

Bioelectrochemical Properties of the ZnO Nanorods Modified by Au Nanoparticles

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Received: 29 October 2014 / Accepted: 24 November 2014 / Published: 2 December 2014

A novel amperometric glucose biosensor based on hybrid nanostructure of ZnO nanorods and Au nanoparticles was investigated, and the morphologies of various self-assembly time of AuNP by self-assembly method on the ZnONR were discussed. The ZnO nanorods (ZnONR) provide a 1-D electron transmission path and the Au nanoparticle (AuNP) was used to enhance the sensitivity. Furthermore, the ZnONR arrays were fabricated by chemical bath deposition which was an aqueous deposition technique on ITO electrode. For the bioelectrochemical properties of glucose sensing, the linear range of the ZnO-Au hybrid nanostructure is 1.38~22.22 mM with sensitivity of 1.44 $\mu\text{A}/\text{mM}$ and the limit of detection (LOD) is 3.51M.

Keywords: ZnO nanorod; Au nanoparticle; Self-assembly; Bioelectrochemical properties.

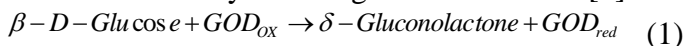
1. INTRODUCTION

The glucose plays an important role for living organisms, especially in the case of patients suffering from diabetes. An accurate measurement of glucose level in blood has been recognized as an important clinical test for diagnosing diabetes mellitus [1-3]. As a result, glucose biosensor plays an important role in diabetes patients' daily life for holding the concentration of glucose in the blood at a normal range. However, since the fabrication of the first glucose biosensor, several of glucose biosensors have been reported, such as fiber optic biosensor [4], LSPR (Localized Surface Plasmon Resonance, LSPR) optic biosensor [5] and fluorescence [6]. Compare of those technical mentions above, electrochemical transducer takes its advantages, for example, simplicity, relatively low cost,

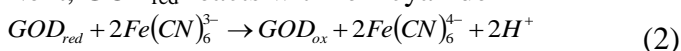
high selectivity and high sensitivity. Therefore, the characteristic of ZnO nanorods modified electrode on electrochemical biosensor have been widely investigated.

Because of the stability and high selectivity of glucose oxidase (GOD), it is currently widely employed in most of the glucose biosensors especially in amperometric glucose biosensor. It contains two flavine adenine dinucleotide (FAD) cofactors and catalyzes.

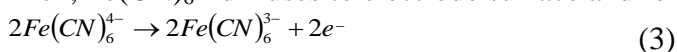
As shown in Fig. 1, the mechanism diagram of glucose biosensor. The common electron transport mediums, ferricyanide (Ferri) and ferrocene (Ferro) which have a better electron transport characteristic were widely used in glucose biosensor [7]. It follows the following reaction equation [7]:



Next, GOD_{red} reacts with ferricyanide



Then, $\text{Fe}(\text{CN})_6^{4-}$ diffuses to electrode surface and releases the electron [7]:



By the above electrochemical reactions, we can get the glucose concentration. Thus, the electrons generated by the redox reaction would be used as electrical signal and then exported to outer current loop.

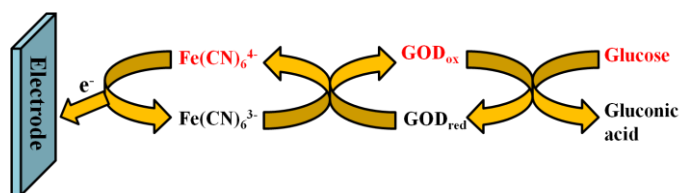


Figure 1. The mechanism diagram of glucose biosensor.

Here in, our strategy for keeping the activity and structure of enzyme and improving the effect of electronics is to modify the electrode with ZnO nanostructure. Therefore, since zinc oxide (ZnO) has many characteristic of semiconductor, such as photoconductive, piezoelectric, electronic and photoelectric. It is a very good optoelectronic material that often used in biosensor. More important, the isoelectric point of ZnO nanorod is around 9.5, and it can carry positive charged in neutral solution. But in buffer solution, the ZnO nanorod can be immobilized the DNA or enzymes with negative charges by electrostatic absorption, and maintaining a high level of biological activity and stability [8].

