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A Glucose Biosensor Based on Glucose Oxidase enzyme and ZnO Nanoparticles Modified Carbon Paste Electrode

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In this study, modified carbon paste electrode (CPE) with zinc oxide (ZnO) nanoparticles used for designing a glucose oxidase enzymatic biosensor to investigation of its electrochemical behavior, and determination of glucose concentration. The size and morphology of synthesized ZnO metal oxide nanoparticles evaluated via chemistry technique like scanning electron microscopy (SEM) and X-ray diffraction (XRD). A pair of clear and stable peaks in PBS (0.1 M, pH = 7) obtained for the direct transfer of electrons from GOD to modified electrode with -340 mV and -385 mV respectively as oxidation and reduction potentials. The enzymatic biosensor exhibited a linear response to the β-D (+) glucose cancentration range from 40 µM to 380 µM with limit of detection equal to 8 µM in a signal to noise ratio of 3. Here, the designed GOD/ZnO/CPE electrochemical biosensor showed high stability and efficiency for glucose sensing.

Keywords: Biosensor, Glucose Oxidase, Zinc Oxide nanoparticles, Carbon Paste electrode

1. INTRODUCTION

Determination of glucose concentrations in clinical samples, biological and chemical as well as in food productions and fermentations is very important [1]. Many chemistry techniques such as fluorescence and electrochemical flow injection for this purpose have been progressed [2]. Among electrochemical detection methods amperometric biosensor based on electron transfer between an electrode and stabilized glucose oxidase enzyme, which can catalyze the oxidation of glucose, is known as a very effective method [3]. This biosensing techniqueis simple and can be realized in two different paths. The First is an indirect way of electron transfer using a mediator to shuttle electrons

[4]. The mediators used in this sensors are ferrocene derivatives, quinones, and etc [5,6]. Another way is by direct electron transfer between the glucose oxidase enzyme (GOD) and electrode (which produces unmediated glucose sensors) [7.8]. Due to the lack of a simple method for stabilizing the enzyme, and the difficulty of direct transfer of electrons between the redox enzyme and the electrode surface, resulting of a thick layer that surrounds the active site of the enzyme, use of these unmediated sensors is limited [9,10]. Therefore, it is necessary to design and develop a new glucose sensor based on direct electrochemistry of GOD. Evaluation of direct transfer of electrons from GOD immobilized on the electrode, during conversion GOD (FAD) to GOD (FADH2), (FAD: flavin adenine dinucleotide) by amperometric and cyclic voltammetry (CV) techniques can be useful in designing new sensors for glucose measurements [11,12,13]. In this work beneficial attributes of zinc oxide nanoparticles and nanotechnology to disign a glucose biosensor based on direct transfer of electrons from GOD were applied. Basis of this study is that the GOD adsorbed on the electrode surface that was modified by zinc oxide nanoparticles performs a rapid transmission electron via the electrode, and GOD was reduced. Reduced form of the glucose oxidase enzyme, GOD (FADH2), can reduce the dissolved oxygen [14]. In the presence of glucose, reductive reaction of oxygen inhibited because the reaction occured between the glucose oxidase oxidative form GOD (FAD) and glucose [15,16]. Which also led to a decrease in the electrocatalytic response [17]. Based on this reduction a method for determining glucose was proposed. This method differs from the conventional amperometric glucose sensors that determined glucose based on oxygen consumption or hydrogen peroxide production [18]. In the case of amperometric biosensors, it requires a high anodic potential which causes the interference with substances such as ascorbic acid and uric acid [19].

2. EXPERIMENTAL

2.1. Reagents and chemicals

β-D (+) glucose, glucose oxidase (GOD), zinc nitrate (Zn $(NO_3)_2$. $4H_2O$), NaOH, ethanol, K_2HPO_4, $KHPO_4$ and graphite powder, were purchased from Sigma and Merck. All solutions were prepared using double distilled water. Standard serum samples were prepared according to the instructions. All chemicals were used with chemical purity and also without further purification. Phosphate buffer solution (PBS 0.1 M pH=7) were prepared from mixing standard solutions of stored K_2HPO_4 and $KHPO_4$ and by adjusting the pH with NaOH or H_3PO_4.

