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Formation of ZnO Nanorods via Low Temperature Hydrothermal Method for Enzymatic Glucose Sensor

(Pembentukan Batang Nano ZnO melalui Kaedah Hidroterma Suhu Rendah untuk Sensor Enzim Glukosa)

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ABSTRACT

In this study, zinc oxide (ZnO) nanorod arrays were synthesized using a simple hydrothermal reaction on a ZnO seeds/ITO substrate and applied for the fabrication of enzymatic glucose sensor. ZnO nanorod matrix provided a favourable environment for the immobilization of glucose oxidase (GOx) and introduced a shuttling way for electronic communication between GOx and electrode. The performance of different aspect ratio of ZnO nanorods that was produced by varying hydrothermal reaction time was studied. The aspect ratio of ZnO influenced the GOx enzyme immobilization. The morphology and structure of prepared ZnO nanorods were characterized by employing scanning electron microscopy (SEM), and X-ray powder diffraction (XRD). Electrochemical measurements of the sensor showed a reproducible sensitivity of 2.06 $\mu\text{A}/\text{cm}^2\text{mM}$ for ZnO matrix grown for 4 h with the aspect ratio of 8.0.

Keywords: Cyclic-voltammetry; glucose oxidase (GOx); hydrothermal; ZnO nanorods

ABSTRAK

Dalam kajian ini, zink oksida (ZnO) batang nano telah disintesis menggunakan reaksi mudah hidroterma pada benih ZnO/substrat ITO substrat dan digunakan untuk fabrikasi sensor glukosa enzim. ZnO matriks batang nano menyediakan persekitaran yang menggalakkan bagi imobilisasi glukosa oksidase (GOx) seterusnya bagi menyediakan laluan komunikasi elektronik antara GOx dan elektrod. Prestasi bagi nisbah aspek batang nano ZnO yang berbeza yang dihasilkan dengan mengubah masa tindak balas hidroterma telah dikaji. Nisbah aspek ZnO mempengaruhi imobilisasi GOx enzim. Morfologi dan struktur ZnO batang nano telah dicirikan dengan menggunakan mikroskop imbasan elektron (SEM) dan pembelauan sinar-X (XRD). Ukuran sensor elektrokimia menghasilkan kesensitifan sebanyak 2.06 $\mu\text{A}/\text{cm}^2\text{mM}$ untuk matrik ZnO yang dihasilkan dengan tindak balas hidroterma selama 4 jam dengan nisbah aspek 8.0.

Kata kunci: Glukosa oksidase (GOx); hidroterma; kitar-voltan; batang nano ZnO

INTRODUCTION

Many efforts have been made to develop an amperometric glucose biosensor based on the use of glucose oxidase (GOx) due to increasing demand for a blood glucose monitoring, environmental monitor, food industry and clinical detection (Ahmad et al. 2012; Anusha et al. 2014; Xia et al. 2014). An enzyme-involved electrochemical glucose biosensor has been intensively studied because of its simplicity, high selectivity and relatively low cost (Kim et al. 2014; Kong et al. 2009). GOx is widely used due to its excellent stability, less expensive, high catalytic properties and real time detection (Anusha et al. 2014; Xia et al. 2014). GOx is highly selective to glucose as it carries one molecule of tightly bound co-enzyme, flavin adenine dinucleotide (FAD) which acts as a redox carrier in catalysis (Wohlfahrt et al. 2004). According to Hwa and Subramani (2014) and Kong et al. (2009) the amount of glucose is proportional to the produced H_2O_2 , thereby the glucose concentration can be determined by measuring the current derived from electrochemical reaction. For this reason GOx is often used as the enzyme in glucose sensor. It is important to consider the choice of nanomaterials

used for enzyme immobilization since GOx modified bare electrodes were hard to achieve its activity and stability (Xia et al. 2014). One dimensional (1D) ZnO has attracted more attention among researchers for bioactive substances due to its high isoelectric point (IEP) ~ 9.5 which makes it suitable for the absorption of low IEP of GOx ~ 4.2 by electrostatic interaction in a proper buffer solution (Lei et al. 2011). Moreover ZnO is biocompatible and has high electron transfer capabilities that will enhance the electron transfer between active sites of enzyme and electrode without the need of electron mediators (Pradhan et al. 2010). According to Ahmad et al. (2012) and Kim et al. (2014), the morphology of ZnO such as shape, size and surface area will greatly influence enzymes loading on the surface of its matrix for a high selectivity, wide linear range and fast sensitivity of glucose detection. Since the glucose level in bloods from diabetic patients can be easily goes up to 15–20 mM, it is important to ensure a sufficient amount of immobilized GOx (Ahmad et al. 2012). Thereby, the degree of GOx immobilization on ZnO nanorods surface is important for a wide linear range of glucose detection. However, limited work on glucose biosensor based on

size and morphology of ZnO nanorods has been reported. In this paper, ZnO nanorods were hydrothermally grown on an indium tin oxide (ITO) seeded substrate for enzyme immobilization in glucose detection. Their glucose sensing performance was investigated on different aspect ratio ZnO nanorods that were produced by varying hydrothermal reaction time. The glucose sensing capabilities were found to be influenced by the surface area of grown ZnO nanorods, suggesting that high aspect ratio and dense grown ZnO are more favourable.

