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The Process of Immobilization of ZnO Nanorods Surface with Galactose Oxidase

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Abstract: ZnO nanorods, with the c-axis orientation used for transparent conductors, solar cells, sensors…, especially the functionalized ZnO nanorods with some kinds of enzymes have been used for biosensor. In this work, we describe the process immobilization of galactose oxidase on ZnO nanorods surface with glutaraldehyde as a cross-linker molecule to make the working electrode in electrochemical biosensor. ZnO nanorods were grown on FTO (Fluorine-doped tin oxide) substrate by solution method at low temperature. The crystalline phase and orientation of ZnO nanorods were identified using X-ray diffraction. The efficiency of the immobilization was calculated by Braford method showed that about 36% enzyme content was immobilized on ZnO nanorods surface. The working electrode based on the immobilized ZnO nanorods was tested in galactose solution by CV (cyclic voltammetry) method indicated the value of current intensity is about 0.14 μA. These results clearly demonstrate the potential of galactose sensor based on ZnO nanorod.

Key words: ZnO, solution method, Braford method, galactose oxidase, galactose biosensor.

1. Introduction

For many applications of nanorods, such as field emission and polymer-inorganic solar cell, catalyst, gas/bio sensor, their 1D structures play an important role [1, 2]. ZnO nanorods have some advantages such as nontoxic, fast electron transfer, high surface to volume, low cost and it has high ionic bonding characteristic, so it is stabile for a long time at biological pH. Furthermore, the IEP (isoelectric point) of ZnO is high (about 9.5) and thus it is a better material to immobilize low IEP such as enzyme or protein. With these characteristic, ZnO nanorods structure is applied in biosensor. In this report, we present the solution method to grow ZnO nanorod and the immobilization process of galactose oxidase enzyme on ZnO nanorods surface. The solution method is used to grow ZnO nanorods because of some advantages as simple method, reaction to form rods is occurred at low temperature (about 50 ^{o}C-120 ^{o}C). Our experiment results show that ZnO nanorods have about 40 nm-50 nm diameter, they aligned vertically on ZnO seed coated FTO substrate and have a preferential orientation of (002) plane. Galactose oxidase enzyme (2mg/mL concentration) was immobilized on ZnO nanorod surface with different time immobilization, the efficiency of this process was about 36.5% with 3 h immobilization time.

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2. Experimental

2.1 The Growth of ZnO Nanorods

The seed ZnO is important in growth process of ZnO nanorods by solution method because this method is simple, low-cost, can be synthesized at low temperature (50 ^{o}C-120 ^{o}C) to grow 1D structured ZnO nanomaterial [3]. RG (reagent grade) zinc acetate dehydrate ($Zn(CH_3COO)_2.2H_2O$) and MEA (monoethanolamine) were first dissolved in an ethanol solvent with Zn^{2+} concentration of 0.75 M to form sol solution. This solution was magnetic stirred in 2 h at room temperature. After that, FTO substrate was coated from sol solution by spin coating and then the annealling process was performanced at 500 ^{o}C to form

ZnO crystal. At the end, this product was cool down at room temperature to have ZnO seed layer.

ZnO nanorods to grow 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 0.02 M. This solution was magnetic stirred in 2 h at room temperature. After that, substrate coating ZnO seed was dipped in that solution and kept at 5 h, 80 ^oC to growth ZnO nanorods following some below chemical reaction. Then, obtained products were cool down at room temperature.

2.2 The Immobilization of Galactose Oxidase Enzyme on ZnO Nanorods Surface

In this part of the experiment work, we prepared two kinds of solution: 2.5% GA (Glutaraldehyde) in 0.1 mM PBS (phosphate buffer solution) as well as galactose oxidase solution in PBS having a concentration of 2 mg/mL of enzyme. These GA and enzyme solutions are mixed in one bottle, then ZnO nanorods substrate is dipped into it for different times in order to investigate the saturation of the ZnO nanorods surface with the enzyme. The efficiency of this immobilization was calculated by Braford method based on the enzyme content adhered to ZnO nanorods surface. With Braford method, to define the enzyme content immobilized on ZnO, we measure OD (optical density) of enzyme solution at 559 nm.