2.2. Apparatus

Cyclic voltammetry experiments were done with an electro-analyzer model (slemilink system EA-201) equipped with a personal computer. A conventional three-pole cell was used throughout the experiments. A carbon paste electrode modified with zinc oxide nanoparticles (diameter 1 mm) as a working electrode, a saturated calomel electrode as a reference electrode, and a platinum electrode as a counter electrode were used. The morphology of the nanoparticles was characterized by Ziess DSM

960A scanning electron microscope (SEM). XRD was obtained using a Phillips PW1800 diffractometer. Electrochemical measurements were performed by PalmSens Potentiostat Galvanostat device, made in Netherland. The device is connected to the computer that requires to work with electrochemical systems data processing software (GPES, software version 4.9, Eco Chemie).

2.3. Preparation of ZnO nanoparticles

For preparing zinc oxide nanoparticles, 0.7 M of sodium hydroxide (NaOH) solution was heated at 55° C, then, 0.3 M of zinc nitrate (Zn $(NO_3)_2$. $4H_2O$) solution was prepared using double distilled water [20,21]. In the next phase solution of (Zn $(NO_3)_2$. $4H_2O$) slowly was droped to the solution on the heat under high speed stirring [22]. The beaker that contains the solution was kept at this condition. After 2 hours, the obtained result was production of sedimented zinc oxide nanoparticles. Then, the production was cleaned by ethanol, washed with double distilled water and dried in room air at temperature of 60° C [23,24].

2.4. Preparation of the modified electrode

Unmodified CPE was prepared by Negahdary et al method [25], in preparing the carbon paste electrode modified with zinc oxide nanoparticles, the 0.7 g carbon powder with 0.1 mg of zinc oxide nanoparticles, 0.8 g of the paraffin prepared in the previous stage and the silicone oil were mixed well for 1h untill homogeneous paste was obtained [26,27]. The remaining steps of preparing of carbon paste electrode modified with zinc oxide nanoparticles, is similar to those of the bare electrode [28].

3. RESULTS AND DISCUSSION

3.1. Characterization of synthesized ZnO nanoparticles

Figure.1 shows the x-ray diffraction (XRD) patterns for ZnO nanoparticles. The diffraction peaks data were gathered at the 2ɵ range. The size of nanoparticles grain was obtained by the Sherrer equation, $D= k\lambda/ (\beta \cos \theta)$, using the relative intensity of (101) peak. In which, k is a constant and is equal to 0.9 for spherical particles, λ is the wavelength (Cu Kα), β is the full width at half height of the line, and ɵ is the diffraction angle [29]. The estimated size of ZnO nanoparticles grain was 20-40 nm and a sharp increase in XRD peaks indicates the crystalline nature of the particles. The reflections of 111, 200, 220, 222 are seen obviously and correlate closely with ZnO reference patterns.

The morphological characteristics of ZnO nanoparticles were investigated by scanning electron microscopy (SEM) [30]. The synthesized nanoparticles are shown in Fig. 2, this image was taken by a scanning electron microscope (SEM) with magnification of 1500 times which indicates that the synthetic nanoparticles have diameters about 20-40 nm. Due to increase of surface to volume ratio, the smaller particles can play an important role during stabilization process [31].

