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HYDROTHERMAL SYNTHESIS AND FABRICATION OF ZINC OXIDE FOR ENZYMATIC GLUCOSE BIOSENSOR

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ABSTRACT

An enzymatic sensor for glucose detection based on zinc oxide nanorods (ZnO NRs) modified indium tin oxide (ITO) electrode was constructed via hydrothermal process. The electrode was characterized by field-emission scanning electron microscopy (FESEM) and X-ray diffractometer. The electrochemical behavior of the produced electrodes was investigated by cyclic voltammetry. Under the optimum condition, the modified electrode displays a linear response to glucose in concentration from 0.05 to 20 mM with sensitivity of 1.602 μA/mM.cm^2 and a limit of detection (LOD) of 0.07 mM. The calculated Michaelis Menten constant, K_m was 3.07. The lower value of K_m means that the immobilized GOx hold a high enzymatic activity, and the proposed modified electrode exhibited a high affinity for glucose. This makes the system is of choice for glucose detection.

Keywords: ZnO nanorods; hydrothermal; glucose oxidase (GOx); cyclic-vaoltammetry;

INTRODUCTION

Glucose biosensor continuously plays a significant role in the field of biological detection, food production and clinical diagnosis, particularly an important role in monitoring blood glucose level in diabetic patients for the diagnosis and treatment of diabetes [1-3]. Several techniques have been developed for a reliable glucose biosensor such as colorimetry, conductometry, optical methods and electrochemical methods [3-5]. The development of electrochemical glucose biosensor based on the use of glucose oxidase (GOx) has been studied due to its high selectivity and low cost [1-3]. Moreover, GOx has been widely used due to its excellent stability, less expensive, high catalytic properties and real time detection [6]. Even though GOx could be immobilized directly on the electrode surface through simple cast method, it is still difficult for enzymes to exchange electron with electrodes surface directly due to its large and complex surface structure of electrode and the change of enzyme shape might be different after being adsorbed on the electrode surface [3]. Therefore, it is important for GOx to be

immobilized on a suitable matrix to ensure the stability of enzymes used in the fabrication of glucose biosensor; hence ZnO has been an attractive candidate.

ZnO NRs has been used as a matrix as is possesses significant sensing surface with high electron mobility (210 cm^2 V^{-1} s^{-1}) and high isoelectric point (IEP) (~9.5), which is suitable for electrostatically adsorbing low IEP of GOx (~4.2) at physiological pH of 7.4 [7, 8]. Moreover ZnO NRs will facilitate a direct electron transfer (DET) of enzyme and minimize the barriers for mass transportation between the substrate and product [8]. Studies have shown that geometric factors such as surface area and shape of ZnO NRs will influence the performance of glucose biosensor when ZnO NRs are used as matrix for enzyme immobilization [5]. Moreover many researches have been focused on the modification of ZnO surface area but not on the crystallinity of grown ZnO NRs. High crystallinity of grown ZnO NRs will lower the resistance of electron transfer between analyte and the electrode surface [3]. Various methods have been employed to synthesis a well align ZnO NRs and by utilizing a buffer layers are commonly used. In this work, we report a simple process to fabricate ZnO NRs on the seeded ITO electrode via hydrothermal process. The influence of number of ZnO seed deposition layer on the morphological and electrochemical properties towards grown ZnO NRs was studied. The glucose sensing capabilities were found to be influenced by the well aligns and good crystallinity of grown ZnO NRs, suggesting the effect of number of seed layers used during hydrothermal process.