MATERIALS AND METHODS

In this paper, ITO substrates were cut and cleaned in NH_4OH , H_2O_2 and distilled water in a ratio of 1:4:20 at 60°C for 20 min and then rinsed with distilled water. In order to eliminate water traces, Isopropyl alcohol was used and dried in a nitrogen gas flow (Besbes et al. 2006). ZnO seed layer was prepared by dissolving a zinc acetate dehydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) in methanol. The mixture was vigorously stirred at 60°C for 20 min. Then 15 mL of monoethanolamine (MEA) was added drop wise into the solution under constant stirring at 60°C for 2 h. The solution was aged at room temperature for 24 h before deposition process (Foo et al. 2013). ZnO seed layer was dropped on the ITO substrate and dried at 150°C for 20 min. This process was repeated 3 times. After deposition, the coated ITO substrates were annealed at 500°C for 2 h in air. Next, the ZnO seeded samples were placed in screw-capped bottles containing 1:1 molar ratio of zinc nitrate ($\text{Zn}(\text{NO}_3)_2$) and hexamethylamine (HMT) as a precursor solution (Ridhuan et al. 2012). The hydrothermal reaction was performed in a pre-heated oven at 80°C at different hydrothermal reaction time (0.5-6 h).

Before the immobilization of GOx, ZnO nanorods were rinsed with phosphate buffer saline (PBS) solution to generate a hydrophilic surface. For immobilization of GOx on ZnO nanorods, 10 μL of GOx (1mg/mL in 0.01M

PBS) solution was dropped onto the surface of ZnO/ITO electrode and kept at 4°C overnight followed by extensive washing step to remove the unimmobilized GOx. Finally, 10 μL of 5% Nafion was dropped to attach GOx/ZnO/ITO tightly on the surface. Nafion is beneficial as a membrane which provides a biocompatible environment to the enzyme without interfering with biosensor (Pradhan et al. 2010). All prepared enzyme electrodes were stored in dry condition at 4°C when not in use. The morphology and structure of prepared ZnO nanorods were characterized by field-emission scanning electron microscopy (FESEM) and X-ray diffractometer (XRD). In order to investigate the electrochemical performance of the fabricated GOx/ZnO/ITO glucose sensor, cyclic voltammetry measurements were carried out from -1.0 to +0.5 V at a scan rate of 50 mV/s. All the measurements were performed at room temperature.

RESULTS AND DISCUSSION

Figure 1(a) shows the surface morphology of ZnO seeded layer with an average diameter of 97.21 nm. ZnO seeds exhibit a homogeneous and uniform in size of nuclei site for further hydrothermal growth. It was crucial to have a ZnO nanocrystal seed layer during the initial stage of hydrothermal as it provides heterogeneous nucleation sites for ZnO formation. ZnO is a polar crystal, with each of Zn atom was tetrahedrally coordinated to four atoms and vice versa (Tan et al. 2014). With the alternate arrangement of Zn^{2+} and O^{2-} ions along the c-axis, an accumulation of charges on the surface of oxide either positively or negatively charged depending on the terminating ions. As a result, an accumulation of $\text{Zn}(\text{OH})_2$ and $\text{Zn}(\text{OH})_2(\text{NH}_3)_4$ can be seen on the existing grains (Lockman et al. 2010; Tan et al. 2014). Figure 1(b)-1(f) shows the FESEM images of ZnO nanostructures grown at different hydrothermal duration. The ZnO nanorods only formed at growth duration of 2 h and more. At growth duration of 0.5 h and 1 h only ZnO nanoparticles were formed. This was due to