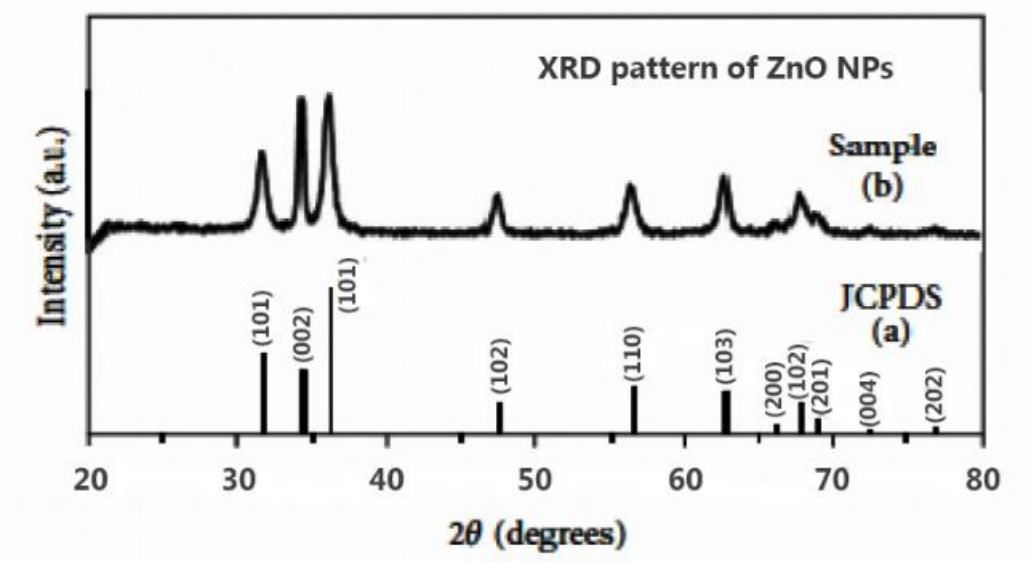

Figure 1. XRD pattern for ZnO nanoparticles

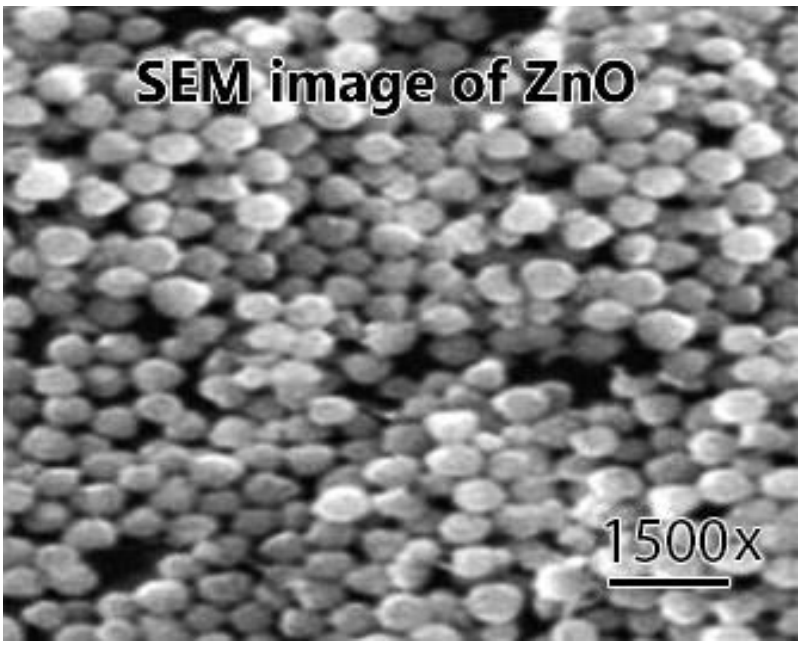

Figure 2. SEM image of ZnO nanoparticles

3.2. Direct electron transfer of GOD/ZnO/CPE

As shown in Fig. 3 (b), a pair of clear and consistent redox peaks have been seen for direct electron transfer between the glucose oxidase enzyme (GOD) and the modified electrode in 0.1 M PBS buffer with pH of 7. The reduction and oxidation peak potentials were -385 mV and -340 mV respectively and no peaks were observed in the unmodified electrode in Fig. 3 (a). The formal potential calculated for the GOD is equal to -362 mV. It was observed that the bare electrode shows no cathodic and anodic peaks in Fig. 3 (a), which implies that ZnO nanoparticles act as facilitator for the electron transferring from the redox proteins to the electrode.

