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The properties of ZnO nanorods modified by Au nanoparticles for galactose biosensor application

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ABSTRACT

Galactose biosensor based on ZnO nanorods were fabricated and modified through the addition of Au nanoparticles. The sol-gel method was utilized to grow ZnO nanorods on indium tin oxide-coated glass substrates (FTO), and the Au nanoparticles were modified on ZnO nanorods by hydrothermal method. Well aligned hexagonal structured ZnO nanorods with a diameter from 40 nm to 60 nm were obtained. The Au nanoparticles were spheres with diameters varied from 6 nm to 8 nm. After these Au NPs were attached to ZnO nanorods electrode, galactose oxidase enzyme (GOx) was immobilized on this electrode in 3.5 hours to form the working electrode in galactose biosensor (GOx|Au-ZnO|FTO). The cyclic voltammetry method was used to test the activity of the working electrode in galactose solution 200 mM concentration. The cyclic voltammetry result showed that the current intensity of the GOx|ZnO|FTO electrode in galactose solution is about 0.09 μA/mm^2. On the other hand, with the GOx|Au-ZnO|FTO electrode, this value increases to 0.2 μA/mm^2.

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1 INTRODUCTION

ZnO nanomaterial exits in a variety of one-dimensional nanostructures such as nanorods, nanotubes, nanowalls, nanowires, etc. (Marie *et al*., 2015, Sung *et al*., 2012). They have potential applications in producing devices such as light emitting diodes, optical waveguides, nanolaser, gas sensor, and biosensor. Especially, ZnO nanomaterial has good characteristics such as high electric transmittance, nontoxic, high biological compatibility, and high isoelectric point (IP 9.5), (Ahmad *et al*., 2012, Sharma *et al*., 2006), etc. so that it could be applied in biosensors. The Au nanoparticles (Au NPs) are good catalytic activity due to their large surface-to-volume ratio. Therefore, it is expected that the modified of Au NPs on ZnO NRs would improve the activity of biosensor (Akbarzadeh *et al*., 2009, Huang *et al*., 2004). In this work, we developed an enzyme electrode biosensor based on ZnO nanorods modified by Au NPs on indium tin oxide-coated glass (FTO) substrate (Au-ZnO| FTO) to test galactose solution by immobilizing galactose oxidase on this electrode (GOx|Au-ZnO|FTO). The result shows that the proposed biosensor has the linear detection ranging from 40 mM to 230 mM galactose solution with a good sensitivity of 100 mV/decade, and the current intensity of the GOx|Au-ZnO|FTO electrode in galactose solution is higher than that of the GOx|ZnO|FTO electrode in this solution.

2 MATERIALS AND METHODS

2.1 The growth of ZnO nanorods on FTO substrate

The seed ZnO is important in the growth process of the ZnO nanorods by solution method. Reagent grade (RG) zinc acetate dehydrate ($Zn(CH_3COO)_2.2H_2O$) and monoethanolamine (MEA) were first dissolved in an ethanol solvent with Zn^{2+} concentration of 0.75 M to form sol solution. This solution was magnetically stirred in 2 hours at room temperature. After that, FTO substrate was coated from sol solution by spin coating, then the annealing process was performced at 500^0C to form ZnO crystal. Finally, this product was cooled down at room temperature to have a ZnO seed layer.

ZnO nanorods were fabricated by solution method from seed ZnO coated FTO substrate. Precursor zinc nitrate dehydrate ($Zn(NO_3)_2.2H_2O$) and hexamethylenetetramine (HMTA, $C_6H_{12}N_4$) were first dissolved in an aqueous solvent with Zn^{2+} concentration of 0.02 M. This solution was magnetic stirred in 2 hours at room temperature. After that, substrate coating ZnO seed was dipped in that solution and kept at 80°C in 5 hours to form ZnO nanorods (La Phan Phuong Ha *et al*., 2017)

2.2 The growth of ZnO nanorods modified by Au NPs

The ZnO nanorods modified by Au NPs (Au-ZnO|FTO) were grown by hydrothermal method with Gold(III) chloride trihydrate ($HAuCl_4.3H_2O$), Sodium borohydride ($NaBH_4.3H_2O$), Sodium citrate ($Na_3C_6H_5O_7.2H_2O$) and deionized water. The hydrothermal temperature is 150°C, and the hydrothermal time changes from 1 hour to 4 hours (La Phan Phuong Ha *et al*., 2017).

