capable of absorbing acidic proteins directly, however; these direct absorption processes are unstable. This is due to the weak electrostatic force between the proteins and the ZnO nanorods that can be easily destroyed by several common conditions [6]. To avoid the prior stated problem, the cross-linking synthesis, which creates strong covalent bonding between the proteins and the ZnO nanorods, is suggested [7, 10]. In this research, carbodiimide cross-linking is chosen for immobilizing proteins. This cross-linking method creates amide bonds between the proteins and ZnO nanorods [7, 10]. To achieve this purpose, the activated carboxylic groups must be functionalized on the ZnO nanorods using Dimercaptosuccinc acid (DMSA) [7, 10, 12, 13]. Finally, the effects of the DMSA concentration on the scattering patterns of the ZnO nanorods were studied.

II. EXPERIMENT

All the chemicals using in this experiment are analytical grade chemicals, which were purchased from Sigma-aldrich, USA. Here, they were directly employed without any further purification. The silica glass slides (1.0 cm x3.0 cm) were cleaned using a series of soap water, acetone, ethanol, and deionized (DI) water, respectively, in ultrasonic bath for 15 minutes.

A. ZnO seeding

ZnO nanoparticles were synthesized for nucleation sites using 1 mM zinc acetate ($Zn(CH_3COO)_2$) in ethanol. The bare glasses were placed on a hot plated at 60°C.

100 µl of zinc acetate solution were dropped on the glass surface and dried. The dropping processes were repeated for 10 times. Afterward, the glass substrates were annealed at 350°C for 2 hours to minimize the oxygen defects within the ZnO nanorods.

B. Hydrothermal growth of ZnO nanorod

After the seeding process, the unseeded sides of the slides were covered by dark masking tapes. Then, the slides were placed on the petri dishes with the processed surface downward. Later, 20 mM Zinc nitrate ($Zn(NO_3)_2$) and 20 mM Hexamine ($C_6H_{12}N_4$) were added to initiate the nanorod growth. The petri dishes were placed inside an oven at 90 °C with different time periods (6, 8, 10, and 12 hours), in order to study the relationship between the incubating time and the growing pattern, in precise, the morphology and the scattering pattern of the ZnO nanorods on the prepared surface. The precursor solutions were changed every 5 hours. Finally, the samples were cleaned by DI water and air-dried at 90 °C for 1 hour.

C. DMSA-ZnO modification

To study the effect of the DMSA concentrations on the scattering patterns of the ZnO nanorods, different concentration of DMSA (0.05 mM, 0.1 mM, 0.3 mM, and 0.5 mM) were prepared in a mixture of Zinc nitrate and Hexamine. Subsequently, the 12-hour period hydrothermal process was employed. After 5 hour, instead of changing the precursor solution, the DMSA solutions were added to the glass substrates. Finally, the samples were cleaned by DI water and air-dried at 90 °C for 1 hour.

D. Light scattering measurement

A nephelometer was built using optical equipment from Thorlabs, USA. Fig.1 shows the nepholometer structure, which includes a handheld laser source (item no.HLS635), an optical power and an energy meter (item no.PM100USB), and a motorized worm drive rotation stage (item no.CR1-Z7). The light source and power meter were placed diagonal to one another.