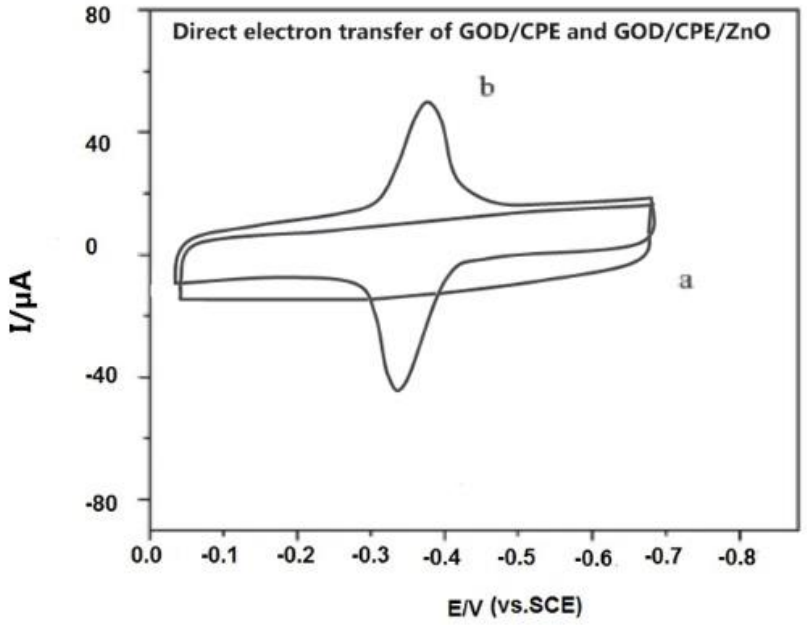

Figure 3. (a) CV of bare CPE, (b) CV of CPE/ZnO in PBS 0.1 M and pH 7 (the scan rate is 50 mv/s)

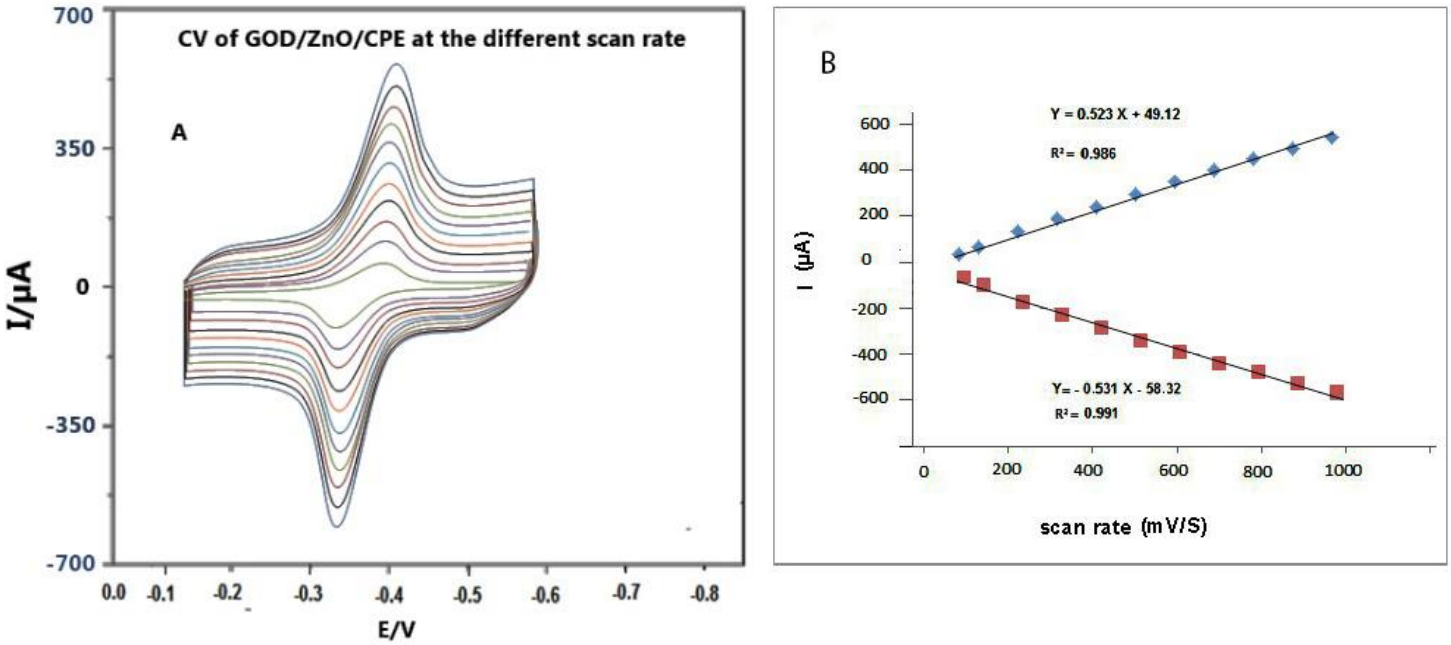

Figure 4. (A) CVs of GOX/ZnO/CPE in PBS at various scan rates, from inner to outer; 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mVs^{-1}, (B) the relationship between the curve currents (ip_a, ip_c) vs the scan rates

These results correlate with the former works which studied the role of nanoparticles in facilitating the electron transferring [32]. It is shown in Fig. 3 (b) that ZnO nanoparticles at the nano level can play a critical role to obtain the cyclic voltammogram of GOD. In an environment where

surface to volume ratio is increased due to the decrease of the size, these nanoparticles have a great effect on the electron transfer between GOD and CPE.