2.3 The immobilization of galactose oxidase enzyme on Au-ZnO| FTO

The 2.5% glutaraldehyde in 0.1 mM phosphate buffer solution (PBS) and galactose oxidase solution in PBS having a concentration of 1mg/mL of enzyme were mixed in one bottle, then rods/rods ZnO substrate was dipped into it in 3.5 hours at room temperature in order to examine the saturation of the ZnO nanorods surface with an enzyme called enzyme immobilized ZnO electrode (GOx|Au-ZnO|FTO) (Fig. 1).

The galactose solutions were prepared with different concentrations, from 30 mM to 230 mM, to test the activity of the enzyme immobilized ZnO nanorods. The electrochemistry response of the proposed biosensor based on the immobilized ZnO nanorods in galactose solution was measured by cyclic voltammetry (CV) method with MPG-2 analyzer using GOx|Au-ZnO|FTO as a working electrode and Ag/AgCl as a reference one (La Phan Phuong Ha *et al*., 2017).

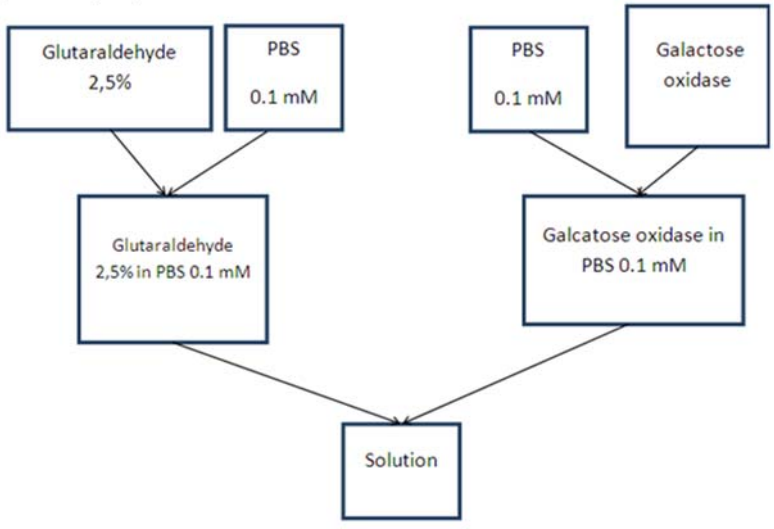


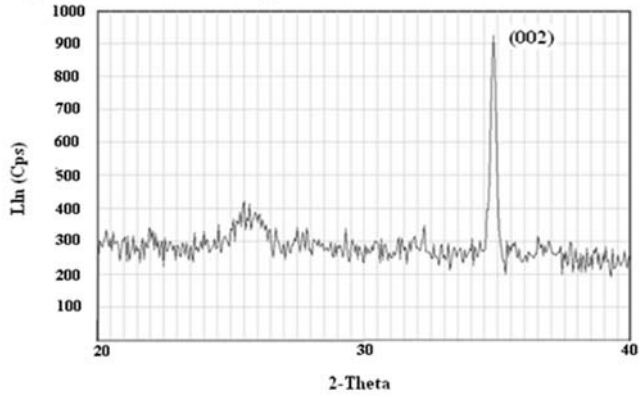
Fig. 1: Flow chart for the immobilization galactose oxidase on ZnO nanorods surface

