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Hydrogen Peroxide Biosensor based on Carbon Paste Modified Electrode with Hemoglobin and Copper(II) Oxide Nanoparticles

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Copper oxide nanoparticles using chemical techniques were synthesized. Characterizations of produced nanoparticles using UV-Vis, XRD and TEM techniques were studied. Hemoglobin immobilized on a carbon paste electrode modified with copper oxide nanoparticles. The used nanoparticles could facilitate electron transfer between the immobilized hemoglobin and the carbon paste electrode. The modified electrode showed a good direct electrochemistry behavior and the hemoglobin due to electrocatalytic reduction of hydrogen peroxide (H_2O_2) displayed a pair of reversible redox peaks of Fe (II) and Fe (III) with a formal potential of -70 mV. The designed Hb/CuO/CPE biosensor showed a linear response to the H_2O_2 concentrations in the range from 0 to 4500 µM with 0.7 µM limit of detection at signal to noise ratio of 3. This sensor exhibited a high stability and a good sensitivity.

Keywords: Hydrogen Peroxide, Biosensor, Hemoglobin, Copper Oxide nanoparticles

1. INTRODUCTION

Hydrogen peroxide is one of substances, that its detection is important in very small quantities in food, cultivation of fungus and algaes, environmental and medical applications [1,2]. One of catalysis product of glucose by glucose oxidase enzyme is hydrogen peroxide [3], which its measure can help to design a glucose biosensor to measure the level of glucose in blood [4]. Also hydrogen

peroxide is toxic product of biochemical reactions in the human body [5], that has destructive and deadly effects on cells and tissues at certain concentrations thus its detection is very important in clinical medicine [6]. So researchs aimed at developing better ways to measure the hydrogen peroxide concentrations in the solution continues [7,8]. One of employed practices is biosensor production [9]. In preparing this biosensors, direct electron transfer of immobilized hemoglobin onto the electrode surface was studied to determine hydrogen peroxide [10,11,12]. Hemoglobin immobilized on the electrode surface catalyzes oxygen reduction and essentially an electrochemical reaction occurs as follow [13,14]:

$$\text{Hb Fe (III)} \rightarrow \quad \text{HbFe (II)}$$

$$\text{HbFe (II)} + O_2 \rightarrow \text{HbFe (II)}\ldots O_2$$

$$\text{HbFe (II)}\ldots O_2 + 4e^- \rightarrow \text{HbFe (II)} + 2H_2O$$

HbFe (II) and HbFe (III) are called Ferro-hemoglobin and Met-hemoglobin, respectively. It is anticipated that the presence of hydrogen peroxide in solution will be affected on combining oxygen with hemoglobin and modulates hemoglobin electrocatalytic behavior [15,16]. This property can be used to measure the hydrogen peroxide in solution. In recent years, many studies have been done on electrochemical biosensors to improve the speed, selectivity and sensitivity [17,18]. Electron transfer mediators that immobilized on the electrodes surface have been widely studied in the past two decades [19]. Different types of quinones, riboflavins and phenoxazines are used to design sensors [20,21,22]. Nanotechnology has caused a massive evolution in biosensors development [23]. So that, use of nanostructures and nanoparticles improved the speed of direct transfer of electrons [24,25]. Recently, mediator-free hemoglobin sensors have been produced for detection of hydrogen peroxide [26]. In this study the electrochemical behavior of hemoglobin by designing a biosensor based on carbon paste electrode modified with copper oxide nanoparticles is studied. Copper oxide is a semiconductor that has many applications [27]. Despite, there are fewer reports about the synthesis and characterization of hydrogen peroxide biosensor based on copper oxide nanoparticles compared to other nanoparticles such as zinc oxide, titanium oxide and iron oxide [28,29].

2. EXPERIMENTAL

2.1. Chemicals

$(CH_3COO)_2Cu.H_2O$, NaOH, NaCl, $Na_2HPO.2H_2O$, CH_3COOH, H_2O_2 and hemoglobin (90%, bovine blood) were purchased from Merck and Sigma. Twice distilled water was used to generate all solutions.