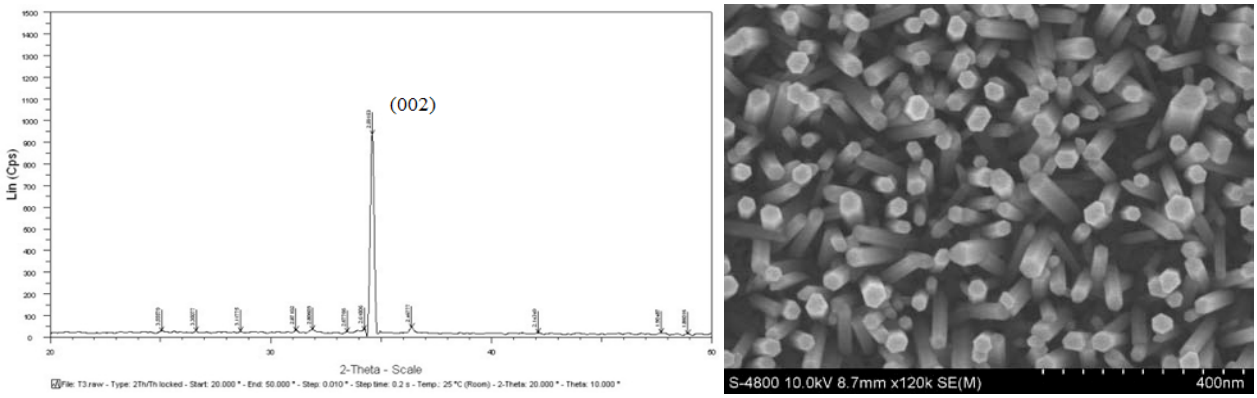
The 200 mM concentration galactose solutions were prepared to test the activity of the enzyme immobilized ZnO nanorods. The electrochemistry response of the proposed biosenor based on the immobilized ZnO nanorods in galactose solution was measured by CV (cyclic voltammetry) method with MPG-2 analyer which used the immobilized ZnO nanorods as a working electrode and Ag/AgCl as a reference electrode.

3. Results and Discussion

3.1 The Growth of ZnO Nanorods by Solution Method

Fig. 1a shows the X-ray diffraction pattern of ZnO nanorods that grew on seed ZnO coated substrate showed the significantly higher intensity from the (002) peak, indicated that the nanorods were preferentially orientated along c axis direction. This obtained result was confirmed that the seed layer ZnO has an important role in alignment growth of ZnO nanorods on ZnO seed layer having c axis orientation. The SEM images of these samples are observed (Fig. 1b) the hexagonal structure of ZnO nanorods with the higher vertical alignment on ZnO seed coated substrate. The average diameter of nanorods in this situation was 40 nm.

These results show that we successfully grow ZnO nanorods with good orientation, high surface area. This structure can be used to immobilize enzyme on it.



(a) (b)

Fig. 1 (a) XRD of ZnO nanorods, (b) SEM of ZnO nanorods.

3.2 The Immobilization of Galactose Oxidase Enzyme on Zno Nanorods Surface

We immobilized galactose oxidase enzyme on ZnO nanorods in 1 h, 2 h, 3 h, 4 h time. The enzyme content immobilized on ZnO will be calculated by Braford method. This method relies on the change of maximum absorption wavelength of Coomassie Brillant Blue reagent when the reagent combines with albumin enzyme. Without albumin enzyme, the maximum absorption wavelength of the reagent in solution is 465 nm. However when we add albumin enzyme to this solution, the maximum absorption wavelength changes to 595 nm. The OD (optical density) of enzyme solution was measured by Spectrophotometer at 595 nm wavelength and this OD value relates to enzyme content in solution. With Braford method, scientists set up the standard curve to calculate enzyme content as in Fig. 2. In this report, the albumin enzyme was replaced with galactose oxidase. The galactose oxidase content immobilized on ZnO was calculated from the OD value of galactose oxidase enzyme solutions after the immobilization, and the efficiency of the immobilization (H%) can be found out based on standard curve of Braford method. The result of OD measurement with different immobilization time is shown in Table 1.