3 RESULTS AND DISCUSSION

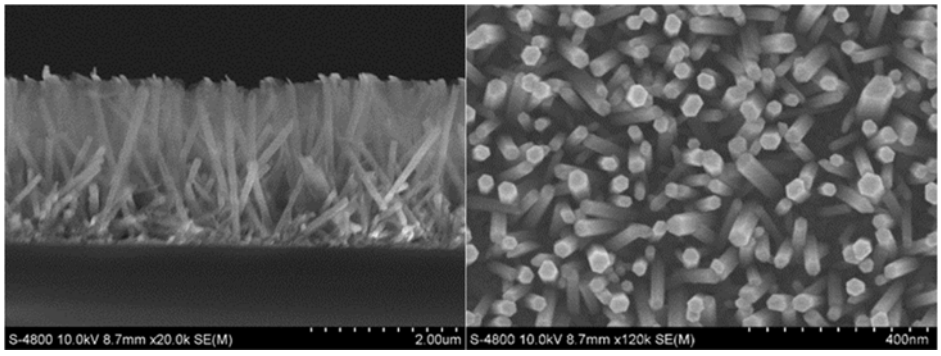
3.1 The growth of ZnO nanorods on FTO substrate by solution method

Fig. 2a shows the X-ray diffraction pattern of ZnO nanorods that grew on seed ZnO coated substrate. The figure showed the significantly higher intensity from the (002) peak, hence, indicated that the nanorods were preferentially orientated along c axis direction. This result confirms that the ZnO seed layer plays an important role in alignment growth of ZnO nanorods on ZnO seed layer having c axis orientation. The Scanning Electron Microscope (SEM) image of ZnO nanorods is observed in Fig. 2b and 2c, showing the hexagonal structure of ZnO nanorods with the higher vertical alignment on ZnO seed coated substrate. The observed average diameter and length of nanorods were 40 nm - 50 nm and 2 μm.

These obtained results show that ZnO nanorods with good orientation, high surface area were successfully grew by solution method . This structure can be used to immobilize enzyme on it.



(a)



(b) **(c)**

Fig. 2: (a) X-ray diffraction of ZnO nanorods, (b) and (c) SEM of ZnO nanorods

3.2 The growth of ZnO nanorods modified by Au NPs

The ZnO nanorods modified by Au NPs (Au-ZnO| FTO) were grown by hydrothermal method where the hydrothermal temperature is kept at 150^{o}C, and the hydrothermal time changes from 1 hour to 4 hours (sample A1, A2, A3, A4). The solutions after the hydrothermal process were analyzed by ultraviolet-visible spectrophotometry. The UV-Vis spectrum of solution of these samples is illustrated in Fig. 3.

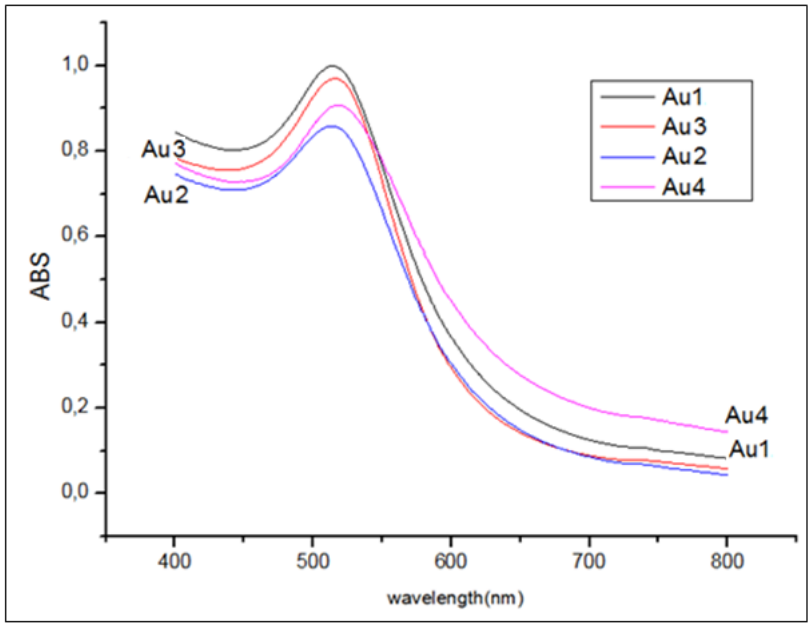


Fig. 3: The UV-Vis spectrum of solution after hydrothermal process with different hydrothermal time (from 1 hour to 4 hours - sample A1 to A4)