2.2. Instrumentation and measuring procedure

Ultrasonic device was used to disperse and separate copper oxide nanoparticles that were synthesized in the laboratory using chemical techniques to achieve better UV-Vis spectra of the nanoparticles using a UV-2120 spectrophotometer (OPTIZEN; South Korea). TEM image was

obtained using CEM 902A microscope (Ziess). XRD was obtained using a PW1800 diffractometer (Philips). Electrochemical measurements were performed with a potentiostat galvanostat device and a conventional three electrode system, where modified carbon paste electrode with copper oxide nanoparticles is as a working electrode, a (Ag / Ag Cl) electrode is as a reference electrode and a platinum rod electrode is as a counter electrode.

2.3. Preparation of CuO nanoparticles

To prepare copper oxide nanoparticles, 10 cc of the 0.2 M ($(CH_3COO)_2$ $Cu.H_2O$) solution was spilled in a flask, then 5 cc of CH_3COOH was added to the above solution and the flask was heated on a heater. Flask with a magnetic stirrer be was stirred. When the temperature reached 100 ° C, 30 cc of 3 M NaOH solution was added to contents of the flask, immediately a large amount of black sediments were formed [30]. The sediments were centrifuged 3 times and washed 3 to 4 times with deionized water and were dried on a plate for 24 hours at room temperature. This way, copper oxide nanoparticles were obtained.

2.4. Construction of CPE(Carbon Paste Electrode)

5 g of graphit powder and 1.5 ml of silicon oil were mixed in a mortar to obtain a hemogeneus carbon paste [31]. A part of the provided carbon paste was filled into a Teflon tube. A copper wire was inserted in the tube to establish an electrochemical contact [32,33]. This produced electrode was used as the working electrode in this study. Before using in laboratory activities, it was polished by a piece of paper.

2.5. Preparing modified CPE(Carbon Paste Electrode)

To prepare a carbon paste electrode modified with copper oxide nanoparticles, in first step 10 mg of provided copper oxide nanoparticles were mixed by 5 g graphit powder to obtain a homogeneous carbon paste. The rest of steps were same as those of providing bare carbon paste electrode. By dissolving 10 mg of hemoglobin in 1000 µl of PBS (pH=7), hemoglobin stock solution was produced [34]. Then for hemoglobin immobilization, 10 µl of this solution gently dropped on the surface of prepared carbon paste electrode modified with copper oxide nanoparticles. The modified electrode with hemoglobin was dried at room temperature for 4 hrs. The modified electrode was kept in 0.1 M PBS (pH=7) at 4º C for the time it was not in use [35].

3. RESULTS AND DISCUSSION

3.1. Using XRD, UV-Vis and TEM for characterization of CuO nanoparticles

XRD pattern of prepared nanoparticles (Figure. 1) showed a single-phase with a *monoclinic* crystalline structure. No peaks were observed for the impurities. Peak intensity (122) was

used to get the grain size using (Debye - Scherrer) equation, $D = K\lambda / (\beta\cos\theta)$ [36]. The grain size was estimated around 30 nm.