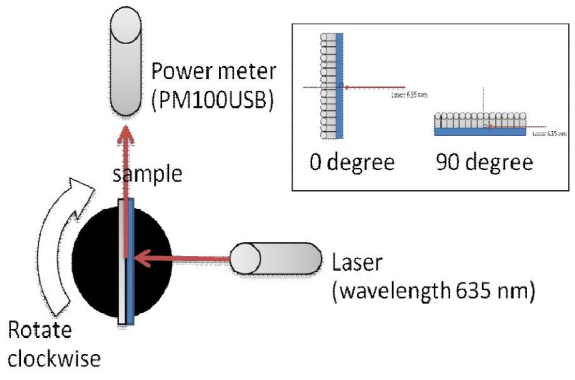


Fig. 1. A schematic diagram of the nephelometer

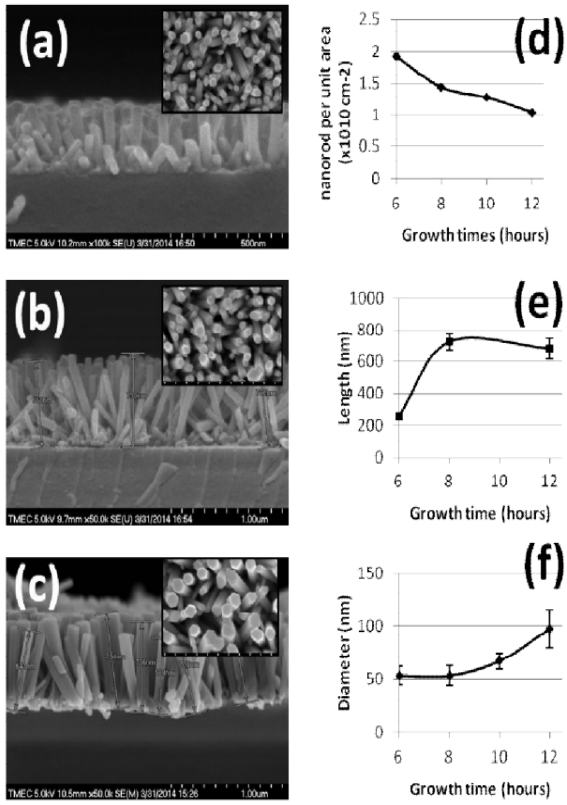


Fig. 2. SEM images of the ZnO nanorods on the glass substrate for (a) 6, (b) 8, and (c) 12 hours, along with the variations in the (d) nanorods per unit area, (e) length, and (f) diameter of the nanorods

To measure the light scattering patterns, the samples were placed at the center of the rotation stage with the processed areas facing outward from the laser source. The approximate power of the laser source was 2.5-5 mW. The samples were rotated 90° clockwise as shown in Fig. 1. The scattering powers corresponding to the scattering angles were automatically recorded.

III. RESULT AND DISCUSSION

A. The effects of the growth time on the morphology of the ZnO nanorods From Scanning electron microscope (SEM) images, the effects of growth time on the number of the nanaorods per unit area, length, and diameter were investigated. As it is depicted in Fig. 2 (d), the numbers of the ZnO nanorods per unit area on the glass substrate were decreased with time. This was because the small nanorods could possibly merge with the nearby nanorods during the synthesizing process. This phenomenon was also reported in our previous work [5]. From Fig. 2(e), the lengths of ZnO crystals were greatly increased in direct proportion to the incubation time. The length from 8 hour incubation time is ~700 nm, which increased up to 300% when comparing with the average lengths of 6 hour growth time (~250 nm). This was due to the polarity on (001) plane of the ZnO crystal structures [1, 5].