The UV-Vis spectrum in Fig. 3 showed that there are absorption peaks of solution after hydrothermal process in the range of 510 nm - 530 nm wavelength so it means Au NPs were successfully synthesized (Chang *et al.*, 2013). Absorption peaks change from 517 nm to 525 nm, and this change is corresponding to that of hydrothermal time. When the hydrothermal time is 2 hours, the absorption peak is at 517 nm wavelength, and the Au nanoparticle size is about 6 nm in this case. Thus, the best hydrothermal time in this experiment is 2 hours to form ZnO nanorods modified Au NPs.

The size of Au NPs in solution after the hydrothermal process was checked by transmission electron microscopy (TEM), and is shown in Fig. 4(a). In this picture, the Au NPs size is approximately 6 nm - 8 nm, and is suitable for the result of Au NPs size calculated from UV-Vis spectrum. The SEM of Au-ZnO|FTO sample in Fig. 4(b) demonstrated the morphology of Au-ZnO| FTO electrode; there are a lot of Au NPs attached to ZnO nanorods surface. This result indicates that the ZnO nanorods modified by Au nanoparticles were successfully grew by hydrothermal method .

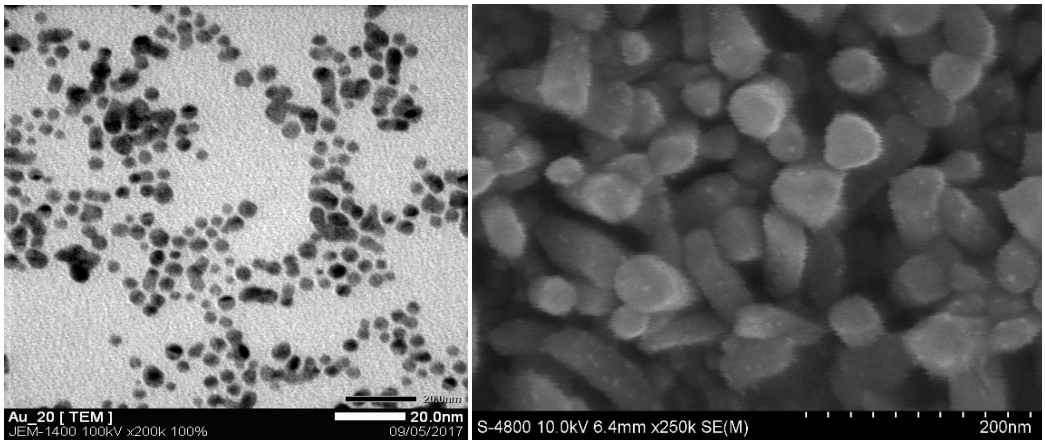


Fig. 4: (a) TEM of Au NPs in solution after hydrothermal process and (b) SEM of the ZnO nanorods modified by Au NPs

3.3 The activity of the GOx|Au-ZnO|FTO electrode in galactose solution

Before testing the activity of the GOx|rods/rods ZnO|FTO in galactose solutions with different concentrations, the activity of the enzyme immobilized ZnO nanorods to pure ZnO nanorods on FTO substrate in 200 mM concentration galactose solution was compared. In this result, the CV curve of pure ZnO nanorods electrode shows that the value of current intensity is about 0.018 µA. On the other hand, with the immobilized ZnO nanorods electrode, this value increases to 0.085 µA (Fig. 5). This result indicates that the GOx|rods/rods ZnO|FTO electrode reacted with galactose in galactose solution to change the value of current intensity. This change can be well explained that based on the oxidation of galactose in the presence of immobilized galactose oxidase, galactohexodialdose and H_2O_2 were produced in the solution, and these products created a potential change at the electrode, as given in the following equation (Sharma *et al*., 2006):

$$\text{Galactose} + O_2 \xrightarrow{\text{Galactose oxidase}} \text{Galactose-hexodialdose} + H_2O_2$$