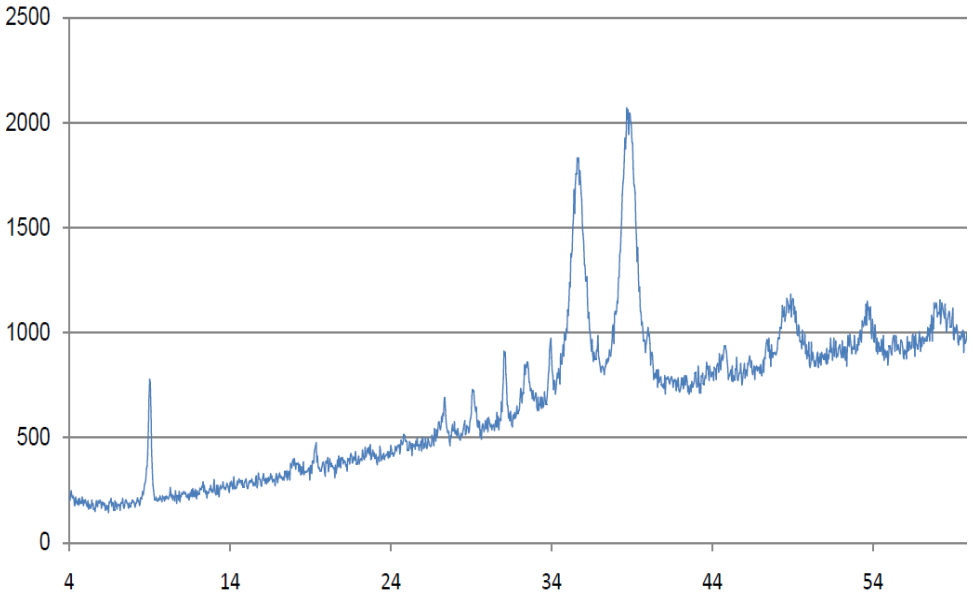


Figure 1. XRD pattern of CuO

Metal nanoparticles have specific characteristics depending on the methods of production, sizes and shapes of nanoparticles [37]. In Fig. 2 UV-Vis spectra of copper oxide nanoparticles is shown. An absorption in 270 nm showed that the nanoparticles are of quantum nature and this property is because of increase of surface to volume ratio of the nanoparticles [38].

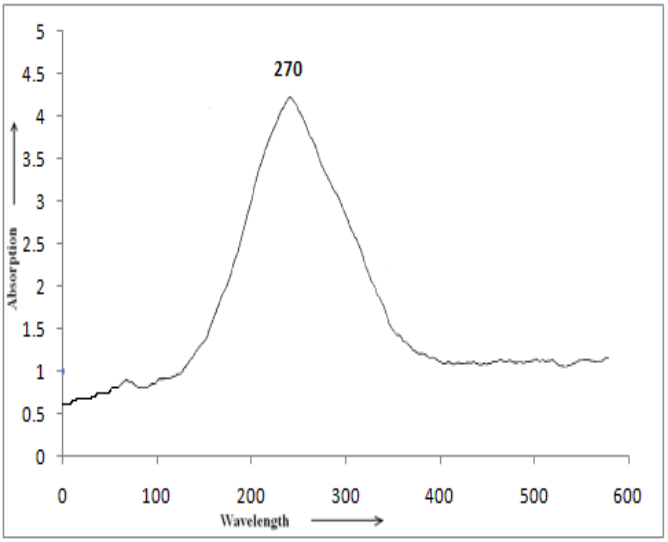


Figure 2. UV-Vis spectra of synthesized CuO nanoparticles

In Fig. 3 transmission electron microscope (TEM) image showed that sizes of copper oxide nanoparticles were about 30 - 40 nm.