EXPERIMENTAL DETAILS

In this work, ZnO NRs were grown on ITO substrates via hydrothermal process assisted with a ZnO seed layer. Prior to the deposition of ZnO seed layer, ITO substrates were cleaned sequentially by ultrasonic agitation in acetone, distilled water and isopropanol for 10 min, followed by drying. In the sol-gel process, the 0.5 M solution of zinc acetate dehydrate was prepared using 2-mehoxylethanol and ethanolamine as solvent and stabilizer, respectively [9]. The sol-gel ZnO was dropped onto ITO substrate and dried at 150 °C for 20 min to remove the organic substance. The ZnO seed were dropped 1 to 5 layers. Finally the coated ITO substrates were annealed at 500 °C for 2 h in air. The samples were referred to as samples 1L, 2L, 3L, 4L and 5L where the numbers indicate the seed deposition layer. Hydrothermal process was used for ZnO NRs growth in aqueous solution containing 1:1 molar ratio of zinc nitrate ($Zn(NO_3)_2$) and hexamethylamine (HMT) as a precursor solution. The hydrothermal process was performed in pre-heated oven at 80 °C for 4 h as or previous work [10]. The morphology and structure of the prepared ZnO seed layer and ZnO NRs were observed by using field emission scanning electron microscope, FESEM while the phase presence and crystallinity of the ZnO NRs were analysed using X-ray diffractometer.

Before the immobilization of GOx, ZnO NRs was rinsed with phosphate buffer saline (PBS) solution to generate a hydrophilic surface. For immobilization of GOx on ZnO NRs, 10 µL of GOx (1mg/mL in 0.01M PBS) solution was dropped onto the surface of ZnO NRs/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 NRs/ITO tightly on the surface. Nafion is beneficial as a membrane which provides a biocompatible environment to the enzyme without interfering with biosensor [11].

RESULTS AND DISCUSSIONS

The surface morphology of ZnO films deposited on ITO substrates with varying number of deposition layers is shown in Figure 1. The thickness of ZnO seed layer determined through the FESEM measurement is 18.2, 30.3, 55.3, 83.4, and 90.4 nm for 1L, 2L, 3L, 4L and 5L, respectively. With increased number of seed deposition layer, the surface of ZnO films was covered with uniform particles with a sufficient space in between. In addition ZnO films surface in thinner thickness was less uniform than those in larger thickness. However, at 5L, the ZnO films showed a densed internal structure, which could hinder the growth of ZnO NRs. The ZnO seeds must not be too compact and be able to provide spaces for ZnO NRs growth during hydrothermal process.