The growth of ZnO nano-film by chemical bath deposition methods have been developed for several years. It was firstly revealed in 2001 from Vayssieres et al. [9]. In 2009, Kong and the others scholar added sodium hydroxide to etch the ZnO nanorod into tube shape by chemical bath deposition, and applied the nanotube on biosensor [10]. In 2009, Yang and the others scholar [9] shows that the ZnO hexagonal nanorod changed into cylindrical nanorod when the pH value changes in the solution. In 2010, Breedon and the others scholar [11] successfully fabricated ZnO nanowire and nanorod structure by variety fabricating process.

On the other hands, the nanometer-sized gold particles are reported to exhibit excellent catalytic activity due to their large surface-to-volume ratio and the presence of active sites on the

surface. Therefore, it is expected that the doped of Au nanoparticles (AuNP) on the ZnO nanorod (ZnONR) arrays would improve the catalytic activity [12]

Among the noble metallic NPs, AuNPs have greater potential to decorate ZnONRs for improving the catalytic activity. These gold nano-particles provided the necessary conduction pathways, besides acting like nanoscale electrodes. AuNPs are relatively stable, catalytically active, water-soluble, optically sensitive and with universal biocompatibility. Many progress [1, 13-15] have been achieved in this research field, but there are remains much room for developing a stable and active catalyst of AuNPs and ZnONRs hybrid nanostructure. And up to now, there is few report about the influence of AuNPs on ZnONRs for the application of glucose biosensors.

In this work, the ZnONR arrays were deposited on ITO glass substrate by employing chemical bath deposition [16] (See Fig. 2(a)). And the AuNP were deposited by self-assembled method (See Fig. 2(b)). The chemical bath deposition provided a more simple, low cost and suitable process for mass production [17-19]. Moreover, the self-assembly method is an easy and directly way for the medication of AuNP on ZnONR [19]. As a result, the ZnONR and AuNP were employed for the glucose sensing. And ZnO/Au hybrid nanostructure deal with the enhancement of glucose sensing via the growth of gold nanoparticle. The concept involves the electrochemical measurement of glucose sensing.

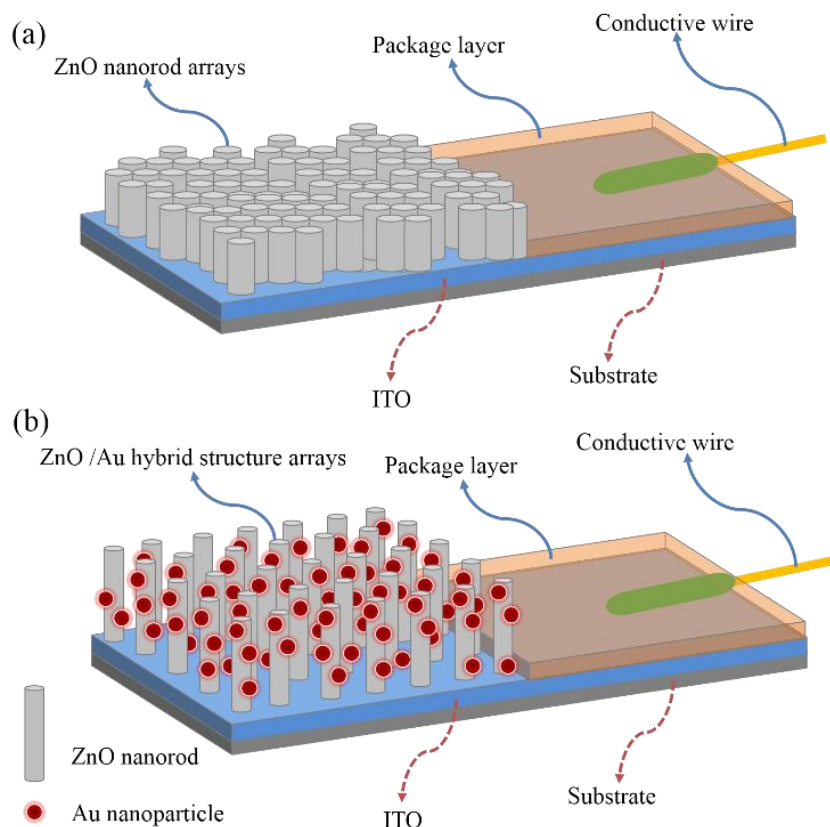


Figure 2. The schematic illustration of modified sensing electrode, (a) ZnO nanorods and (b) ZnO-Au hybrid nanostructure.