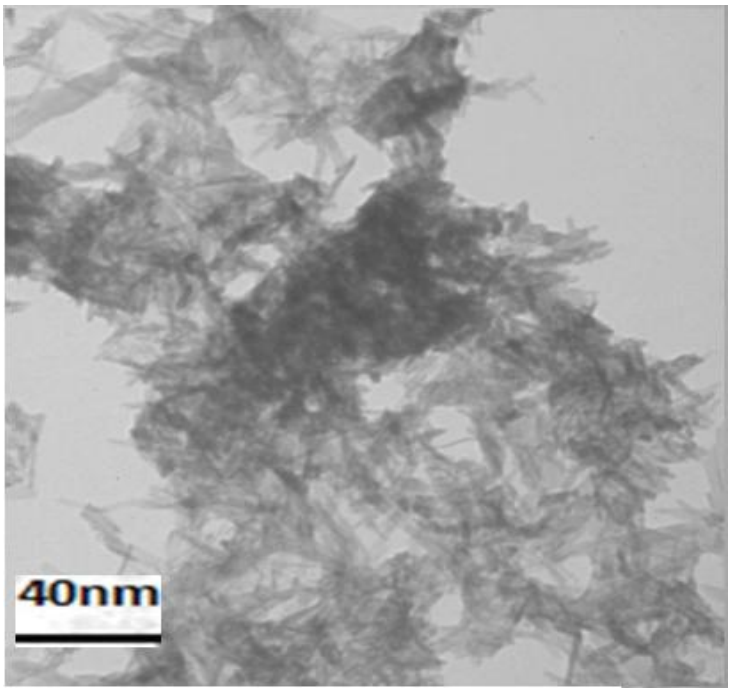


Figure 3. TEM image of CuO nanoparticles

3.2. Direct electrochemistry of Hemoglobin/Carbon Paste Electrode(Hb/CPE) and Hemoglobin/CuO/Carbon Paste Electrode(Hb/CuO/CPE)

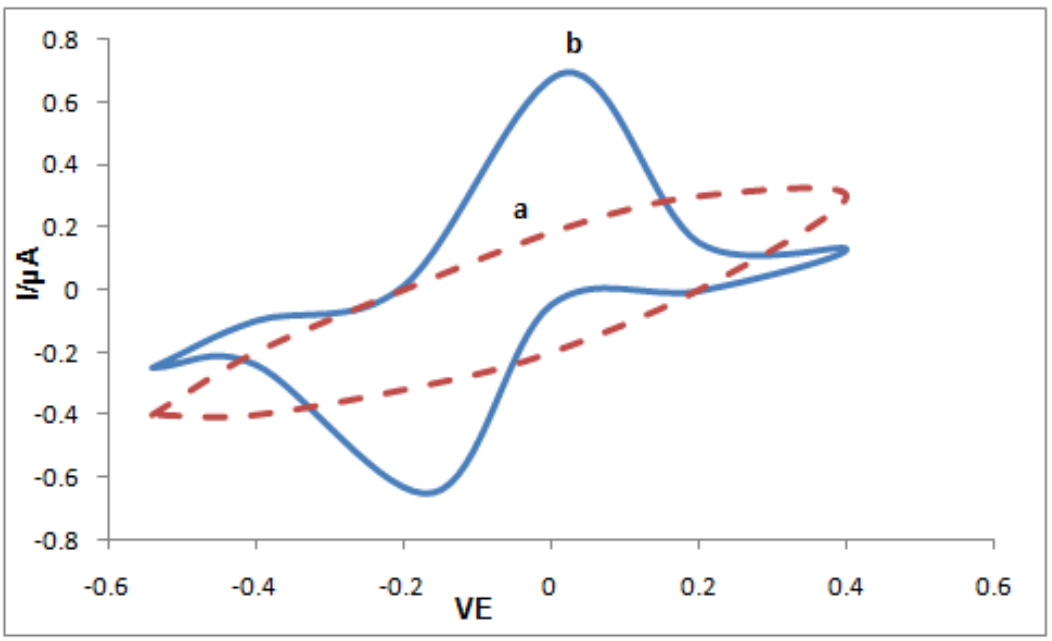


Figure 4. Cvs of Hb/CPE (a) and Hb/CuO/CPE (b) in 0.1 M PBS (pH=7) at a scan rate of 700 mV s^{-1}

Cyclic voltammogram of unmodified electrode with scan rate of 700 mV s^{-1} in 0.1 M PBS (pH =7) was obtained, as shown in Fig. 4 (a) no peaks were observed for unmodified electrode. In the next experiment we modified carbon paste electrode with copper oxide nanoparticles and hemoglobin then obtained cyclic voltammogram of it in the same conditions. As shown in Fig. 4 (b) a couple of anodic and cathodic peaks were observed in 20 and - 160 mV against (Ag / Ag Cl) electrode, respectively. The formal potential was calculated - 70 mV. This experiment showed that direct electron transfer of immobilized protein on the unmodified electrode surface was very slow, so using nanoparticles to modify the electrode played a important role in direct electron transfer of hemoglobin, and increased the redox peak currents which is a significant goal in designing the biosensor [39].