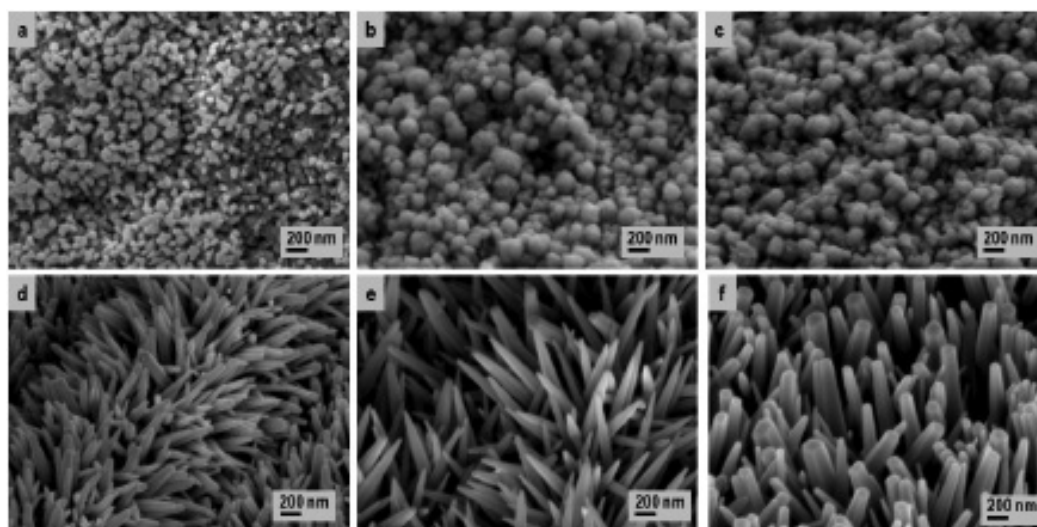


FIGURE 1. FESEM images of (a) ZnO seed layer, different hydrothermal growth duration of (b) 0.5 h, (c) 1 h, (d) 2 h, (e) 4 h and (f) 6 h

insufficient time for a complete thermal decomposition of precursor solution to provide OH^- and Zn^{2+} that result in less formation of $\text{Zn}(\text{OH})_2$. As a result, relatively slow growth rate took place at the nucleation site that causes no formation of nanorods. This result was in agreement with Khusaimi et al. 2010. Homogeneous and denser ZnO nanorods could be observed when we prolonged the growth duration with an average diameter of 83.2 and 90.1 nm for growth duration of 2 and 4, respectively. As the hydrothermal growth duration was increased to 6 h, the morphology consisted of a mixture of small tips and large tapered hexagonal rods with an average diameter of 75 and 197 nm, respectively. The formation of large nanorods was due to the possible coalescence of the single nanorods forming larger nanorods. This was in agreement with our previous work that the coalescing of nanorods leads to formation of larger nanorods at longer time of exposure to hydrothermal solution due to the Oswald ripening mechanism (Lockman et al. 2010).

XRD patterns for samples prepared at 0.5, 1, 2, 4 and 6 h are shown in Figure 2. Except for the peaks caused by the ITO substrate, the patterns exhibit characteristic peaks of wurtzite ZnO hexagonal structure that is ascribed to JCPDS #36-1415. The inset in Figure 2 shows a (100), (002) and (101) peaks of ZnO nanorods at 2θ of 31.7° , 34.4° and 36.2° , respectively. The intensity of all peaks increased with increasing growth duration. This was due to the good crystallinity of the grown ZnO nanorods. The presence of (100) and (101) peaks in the XRD pattern, implied poor upright alignment and slanted of grown ZnO nanorods which was consistent with the cross section of FESEM analysis.

A 3-electrode system consisting of ITO electrode with as grown ZnO as a working electrode, Pt electrode as auxiliary electrode and Ag/AgCl as reference electrode

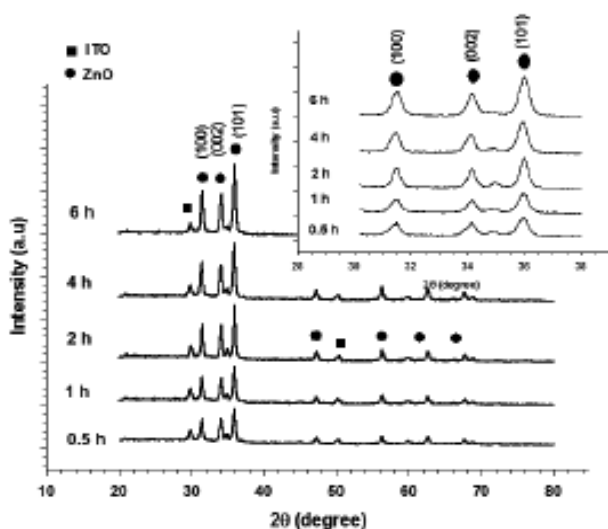


FIGURE 2. XRD patterns of the grown ZnO at different hydrothermal growth duration. The insets show the (100), (002) and (101) diffraction peaks of ZnO formation at $2\theta = 30\text{--}38^\circ$

were used for electrochemical studies, which were carried out in 15 mM glucose in 0.01 M PBS buffer. Figure 3 shows the cyclic voltammetry characteristics of ITO/ZnO/GOx/Nafion electrodes with different hydrothermal growth duration of ZnO. It was noticeable that the oxidation current increased gradually with the increase of growth duration up to 4 h growth and decreased back at growth duration of 6 h. The current increase was due to the oxidation of H_2O_2 which released two electrons. As agreed by many authors, the electrochemical reactions can be described as follows (Kim et al. 2014; Lei et al. 2011; Pradhan et al. 2010):