2. EXPERIMENTAL

2.1. Reagents and Materials

All the reagent is analytical grade and without further purification. The $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and hexamethylene-teramine ($\text{C}_6\text{H}_{12}\text{N}_4$, HMTA) were purchased from Showa Chemical Industry (Tokyo, Japan). The glucose oxidase (123400 u/g) and phosphate buffered saline (PBS, pH 7.4 at 25°C) were purchased from Sigma. The ferricyanide (Ferri, $\text{Fe}(\text{CN})_6^{3-}$), ferrocyanide (Ferro, $\text{Fe}(\text{CN})_6^{4-}$) and β -D(+)-glucose were purchased from Avantor. The (3-Mercaptopropyl)trimethoxysilane (MPTMS) ($\text{C}_6\text{H}_{16}\text{O}_3\text{SSi}$) is purchased from Acros. The synthesized and fabrication of Au nanoparticles colloidal is referred to *Chen et al.* research [20] and have a particles size in diameter of 5 nm.

2.2. Measurement and apparatus

The electrochemical experiments were performed on a Potentiostat SP-150 (Bio-logic, USA). All experiments were carried out with modified electrode as the working electrode, a platinum plate as the auxiliary electrode, and a silver / silver chloride electrode (Ag/AgCl) as the reference electrode. The Field Emission Scanning Electron Microscopy (FE-SEM) was performed with S4800-I (Hitachi, Japan); UV spectrum was obtained at ambient temperature using a V-550 UV-Visible Spectrophotometer (JASCO, USA).

2.3. Fabrication of ZnO nanorods sensing electrode

ZnO nanorods were synthesized by the chemical bath deposition [16]. Briefly speaking, the ITO electrode was firstly coated ZnO seeds layers for the preparation of growth of ZnONR. Then the ZnO seeds layer coated electrodes were annealed at 350°C for 30 minutes. The $\text{Zn}(\text{NO}_3)_2$ and HMTA were dissolved in equal molar ratio with a concentration of 0.025M in distilled water. Then the solution was heated and stirred at 90 °C for 60 minutes and became white and cloudy. After ultrasonic treatment for 1 min in water bath, the solution was dried with nitrogen gas. Then the as-synthesized ZnONR electrode was preparation for self-assembled of AuNPs.

2.4. Modification with Au nanoparticle on the ZnO nanorods

The modified ZnO electrode with MPTMS was soaked in the Au nanoparticle colloidal solution for several hours. Then the Au nanoparticles was attached on the surface of ZnO modified electrode. After several hours, the electrode was picked up from Au colloidal solution. The ZnO-Au modified electrode is cleaned by toluene and ethanol, then it was dried by nitrogen gas and backed with oven at 60°C for 30 minutes. The modified electrodes were annealed at 350°C for 30 minutes. The electrode finally covered with sliver glue and epoxy and determined a sensing area of $0.6 \times 0.5 \text{ cm}^2$.

2.5. Preparation of the electrolyte

In this step, the electrolyte was synthesized from 0.1M phosphate buffer solution (PBS), 1mM $\text{Fe}(\text{CN})_6^{3-/4-}$. Then GOD was dissolved in electrolyte to form 200 U/ml GOD solution. Similarly, the glucose electrolyte (200mg/dl glucose) was mixed with GOD solution to obtain the GOD catalysis solution.

3. RESULT AND DISCUSSION

3.1. Morphology of modified electrodes

The morphology of modified electrodes are shown in this section. As shown in Fig. 3(a), the ZnO seeds were coated on the ITO surface and ready for the growth of ZnO nanorods. The Fig. 3(b) and (c), the ZnO nanorods were grown on ITO electrode successfully by chemical bath deposition and the diameter of ZnO nanorods is about to 24~42nm which is smaller than the results of our research group [16] (50nm, higher concentration); as shown in the Fig. 3(b), the well aligned ZnO nanorod arrays were with a height of 679nm. The well aligned ZnO nanorod arrays could attribute to the annealed treatment of ZnO seeds [21]. The 1-D structure of ZnO nanorods supply a linear path for the transfer of electron. In the Fig. 3(d), the Au nanoparticles were grown on the ZnO nanorods (ZnONR) uniformly and the diameter of ZnONR is about to 47~51nm. This morphology are similar to the research results of Lanlan Sun et al. [14] that the Au nanoparticles are prepared by hydrothermal method and with a gold particle size is approximately 20 nm [14].