3.3. Effecs of different scan rates on cyclic voltammograms of Hemoglobin/CuO/Carbon Paste Electrode(Hb/CuO/CPE)

As shown in Fig. 5 (a) effects of various scan rates on direct electron transfer of hemoglobin were studied. In Fig. 5 (b) a linear dependence between the anodic and cathodic peak currents of hemoglobin by increasing scan rates was obsereved. The correlation coefficients were equal to 0.997 and 0.990 for cathodic and anodic peaks, respectively. It shows that the immobilization of hemoglobin was stable on the electrode surface [40].

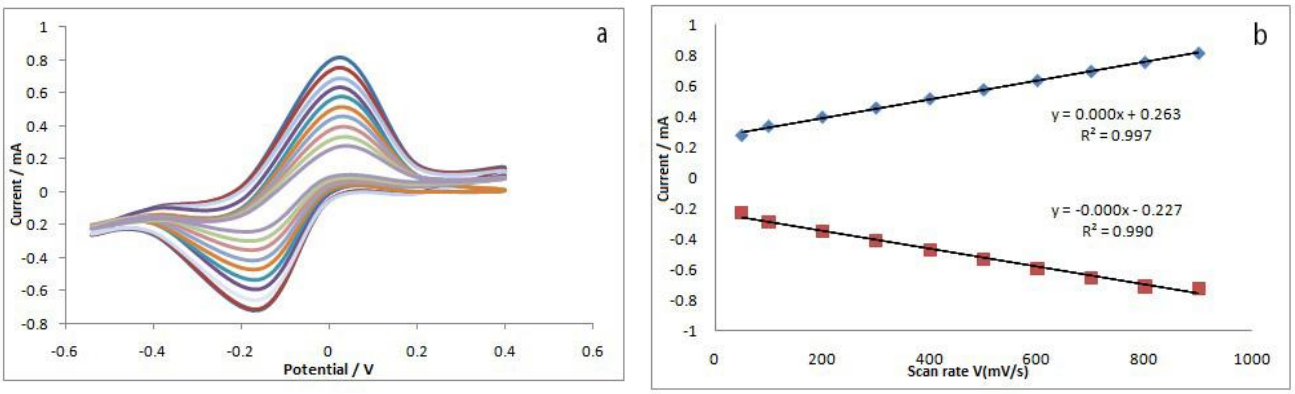


Figure 5. (a) CVs of Hb/CuO/CPE in 0.1 M PBS at different scan rates, from inner to outer: 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 mV s^{-1}**.** (b) Plots of peaks currents vs scan rates.

3.4. Effect of scan rate on the cathodic and anodic ptentials

In Fig. 6 (a), the relationship between peak potentials and natural logarithm of scan rates for Hb/CuO/CPE is shown. It was observed that in scan rates range of 0.3 to 0.9 V s^{-1}, anodic and cathodic peak potentials are changed linearly with natural logarithm of scan rates. The cathodic linear equation was y = 0.326 x + 0.820 with correlation coefficient of 0.975, and anodic linear equation was y = - 0.301 x - 0.755 with correlation coefficient of 0.984, Fig. 6 (b).