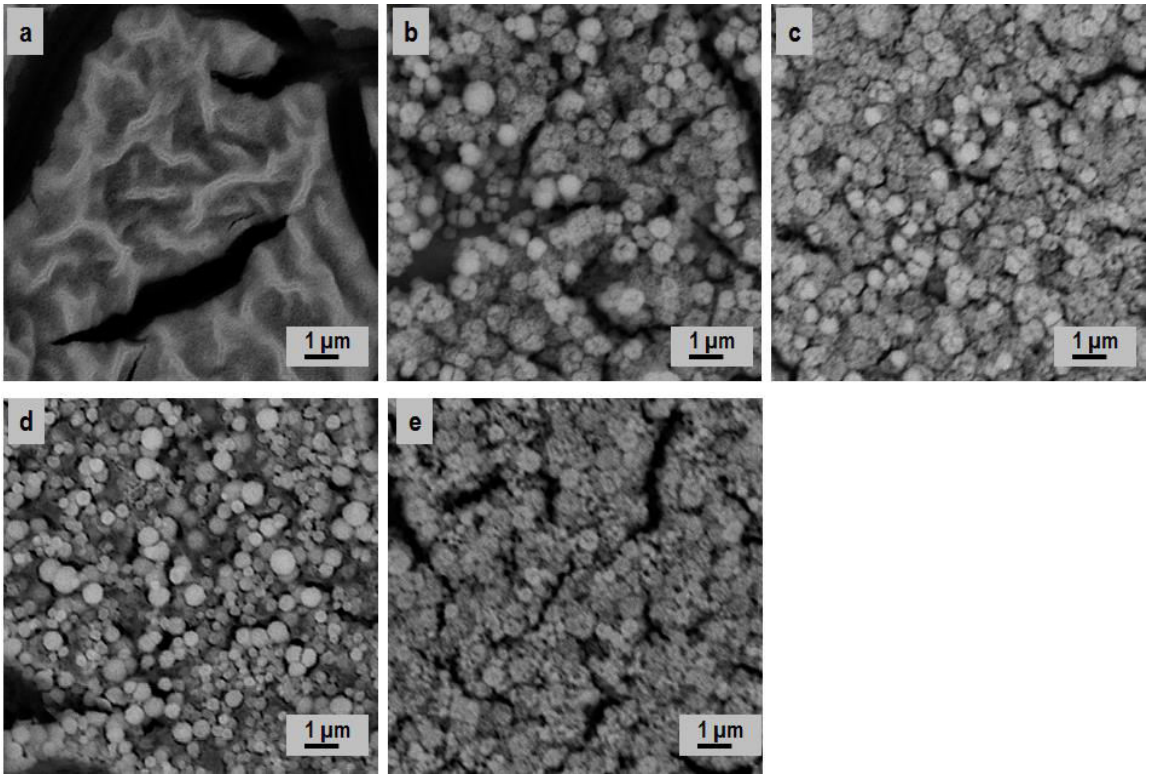

Figure 1: Surface morphologies of ZnO seeds layer at different deposition layer; a) 1L, b) 2L, c) 3L, d) 4L, and e) 5L

Different numbers of seed layer deposition were then subjected to hydrothermal growth in order to investigate the effect of ZnO films layer on ZnO NRs formation. Figure 2 displays a cross sectional FESEM images of ZnO NRs at different numbers of seed

layer deposition. From the figure, for 1L ZnO films, the morphology of grown ZnO NRs consisted of small and several large NRs which is due to the use of thin seed layer that consisted of small grains. This is in agreement with Ghayour et al. [12] that with thin seed layer a small diameter of NRs will form due to its high average roughness, low crystallinity and small grain size. As number of ZnO seed layer was increased, ZnO nanorods growth became uniform and dense with an average length of 750-780 nm and an average diameter of 70-124 nm. In addition, the degree of vertical alignment of ZnO NRs was found to be dependent on the number of seed layer deposition. As the number of seed layer deposition increased from 1L to 3L, the degree of alignment of grown ZnO NRs enhanced as shown in Figure 2(a)-(c). However, further increased the number of seed layer deposition to 4L and 5L resulted in the tilting of nanorods as shown in Figure 2(d) and (e), respectively. As agreed by Zhang et al. and Ye et al. [9, 13], the thickness of seed layer could affect in producing well-aligned nanorods. Therefore, a threshold of number of seed layer deposition for ZnO films could be due to preferred orientation of seed layer which then influenced the alignment of grown nanorods existed.