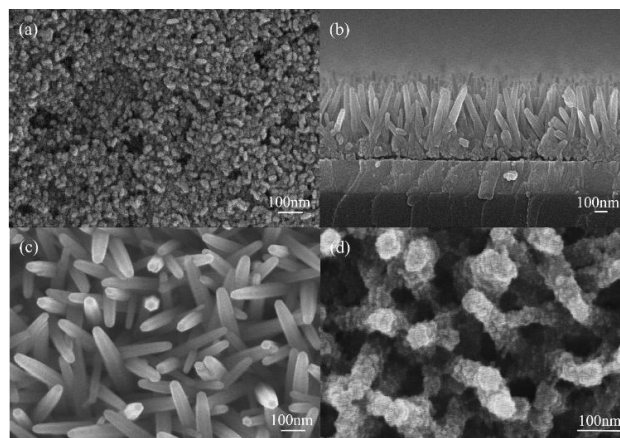


Figure 3. The FE-SEM analysis of (a) ZnO seed layers; (b) cross section of ZnO nanorods arrays; (c) top view of ZnO nanorods; (d) ZnO-Au hybrid nanostructure.

3.2. Optical absorption spectrum of ZnO modified electrode

The absorption spectrum is measured by UV/VIS spectroscopy. The electrons of molecules absorb specific energy when they are irradiated with light. In this section, the UV-Vis spectroscopy is

employed to verify the existence of Au nanoparticles since the LSPR effect of the particles with a resonance wavelength in the visible region. As shown in Fig. 4.3.2, it is shown that Au nanoparticle has been attached on the ZnO nanorod, which shows an obvious absorption peak at the range of 500~550 nm in the visible spectrum which is consistent with the results of Lanlan Sun et al. that AuNP modified on the ZnO will show an obvious peak at 500nm ~ 550nm in the absorption spectrum [14]. The AuNPs is self-assembled on ZnONR for 12 hours hence the sample is in a color of slight pink. It could be attributed to the localized surface plasmon resonance of Au nanoparticle. The result is corresponding to the SEM image, Fig. 3(d), that the AuNPs are attached on the ZnONR surface. The rough surface of ZnONR is a result with the attachment of AuNPs on the ZnONR surface. And the AuNPs show an obvious absorption peak at the range of 500 ~ 550 nm in the visible light region.

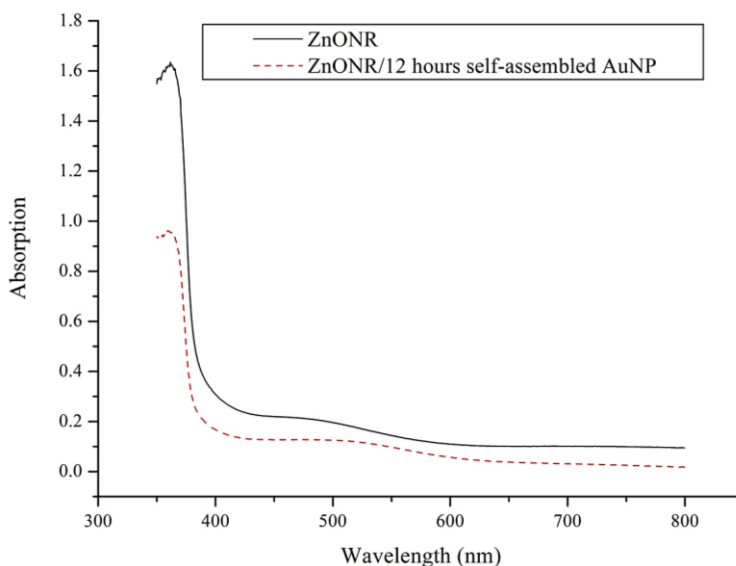


Figure 4. The UV-VIS spectrum of ZnO/Au hybrid nanostructure.

3.3. Electrochemical behavior of ZnO-Au modified ITO electrode

The CV results of ZnONR and ZnO/AuNP modified electrodes are also showed in Fig. 5 and the insert Table. The ZnONR were self-assembled with 9~18 hours which were denoted as ZA9, ZA12, ZA15 and ZA18. Basically, the current response is due to the redox reaction of $\text{Fe}(\text{CN})_6^{3-/4-}$, and the linear structure is an important factor to enhance the electrons transfer. From the comparison of the sample between ZnONR and samples ZA9~ZA18, the sample ZA12 shows a poor catalyst activity, but the sample ZA9 shows an excellent catalyst activity. Where, the current response of the sample ZA12 is the lowest of 20.60 μA , and the highest between ZA9~ZA18 is the sample ZA9 with a current response of 46.78 μA . Moreover, the ΔE_p did not change a lot between each samples that means the electrons transfer rate does not make much difference. It is said that the different assembles time of Au nanoparticles show different catalyst activity. An there is an appropriate AuNP self-assembly time is 12 hours for modification of ZnO nanorods.