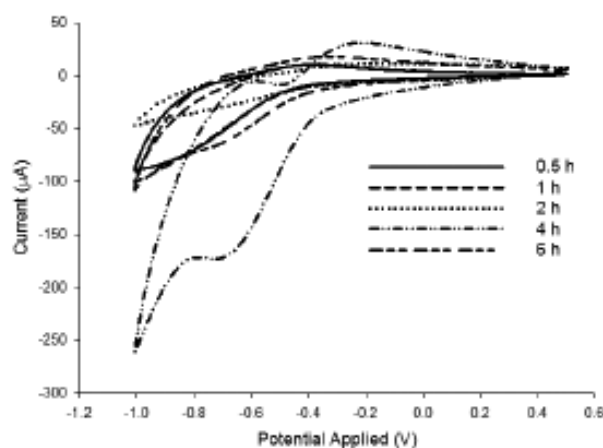
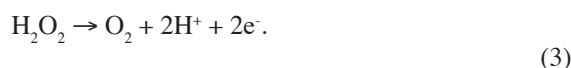
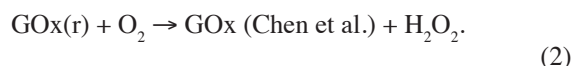
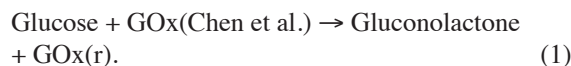


FIGURE 3. Cyclic voltammetry of ITO/ZnO/GOx/Nafion electrodes in 15 mM glucose in 0.01 M PBS at different hydrothermal growth duration

Moreover, the oxidation peak potential for H_2O_2 shifts positively with the increase of growth duration up to 4 h, then negatively shifts at 6 h. This finding suggests a kinetic control over the redox at the active sites of GOx/ZnO electrode (Lei et al. 2011). The sensitivity as a function of the hydrothermal growth duration is shown in Figure 4 where the sensitivity of GOx/ZnO electrode increased up to 4 h then decreased at 6 h. This was due to the high aspect ratio of ZnO nanorods grown at 4 h compared to 6 h which contributed to the highest sensitivity of glucose sensors. The average aspect ratio of grown ZnO nanorods is 6.3, 8.0 and 6.7 for the samples at growth of 3, 4 and 6 h, respectively. According to Kim et al. 2014, the glucose sensing capability was found to be strongly associated with the surface area of nanorods in which GOx molecules cover the entire surface of ZnO nanorods not only on the

tip of nanorods. Even though the ratio of the amount of GOx that attached to the surface of ZnO nanorods is not yet known, the high aspect ratio of ZnO nanorods grown at 4 h was likely to have the highest amount of GOx molecules. As a result, the signal of glucose sensing increased. This result was in agreement with Ahmad et al. (2012) that high aspect ratio of grown ZnO nanorods provides high electron communication features that enhance the direct electron transfer through ZnO nanorods to electrode. Therefore, hydrothermally grown ZnO nanorods on ITO seeded substrate can be effectively applied for a high performance of glucose sensing.

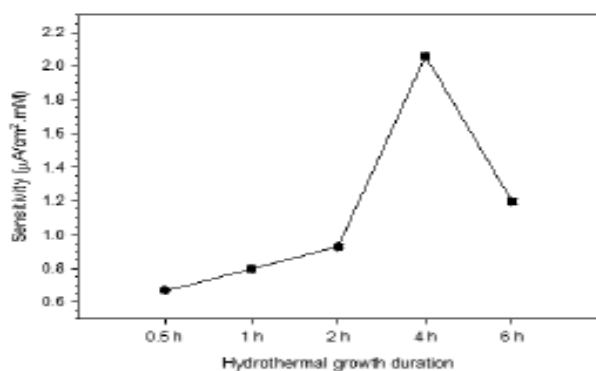


FIGURE 4. Sensitivity of prepared ZnO/ITO electrode as a function of different hydrothermal growth duration of ZnO

CONCLUSION

In conclusion, ZnO nanorods arrays were successfully formed on ITO seeded substrates with different aspect ratio by hydrothermal synthesis and used as a matrix for glucose sensing. A uniform distribution of ZnO nanorods were grown for 4 h with aspect ratio of 8.0 exhibited the best result in glucose with sensitivity of $2.06 \mu\text{A}/\text{cm}^2 \text{mM}$.

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