In another study, the electron transport properties of GOD on the modified CPE by ZnO nanoparticles were evaluated, and scanning rates effects on cyclic voltammogram of glucose oxidase were also studied. Fig. 4 (A) shows a dependence between the cathodic and anodic current peaks of GOD with scan rate and Fig. 4 (B) also shows that redox peak currents increase linearly with scan rate. The correlation coefficient is equal to 0.986 for cathode peak and 0.991 for anode peak. This finding refers to the fact that process is under control of absorption of the species of redox and indicates the stable immobilization of GOD enzyme on the electrode surface [33].

3.3. Dependence of GOD solution pH on direct electron transfer

In Fig. 5 it is observed that changes caused in cycle voltammogram peak potentials and currents by pH were reversible at pH limits between 5 and 7. This says that if an electrode of a sample solution with various pHs is transferred to its main buffer, the same cyclic voltammogram can be obtained. Increase of solution pH caused a translocation in potential of both anodic and cathodic peaks. The diagram of pH (from 4 to 11) Vs potential created a pH line with slope equal to -46.5 mV which is also close to the expected value -58 mV. This shows that 2 protons and 2 electrons are involved in electron transfer. Irreversible decrease in peak current at pH=4 was resulted from protein denaturation that was because of separation of FAD group in this region of pH [34].

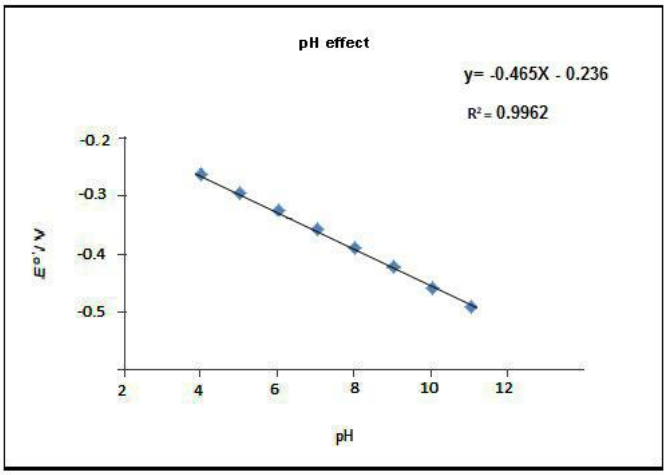

Figure 5. Effect of pH of buffer solutions on the current response of GOX/ZnO/CPE.

3.4. Electrochemical characterization of the glucose biosensor

The cyclic voltammogram resulting from electron transfer by glucose oxidase in the presence of the dissolved oxygen changes significantly. Increase of reduction peak current and decrease of oxidation peak current in Fig. 6 (b) shows a great current response occured in potential -0.5 v.