$$H_2O_2 \longrightarrow O_2 + 2e^- + 2H^+$$

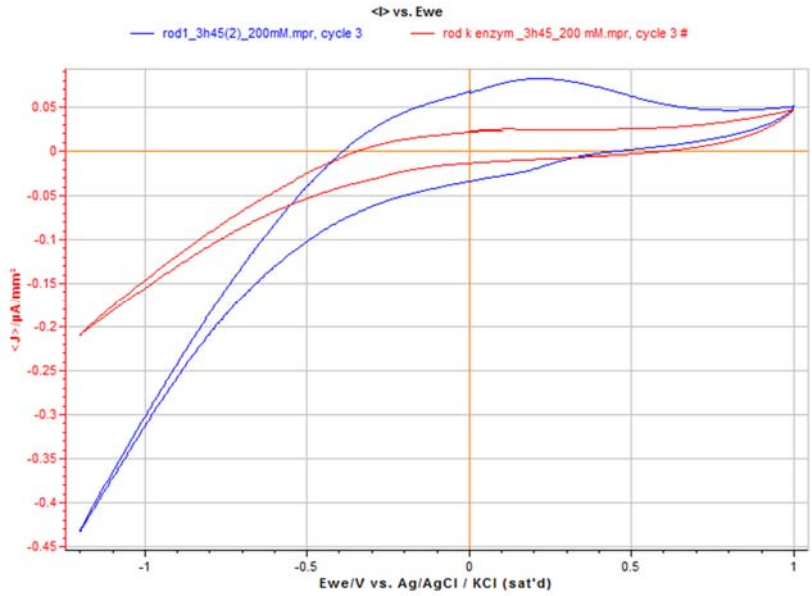


Fig. 5: The CV curve of (a) the pure ZnO nanorods and (b) the immobilized ZnO nanorods in 200 mM galactose solution

Next, we compared the activity of the GOx|ZnO|FTO to the GOx|Au-ZnO|FTO in 200 mM concentration galactose solution. In this comparison, the CV curve of GOx|ZnO|FTO electrode shows that the value of current intensity is about 0.085 µA. On the other hand, with the GOx|Au-ZnO|FTO electrode, this value increases to 0.2 µA (Figure 6).

This result can be explained that Au NPs were used as biosensitive particles, effectively facilitated direct electrochemistry of galactose oxidase in galactose solution, so the value of current intensity increases (Peh *et al*., 2010, Hsueh-Tao *et al*., 2015).

From the above result, the activity of the GOx|Au-ZnO|FTO in galactose solutions is tested with different concentrations. Six galactose solutions are prepared with different galactose concentrations, from 40 mM to 230 mM. The GOx|Au-ZnO|FTO electrodes were dipped in these solutions at the same time, and the CV response is given in Table 1.

Table 1: The value of current intensity follows the galactose concentration

Galactose concentration (mM)	The average current density (J) (10^{-2} µA/mm^2)	The error (10^{-2} µA/mm^2)
40	15.73	0.33
60	16.39	0.45
80	17.16	0.47
110	18.05	0.37
140	19.02	0.49
170	19.61	0.35
200	20.15	0.45
230	20.96	0.41