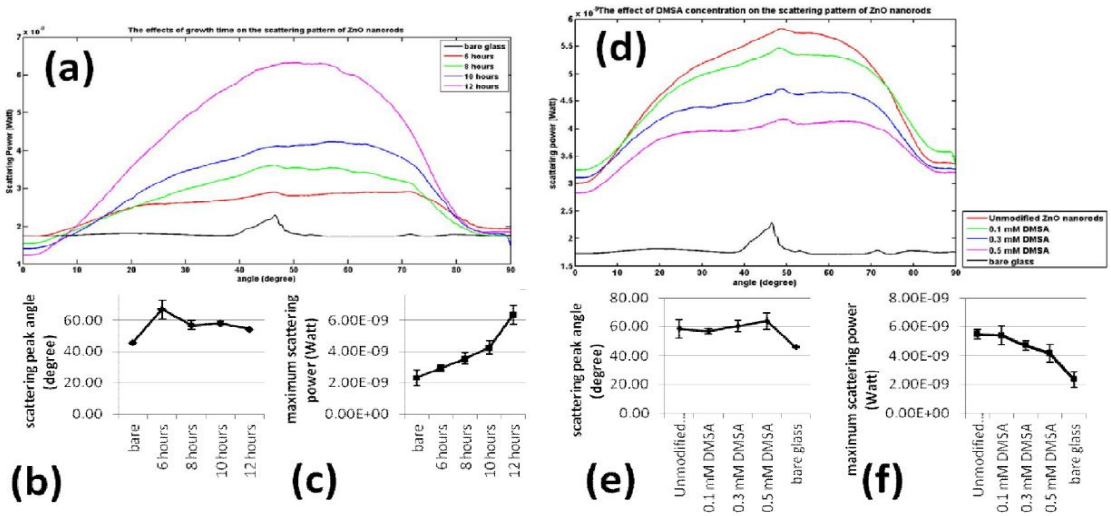


Fig. 3. (a) The effect of the growth time on the scattering patterns of the ZnO nanorod along with the (b) average scattering angles and the (c) average scattering powers, and (d) the effect of DMSA concentration on the scattering patterns of the ZnO nanorods along with the (e) average scattering angles and the (f) average scattering powers

Furthermore, the growth time also played a minor role in increasing the diameters of the ZnO nanorods from ~50 nm to ~100 nm. This was resulted from the attachment of hexamine around the non-polar surfaces, which controlled the growth direction of the ZnO nanorods [1].

B. The effect of the growth time on the scattering patterns of the ZnO nanorods

As aforementioned, the ZnO nanorods could enhance the light scattering on the treated glass surfaces. The scattering powers gradually increased from 0 to 45 degree. After 45 degree, the powers of the scattered light reached the maximum and saturated until around 50 to 70 degrees depending on the samples. Afterward, the powers of the scattered light were rapidly decreasing as shown in Fig.3 (a). From Fig.3 (b) and (c), the growth time showed no effect on the scattering angles of the ZnO nanorods. The average scattering peak angles were approximately 60 degree. However, the growth time demonstrated a relationship in increasing the scattering powers. The maximum scattering power was measured from the samples with 12 hours growth time, which was 6.35 nW with 9.76% relative standard deviation (RSD). This power was dramatically higher than the untreated glasses, which had an average scattering power equal to 2.34 nW with 22.32 %RSD.

C. The effect of the DMSA concentration on the scattering of the ZnO nanorods

Because of the dissolved ZnO nanorod in acidic solution, the results of the scattering patterns in Fig. 3(d) demonstrated that a high concentration of DMSA solution resulted in a decreasing of the scattering power of the ZnO nanorods. This result are reported in another research study [14]. The optimized concentration of the DMSA solution should not be extremely low or high. In precise, a low concentration of DMSA was inadequate to create the ZnO nanostructure, while a high concentration of DMSA would dissolve the ZnO nanorods.

For the scattering patterns, the DMSA concentrations showed no effect on the scattering angles (Fig. 3(e)). However, an increasing in the DMSA concentration dramatically reduced the scattering powers as shown in Fig. 3(f). When 0.1 mM DMSA was added to the solution, the scattering power was declined to 5.38 nW with 12.24%RSD. This concentration contributed to the lowest scattering power reduction, which was -1.5%. In case of 0.3 mM and 0.5 mM DMSA solution, the maximum scattering powers were reduced by -13.74% and -23.35% respectively.

Therefore, 0.1 mM DMSA was uitilized to functionalize carboxylic group on the ZnO nanorod surfaces for further immobilizations.

CONCLUSION

As a summary of this study, the DMSA-ZnO nanorod platforms were successfully developed. In order to maximize the light scattering, the optimal hydrothermal growth time and the concentration of DMSA was 12 hours and 0.1 mM, respectively. This DMSA-ZnO platform could possibly be applied to develop an optical biosensor based on the light scattering measurement in the nearly future.

REFERENCES

[1] S. Baruah and J. Dutta, "Hydrothermal growth of ZnO nanostructures," Science and Technology of Advanced Materials, vol. 10, Mar 2009.