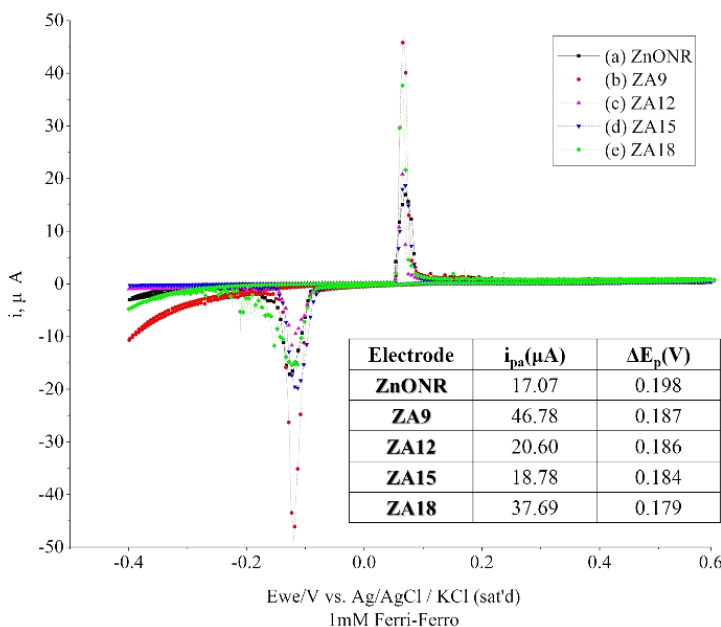


Figure 5. The CV results of AuNP modified ZnO electrode with different self-assembled time in 1mM Ferri-Ferro solution.

The increased current by catalyzed reaction was shown in Table I, where $\Delta I_{pa} (\mu A) = I_{pa, G} - I_{pa, B}$, the variables of $I_{pa, B}$ and, $I_{pa, G}$ represent oxidation peak currents in the absence and presence of GOD, respectively.

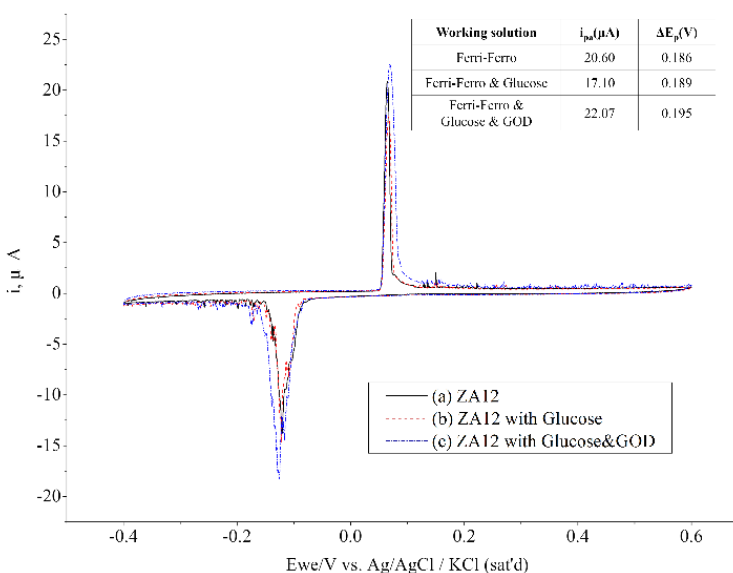


Figure 6. The CV results of AuNP modified ZnO electrode which immersed in AuNPs colloid for 12 hours with different working solution, (a) In Ferri-Ferro solution, (b) with glucose added, (c) with both glucose and GOD added.

As shown in Fig. 6, the catalyst activity of glucose redox reaction is carried out with the sample ZA12 in three different working solutions since the sample show a stable performance in the current

response and potential difference. The results show that the current response is increased when the glucose and GOD are added in the working solution. There is one thing should be mentioned that the ΔE_p is increased when the GOD is introduced into the solution. The reason could be attributed to that the enzyme isn't fixed on the electrode that lead the electron transfer path be to more complicated and the electron might be recombined with other materials. Besides, the AuNPs modified ZnO electrode shows poor catalytic activity, the possible reason could be attributed to that the ZnO thin film is not grown homogenous that different amounts of nanoparticle adhered on the electrode and cause poor catalytic response.