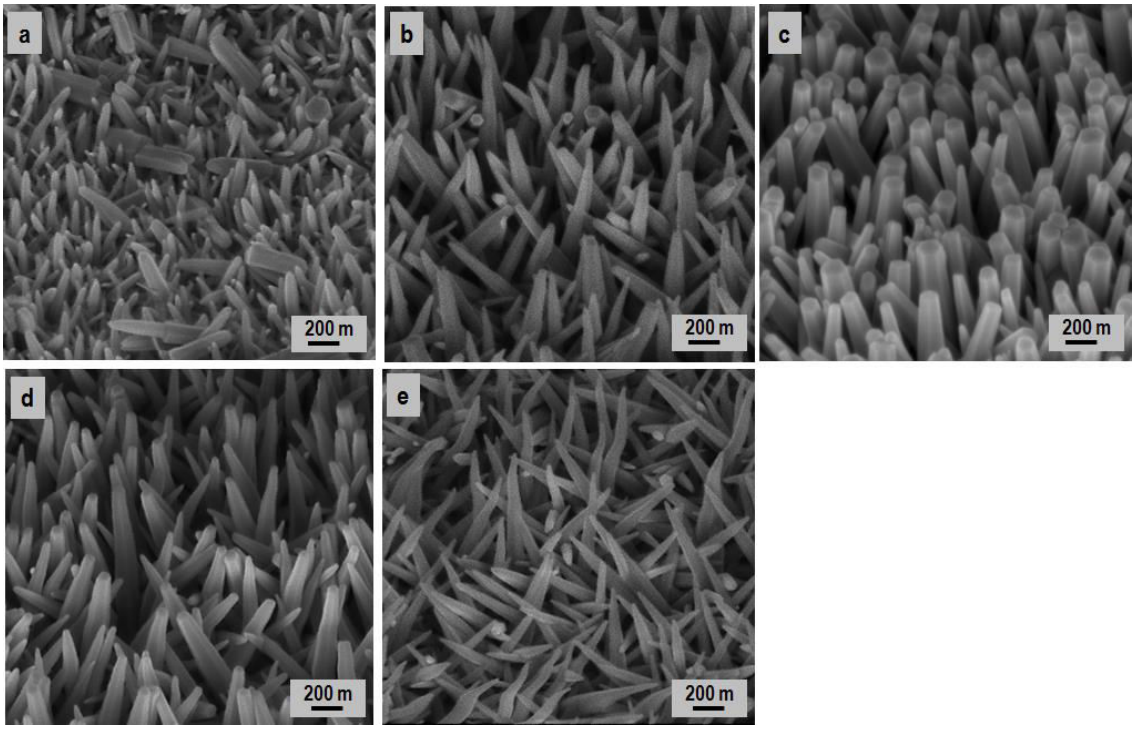

Figure 2: FESEM images of ZnO NRs grown on seeds at different deposition layer; a) 1L, b) 2L, c) 3L, d) 4L, and e) 5L. The samples were hydrothermally grown for 4h at 80°C

Figure 3 shows XRD patterns of ZnO seed layers and ZnO NRs samples. Besides the ITO substrate peaks, all other peaks are well consistent with hexagonal wurtzite structure of ZnO (JCPDS # 36-1451). As shown in figure 3(a), the intensity of (002) peak increased first as the deposition seed layer increased from 1L to 3L, and then

decreased at 4L and 5L, revealing the dependence of preferred orientation on ZnO seed layers. Moreover, the decrease in (002) peak intensity showed that at 4L and 5L samples, a ZnO film was low in degree of crystallinity. Figure 3(b) shows XRD patterns of ZnO NRs grown on different number of seed layer. As observed in the figure, (002) peak intensity of ZnO NRs increased compared to ZnO seeds layer. These results confirmed a preferred growth in c-axis direction of the NRs and the increase in the NRs density which was consistent with the FESEM images in Figure 2.