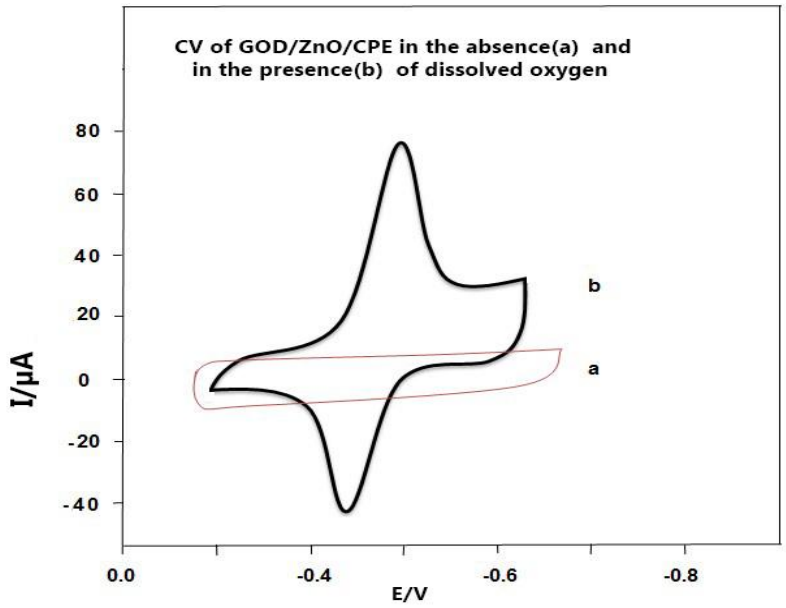

Figure 6. CV of GOD/ZnO/CPE in the absence (a) and in the presence (b) of dissolved oxygen in PBS 0.1 M and pH 7 (scan rate is 50mv/s)

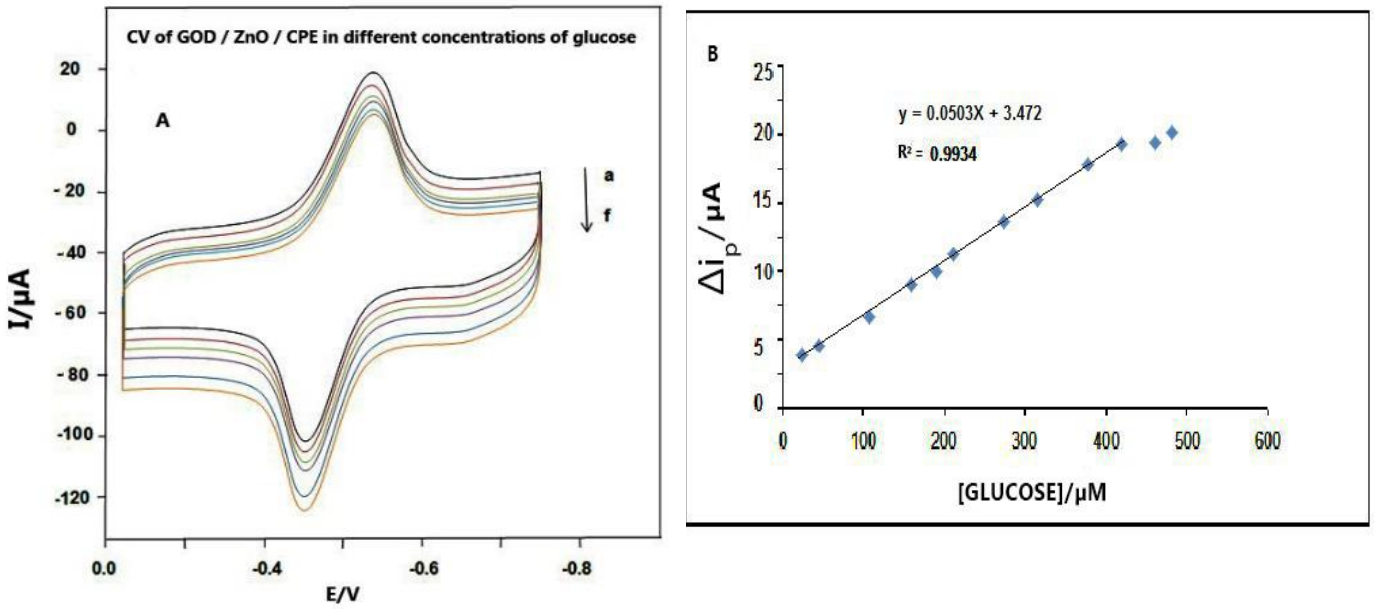

Figure 7. (A) CVs of GOD / ZnO / CPE in PBS 0.1M & pH 7 containing dissolved oxygen concentrations of 40, 60, 120, 200, 280, 380 µM glucose (a to f) at a scan rate of 50 mv/S. (B) A linear calibration curve

The difference in Fig. 6 (a) and (b) indicates that absorbed GOD on the electrode caused reduction of dissolved oxygen and a significant increase in reduction peak current observed [35].

$$GOD\ (FAD) + 2e^- + 2H^+ \leftrightarrow GOD\ (FADH_2) \quad (1)$$

$$GOD\ (FADH_2) + O_2 \rightarrow GOD\ (FAD) + H_2O_2 \quad (2)$$

Soon after adding β-D (+) glucose to PBS solution saturated with air, the reduction current response of GOD/ZnO/CPE was dropped in Fig. 7 (A). It was more decreased after readding β-D (+) glucose.