$$H\% = \frac{beginning\ enzyme\ content - \ enzyme\ content\ after\ the\ immobilization}{beginning\ enzyme\ content} \cdot 100\%$$

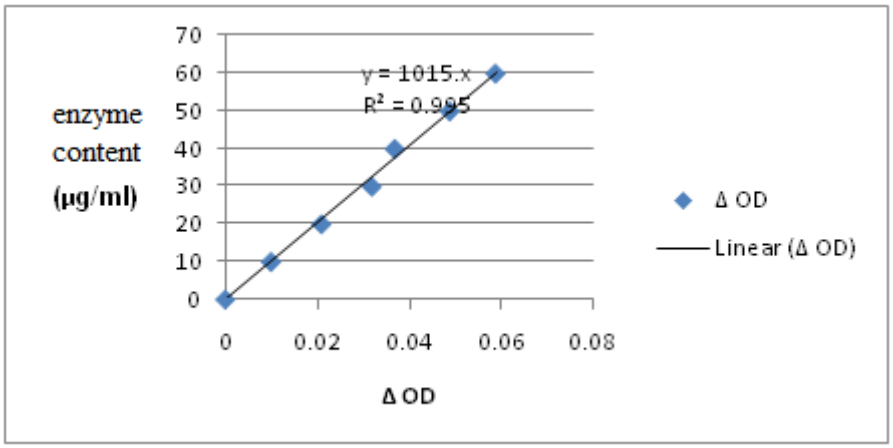


Fig. 2 The standard chart of Braford method.

Table 1 OD value and the efficiency of the immobilization with different immobilization time.

Immobilization time	1 h	2 h	3 h	4 h
OD value	0.095	0.091	0.086	0.088
ΔOD	0.034	0.03	0.025	0.027
Enzyme concentration (find out from Braford standard graph) (µg)	34.5	30.45	25.38	27.5
Enzyme content after the immobilization (µg)	1,725.5	1,522.5	1,269	1,440
Immobilized enzyme content (µg)	274.5	477.5	731	560
H%	13.8	24	36.5	28

In Table 1, the efficiency of the immobilization is 13.8%, 24%, 36.5% and 28% corresponding to 1 h, 2 h , 3 h, 4 h immobilization time, and it shows that we successfully immobilized galactose oxidase on ZnO nanorods with the efficiency of the immobilization is 36.5% corresponding to 3 h immobilization time.

3.3 The Activity of the Immobilized ZnO Nanorods

To test the activity of the enzyme immobilized ZnO nanorods with 3 h immobilization time, we compared the activity of the enzyme immobilized ZnO nanorods to pure ZnO nanorods on FTO substrate in 200 mM concentration galactose solution. In this solution, the CV curve of pure ZnO nanorods electrode showed that the value of current intensity is about 0.011 µA. On the other hand, with the immobilized ZnO nanorods electrode, this value increases to 0.14 µA (Fig. 3).

This result indicated that the immobilized ZnO nanorods electrode reacted with galactose in galactose solution to change the value of current intensity. This change can be explained base on the oxidation of galactose in the presence of immobilized galactose oxidase, galactohexodialdose and H_2O_2 were produced into the solution and these products created a potential change at the electrode, as given in the following equation [4, 5]:

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

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

With these achieved results above, it shows that we successfully immobilized galactose oxidase on ZnO nanorods and this immobilized ZnO nanorods electrode has good operation ability in galactose solution.