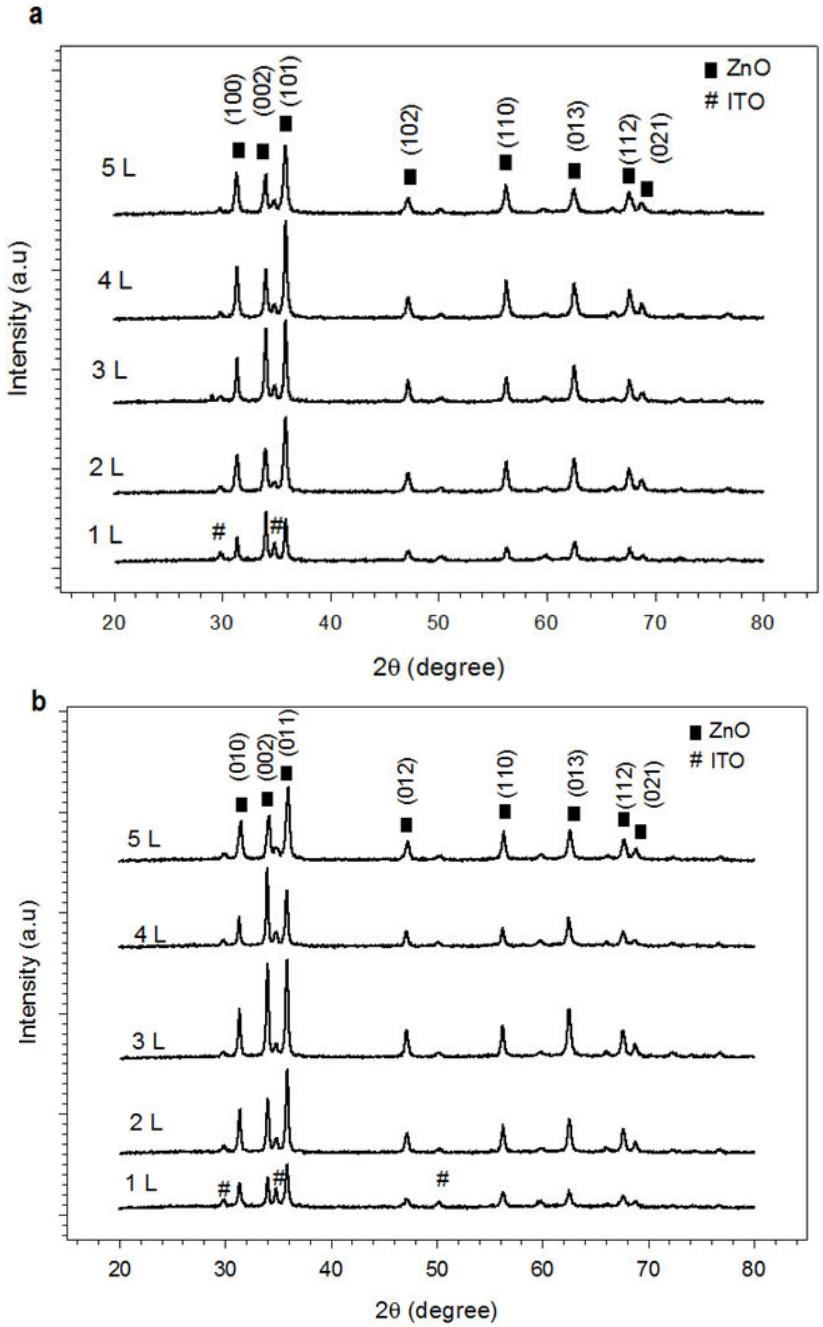

Figure 3: XRD pattern of (a) ZnO seed layer and (b) ZnO NRs grown at different deposition layer; a) 1L, b) 2L, c) 3L, d) 4L, and e) 5L, respectively

Inset in Figure 4 shows cyclic voltammetry (CV) curves of Nafion/GOx/ZnO NRs/ITO electrode of the 3L sample between potential of -1.0 and +0.5V at scan rate of 50 mV/s without glucose and upon addition of 3 mM glucose. Upon addition of 3 mM glucose, the shape of CV changed with an increase in oxidation peak current and decreased of reduction peak current, indicating that GOx enzyme immobilized on ZnO NRs was sensitive in glucose environment. Moreover, the result demonstrated that grown ZnO NRs enhanced the enzyme catalytic sites assessable to substrate molecules and was able to shuttle electrons between GOx and ZnO NRs matrix and able to remain their activities on ZnO NRs matrix. The mechanism of the biosensor are summarized as follow [8, 14] :

Nafion/GOx/ZnO NRs/ITO (working electrode):

$$GOx\ (FAD) + glucose \rightarrow GOx\ (FADH_2) + glucolactone \quad (1)$$

$$Glucolactone + H2O \rightarrow gluconic\ acid \quad (2)$$

$$GOx\ (FADH_2) \rightarrow GOx\ (FAD) + 2e^- + 2H^+ \quad (3)$$

Pt (counter electrode)