As we know GOD in the presence of β-D (+) glucose catalyses a reaction according to equation 3, and decreases the amount of oxidated GOD on the electrode surface. Therefore, adding glucose restrains the reaction in equation 2 and decreases reduction current [36]. The GOD/ZnO/CPE cyclic voltammogtam shows in Fig. 7 (A), that by continuous adding β-D (+) glucose to PBS solution saturated with air decreases peak current. The sensor exhibited linear response range to the concentration of β-D (+) glucose from 40 to 380 µM with the correlation coefficient of 0.9934 and limit of detectionequal to 8 µM in a signal to noise ratio of 3. The obtained results of the proposed paper were compared with those of the other works. For the results refer to Table 1.

Glucose + GOD (FAD) → gluconolactone + GOD ($FADH_2$) (3)

Table 1. Comparison of biosensor sensitivity with some reported glucose biosensors

Nano structure of Glucose biosensor	Limit of detection	Stability	Matrix	Ref No
ZnO:Co nanoclusters	20 µM	2 week	In solution	37
ZnO nanorod	10 µM	1 week	In solution	38
Graphene/AuNPs/chitosan	180 µM	2 week	In solution	39
Carbon nanotubes	200 µM	1 week	In solution	40
ZnO nanoparticle	8 µM	4 week	In solution	This Work

3.5. Determination of glucose in serum samples

Serum glucose was determined using the standard sample adding on the sensor. The current response in 5 mL of PBS 0.1 M pH 7 solution that contains 40 µL serum samples was recorded. Then four solutions each containing 10 µL β-D (+) glucose with concentration of 20 mM to determine, successivly were added to the system. All concentrations of glucose in detection solution were in a linear range of answer. Glucose level was determined equal to 8.64 µM, that was close to 8.74 µM obtained by spectrometry. Interference effects in the presence of uric acid or ascorbic acid were studied by detection test of 1 mM glucose. 0.18 mM uric acid or 0.32 mM ascorbic acid increase the reduction currents to 3.7% and 4.5%. Therefore, these materials difficultly cause any interference in biosensor response respectively.

3.6. Selectivity, stability and longevity of GOD biosensor designed

The sustainability of a biosensor, specifically depends on the preparation procedure, receptor and transformer. In addition, it severely depends on the response of speed limiting factor like an external substrate or an internal diffusion or a biological diagnostic reaction. Finally it must be mentioned that stability depends on the condition as well. To determine the practical stability, we recommend to consider analyte concentration, continual or serial contact of biosensor with analyte solution, temperature, pH, buffer structure, presence of organic solvents and the composition of sample material. The stability of biosensors in laboratory conditions may be much better than their usage status, and depends on the industrial limitation, and the targets. To evaluate the stability of the stored biosensor, dryness, humidity, buffer composition, atmosphere composition and pH parameters in the presence of additive materials are considered. When an enzymatic electrode was kept at 4º C, 95% of its primary current response for determination of glucose was observed after temporary usage during a 4-week period. This sensor can maintain a constant flow, after 170 continual cycles in presence of glucose. The sensor is renewable by adding the GOD solution on electrode`s surface after cleaning its top. The response of renewed current was experimented at 0.2 mM concentration of β-D (+) glucose. For 7 times of continuous renewing the relative standard deviation was 4.7%. Therefore this method is fast, easy, and repeatable for elimination of separated surface from GOD membrane. The capability of reproducing of 6 electrodes showed an acceptable repeatability with standard deviation of 5.5% for recorded flow in 0.2 mM concentration of β-D (+) glucose.

4. CONCLUSIONS

Convergence of bioelectrochemistry with nanotechnology resulted in designing of efficeient third generation enzymatic biosensor for glucose determination. Although the designed GOD/ZnO/CPE biosensor showed appropriate reproducibility, sensitivity, stability and low detection limit, however more investigation in this case may needed to introduce it as excellent potential candidate for application in biomedical and biotechnology industries.

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