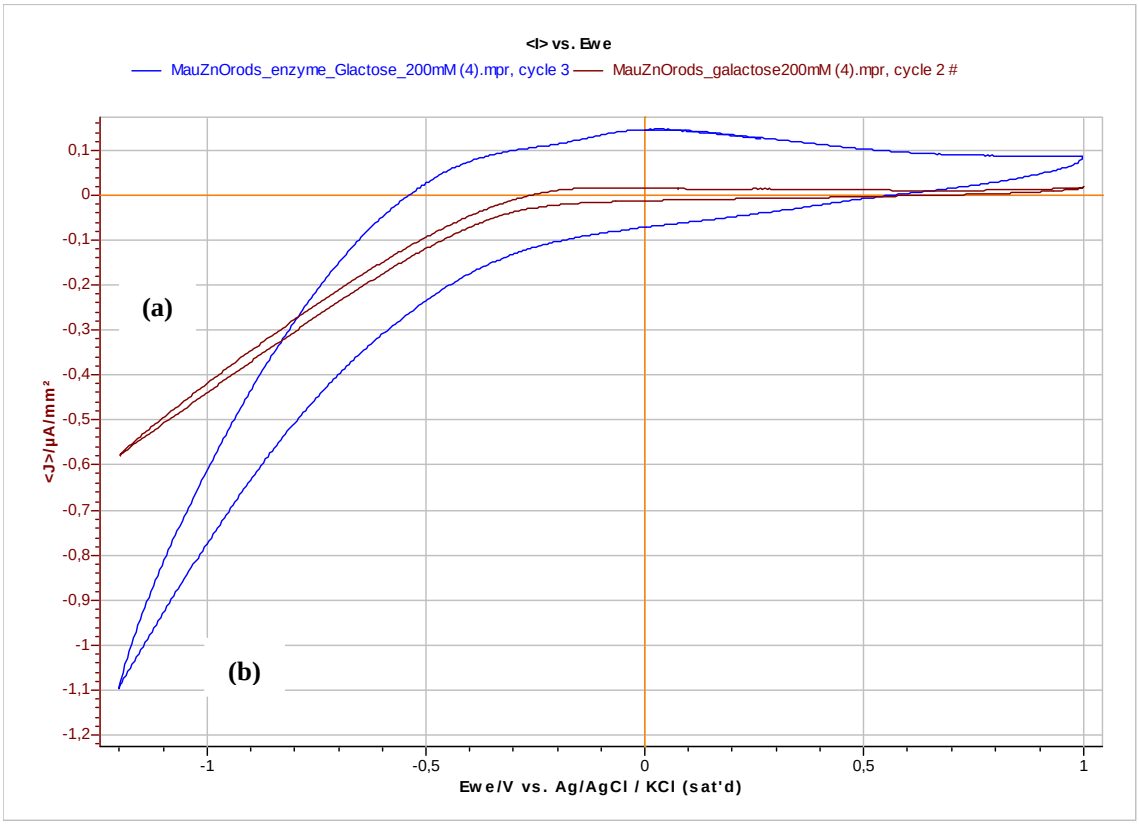


Fig. 3 The CV curve of (a) the pure ZnO nanorods and (b) the immobilized ZnO nanorods in 200 mM galactose solution.

4. Conclusions

We have successfully grown up the ZnO nanorods on seed ZnO coated FTO substrate by solution method, the nanorods were preferentially orientated along c axis direction, the average diameter of nanorods in this situation was 40-50 nm. We also successfully immobilized galactose oxidase on ZnO nanorods with the efficiency of the immobilization is 36.5% corresponding to 3 h immobilization time. These results are basic for galactose sensor application based on ZnO nanorods in the next step to test some parameters influenced the working of sensor, such as the pH, the temperature, the concentration … of galactose solution.

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