$$2H^+ + O_2 + 2e^- \rightarrow H_2O_2 \quad (4)$$

The effect of the grown ZnO NRs grown on different number of seed layer on the electrochemical signal of the biosensor in 3 mM of glucose concentration environment was investigated and the results are shown in Figure 4. It is known that the morphology of ZnO such as shape, size and surface area will influence the enzymes loading on the surface of its matrix for a high sensitivity and selectivity of glucose detection [5, 15]. In this work we found that beside high surface area of ZnO NRs, crystallinity and alignment of grown NRs also influenced the performance of glucose detection. It is noticeable that the oxidation current increased gradually from sample 1L to 3L and decreased back in 4L and 5L samples. This is due to the good alignment and high crystallinity of the grown ZnO NRs used as matrix as shown in FESEM image Figure 2 and XRD pattern Figure 3(b), respectively. This result is in agreement with Sarkar and Bhattacharyya [14] that observed good alignment of nanorods induced a direct electron transfer. As a result, the produce current increased. Additionally a good crystallinity of grown ZnO NRs will ensure a low resistance for electron transfer between the active site of GOx and the ZnO NRs matrix surface [3]. Hence 3L sample was selected as an optimum to modify matrix.

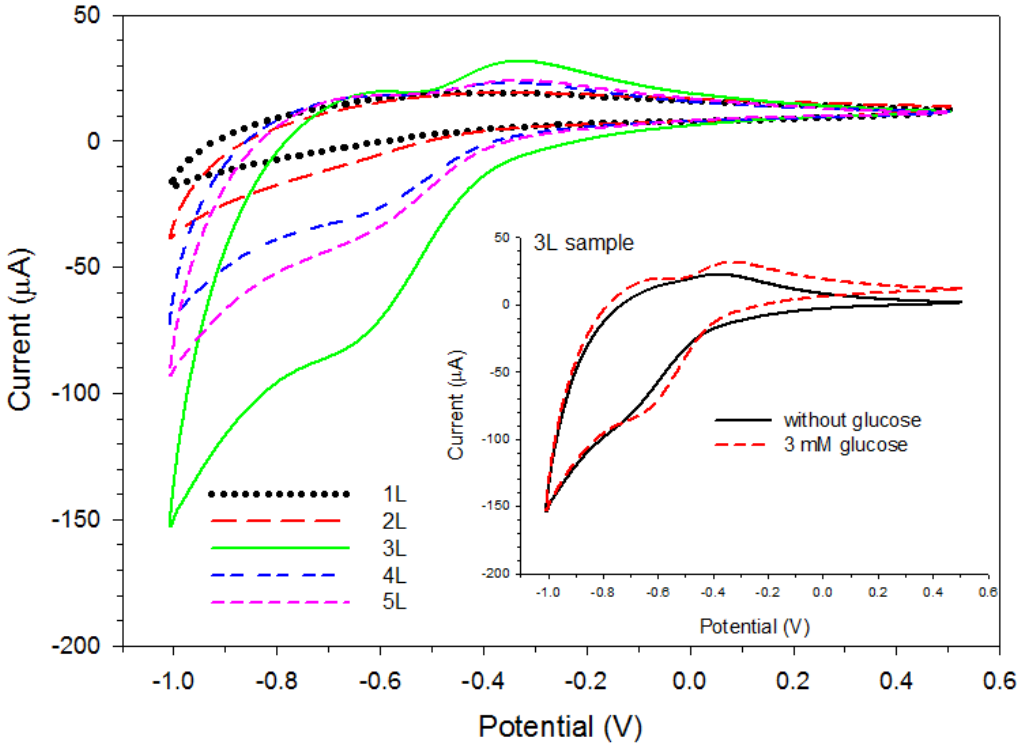

Figure 4: CV of Nafion/GOx/ZnO NRs/ITO electrodes in 3 mM glucose solution at different deposition seed layer. Inset CV response of sample 3L ZnO NRs biosensor without glucose and with 3 mM glucose