Table 1. Response Current of Virous ZnO Nanorods Modified ITO Electrode Table Type Styles

Electrode	$I_{pa, B}$	$I_{pa, G}$	ΔI_{pa}
ZnONR	18.14	20.25	2.11
ZA9	47.68	48.34	0.66
ZA12	17.11	22.08	4.97
ZA15	26.45	28.83	2.38
ZA18	39.71	43.64	3.93

3.4. The application for glucose sensing

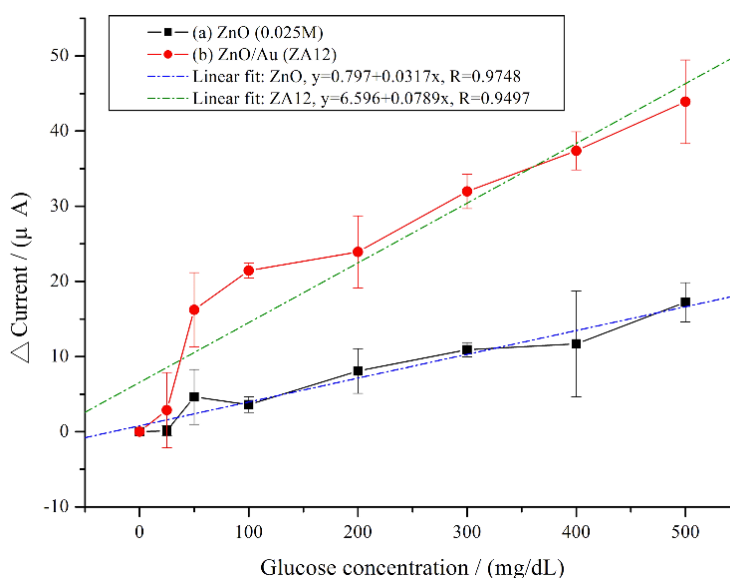


Figure 7. The glucose calibration curve of (a) ZnO nanorods electrode, (b) ZnO-Au (ZA12) hybrid electrode.

As shown in Fig. 7 and Table II, the ZnONR and ZA12 sensing electrode were applied to the glucose sensing. Also, the calibration of glucose were determined in this section. Furthermore, the sensitivity, limit of detection and liner range of the sensing electrodes were also determined. With the absence of Au nanoparticles, the sensitivity of sensing electrode was increased form 0.58 $\mu\text{A}/\text{mM}$ to 1.44 $\mu\text{A}/\text{mM}$, which was increased 148%. This enhancement result could fit the research of *Jin et al.*

[1]. But, since the sheet resistance of AuNP modified electrode was slightly increase, the limit of detection was also increased 48% to 3.51M. However, the result shows an interesting characteristic such as sensitivity and wide linear range and prove a potential of such ZnO-Au hybrid nanostructure as good glucose biosensor.

Table 2. The properties of glucose biosensor (a) ZnO nanorod electrode, (b) ZnO-Au (ZA12) electrode.

Electrode	Sensitivity ($\mu\text{A}/\text{mM}$)	Linear range (mM)	LOD (M)
(a) ZnONR	0.58	1.50~27.78	2.36
(b) ZA12	1.44	1.38~22.22	3.51

4. CONCLUSION

In this study, the ZnONR and AuNP are successfully modified on ITO by chemical bath deposition and self-assembly method which are formed on ZnONR electrodes and ZnO-Au hybrid electrode. The experiment results show that there is a better current response and glucose catalyst activity with the appropriate self-assembly time of AuNPs modified on the ZnONR electrode. The ZnONR electrode with the self-assembled time of AuNPs for 12 hours which shows a better performance in the glucose catalyst. The ZnO-Au hybrid sensing electrodes show a good sensitivity of 1.44 $\mu\text{A}/\text{mM}$, a linear range through 1.38~22.2mM and the LOD is 3.51M. The results show the fabrication processes are easy, convenient and low cost for the fabrication of ZnO-Au hybrid electrode on the normal temperature and pressure. The ZnO-Au hybrid electrode has a potential as the high sensitive glucose sensing electrode.

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