[2] Y. J. Liu, Y. Zhang, H. X. Lei, J. W. Song, H. Chen, and B. J. Li, "Growth of well-arrayed ZnO nanorods on thinned silica fiber and application for humidity sensing," Optics Express, vol. 20, pp. 19404-19411, Aug 13 2012.

[3] A. O. Dikovska, P. A. Atanasov, A. T. Andreev, B. S. Zafirova, E. I. Karakoleva, and T. R. Stoyanchov, "ZnO thin film on side polished optical fiber for gas sensing applications," Applied Surface Science, vol. 254, pp. 1087-1090, Dec 15 2007.

[4] A. O. Dikovska, G. B. Atanasova, N. N. Nedyalkov, P. K. Stefanov, P. A. Atanasov, E. I. Karakoleva, et al., "Optical sensing of ammonia using ZnO nanostructure grown on a side-polished optical-fiber," Sensors and Actuators B-Chemical, vol. 146, pp. 331-336, Apr 8 2010.

[5] T. Bora, H. Fallah, M. Chaudhari, T. Apiwattanadej, S. W. Harun, W. S. Mohammed, et al., "Controlled side coupling of light to cladding mode of ZnO nanorod coated optical fibers and its implications for chemical vapor sensing," Sensors and Actuators B: Chemical, vol. 202, pp. 543-550, 2014.

[6] X. W. Liu, Q. Y. Hu, Q. Wu, W. Zhang, Z. Fang, and Q. B. Xie, "Aligned ZnO nanorods: A useful film to fabricate amperometric glucose biosensor," Colloids and Surfaces B-Biointerfaces, vol. 74, pp. 154-158, Nov 1 2009.

[7] T. Y. Liu, H. C. Liao, C. C. Lin, S. H. Hu, and S. Y. Chen, "Biofunctional ZnO nanorod arrays grown on flexible substrates," Langmuir, vol. 22, pp. 5804-9, Jun 20 2006.

[8] J. W. Zhao, L. Z. Wu, and J. F. Zhi, "Fabrication of micropatterned ZnO/SiO(2) core/shell nanorod arrays on a nanocrystalline diamond film and their application to DNA hybridization detection," Journal of Materials Chemistry, vol. 18, pp. 2459-2465, Jun 7 2008.

[9] J. W. Zhao, D. H. Wu, and J. F. Zhi, "A novel tyrosinase biosensor based on biofunctional ZnO nanorod microarrays on the nanocrystalline diamond electrode for detection of phenolic compounds," Bioelectrochemistry, vol. 75, pp. 44-49, Apr 2009.

[10] X. Yan, Z. Huang, M. He, X. Liao, C. Zhang, G. Yin, et al., "Detection of HCG-antigen based on enhanced photoluminescence of hierarchical ZnO arrays," Colloids Surf B Biointerfaces, vol. 89, pp. 86-92, Jan 1 2012.

[11] H. Fallah, M. Chaudhari, T. Bora, S. W. Harun, W. S. Mohammed, and J. Dutta, "Demonstration of side coupling to cladding modes through zinc oxide nanorods grown on multimode optical fiber," Opt Lett, vol. 38, pp. 3620-2, Sep 15 2013.

[12] F. Lu, W. Cai, and Y. Zhang, "ZnO Hierarchical Micro/Nanoarchitectures: Solvothermal Synthesis and Structurally Enhanced Photocatalytic Performance," Advanced Functional Materials, vol. 18, pp. 1047-1056, 2008.

[13] Z. b. H. G.Y. Mei Yang, Yun qing Kang, Xiao ming Liao, Hui Wang, "Preparation and Optical Properties of Biomimic Hierarchical ZnO," Crystal Growth and Design, vol. 9, pp. 707-714, 2009.

[14] G. Y. Mei Yang, Zhongbing Huang, Yunqing Kang, Xiaoming Liao, Hui Wang, "Preparation and Optical Properties of Biomimic Hierarchical ZnO Column Arrays," Crystal Growth and Design, vol. 9, pp. 707-714, 2009.

★★★