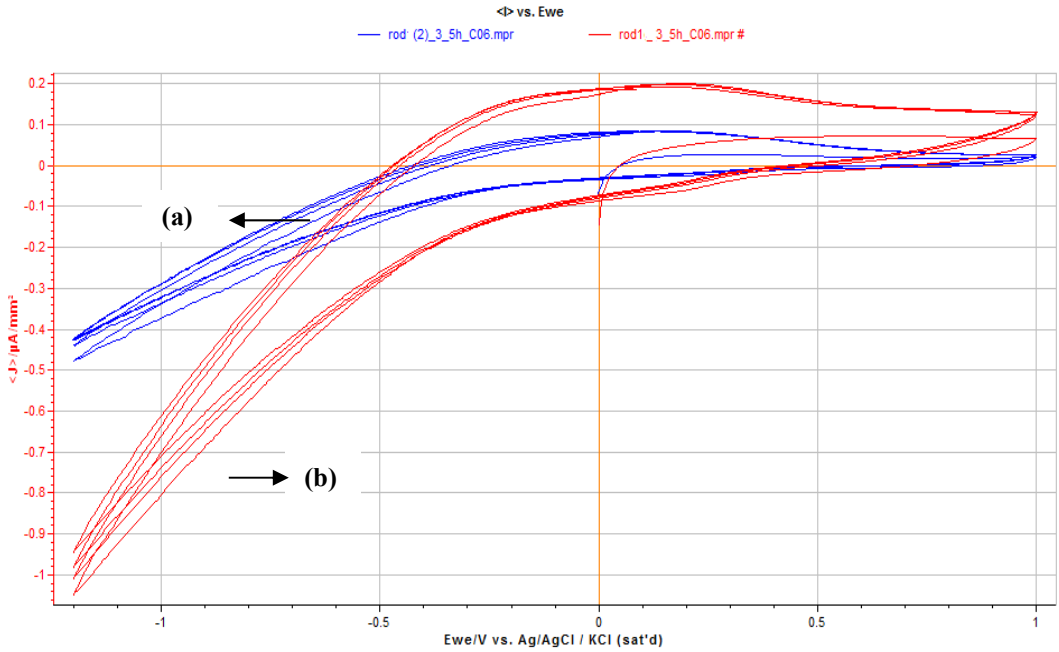


Fig. 6: The CV curve of (a) the GOx|ZnO|FTO and (b) the GOx|Au-ZnO|FTO in 200 mM galactose solution

The average J following different galactose concentrations were demonstrated in Fig. 7. The observed chart shows that the average current density linearly increases with the increase of galactose concentration from 40 mM to 230 mM, and this can be obtained from the equation y = 0,03629x + 3,28223 (with R^2 = 0,99791). This is an important characteristic of a biosensor.

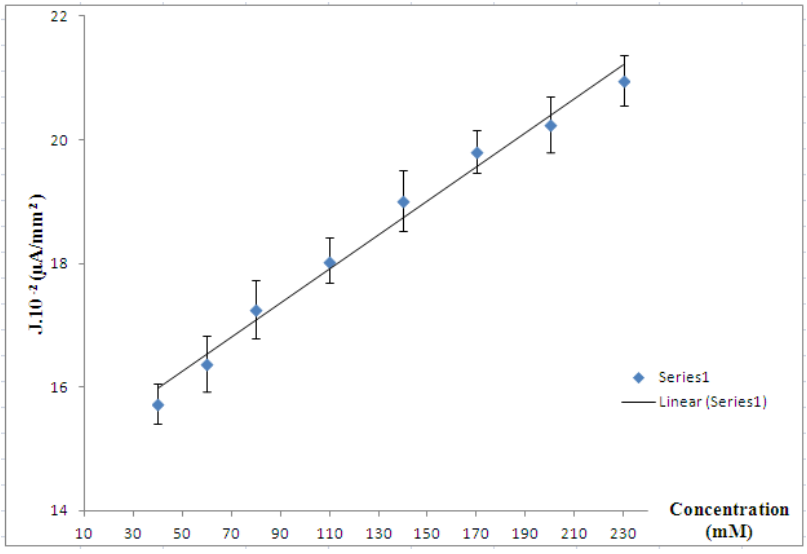


Fig. 7: The chart of the average current density following the galactose concentration

4 CONCLUSIONS

The ZnO nanorods on seed ZnO coated FTO substrate were sucessully synthesized by solution method with 50 nm average diameter of nanorods, the ZnO nanorods modified by 6 - 8 nm of Au NPs by hydrothermal method. Besides that, the galactose oxidase was well immobilized on Au-ZnO| FTO electrode. The experimental results show that there is a better current response with the GOx|Au-ZnO| FTO electrode. The galactose biosensor based on this electrode well operated in galactose solution, the average current density lineally increases with the increase of galactose concentration from 40 mM to 230 mM. These results are basic to the galactose sensor applications based on ZnO nanomaterial.

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