Using the optimum conditions established in the above study (sample 3L), the chrono-amperometric response of the glucose biosensor was investigated by increment of glucose (from 0.05 to 20 mM) into the continuously stirred 0.01 M PBS solution. The chrono-amperometric response (current versus time) of sample 3L is presented in Figure 5(a). The result showed an increase in current upon successive addition of glucose and the time required to reach a maximum steady-state current was within 6 s. The corresponding calibration curve of the biosensor is shown in Figure 5(b) with the steady-state currents gradually increased with increasing concentration of glucose with a correlation coefficient of 0.9896. This indicated that GOx was successfully immobilized on ZnO NRs and retained its bioactivity perfectly. In agreement with Gu et al. [16] that ~1 of correlation coefficient indicated that the redox reaction is a surface process and the electrons easily transfer between GOx and ZnO NRs/ITO electrode. The regression equation is $I(\mu A) = 1.602C_{glucose} + 1.15$ with a limit of detection (LOD) of 0.07 mM was obtained. The calculated sensitivity of Nafion/GO/ZnO NRs/ITO electrode was 1.602 µA/ mM cm^2 which is slightly higher than Nozaki et al. [18] which is 1.4 µA/ mM cm^2. The sensing range of the sensor is weel suitable for a glucose sensor of human blood corresponding to 9-360 mg/dL.

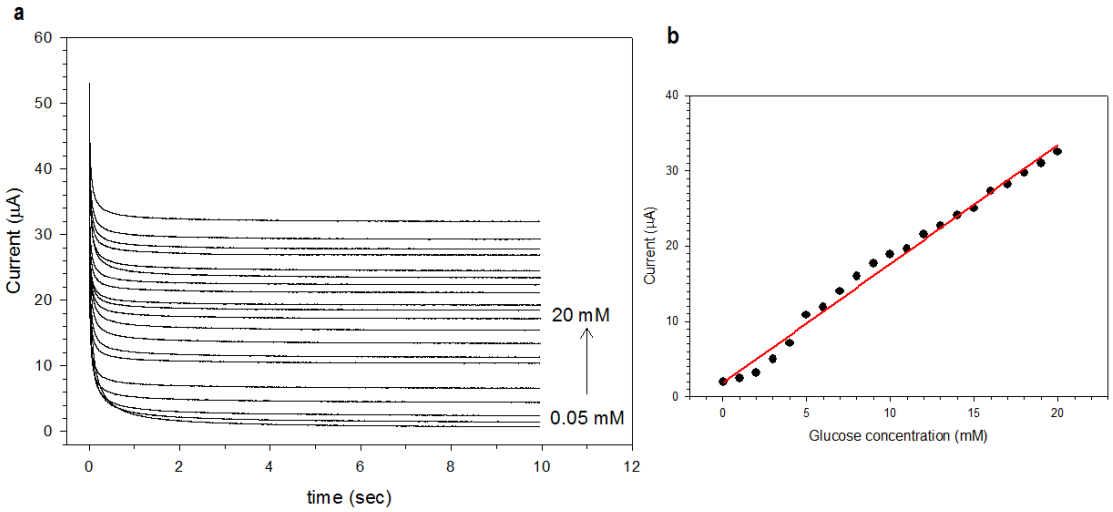

Figure 5: (a) Chrono-amperometry of Nafion/GOx/ZnO NRs/ITO electrode with addition of different concentration of glucose solution and (b) Calibration curve of fabricated electrode

CONCLUSION

In this work, we report an enzymatic glucose sensor based on ZnO NRs modified on ITO electrode. ZnO NRs were successfully grown on ITO by employing a ZnO seed layers. The effects of different number of seed deposition layer on morphology and its performance in glucose biosensor have been investigated. Results show that 3 layers of seed deposition layer produced a highly oriented grown and high crystallinity of ZnO NRs. The electrochemical results of sensor showed a sensitivity of 1.602 μA/ mM cm^2, with a wide linear range from 0.05 mM to 20 mM. Additionally, the LOD and K_m of proposed sensor was calculated to be 0.07 mM and 3.07, respectively.

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