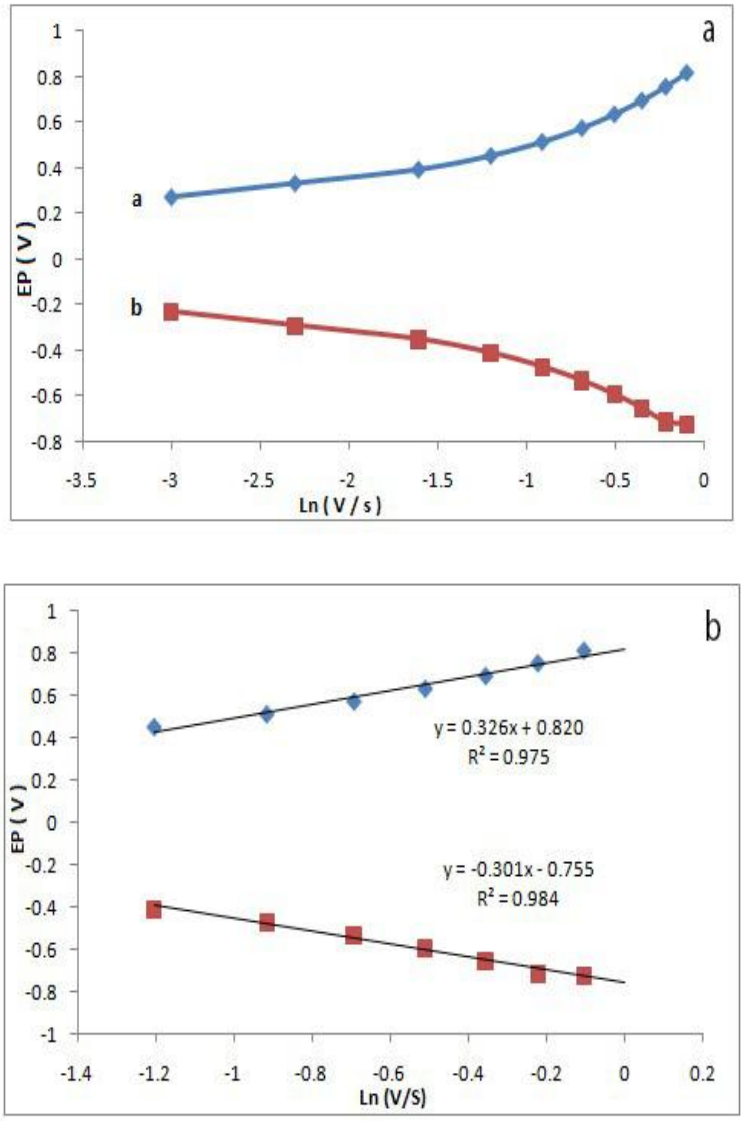


Figure 6. (a) The relationship between cathodic and anodic peaks potentials with natural logarithm of scan rates. (b) The linear dependence of the peak potentials vs natural logarithm of scan rates.

3.5. Effect of pH values on direct electron tranfer of Hb immobilized on the modified electrode

In order to prepare an efficient H_2O_2 biosensor, effects of pH values on direct electron transfer between hemoglobin and carbon paste electrode modified with copper oxide nanoparticles were studied, Fig. 7. This experiment shows that hemoglobin immobilization on the modified electrode is highly dependent on pH values. The results showed a linear dependence between formal potential of the electrode and pH values from 3 to 10. The changes in this range of pH were reversible. Any increase in the pH of the solution caused a negative shift in the potentials of the anodic and cathodic peaks.

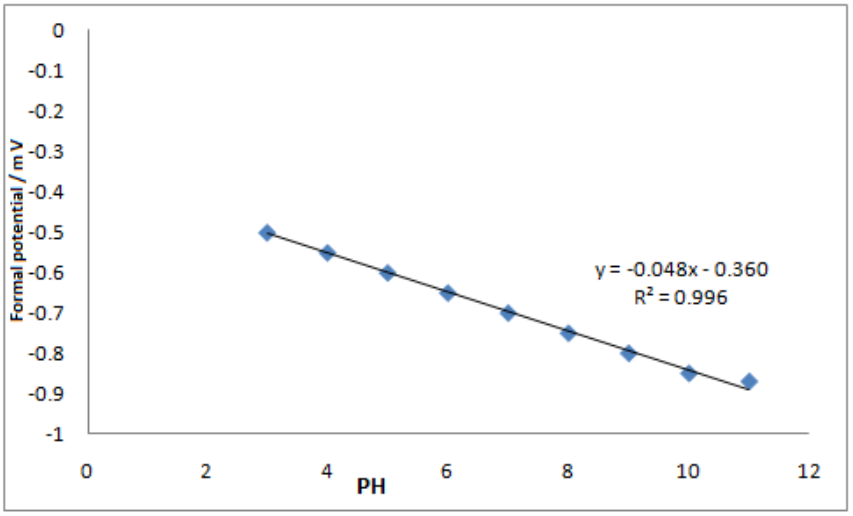
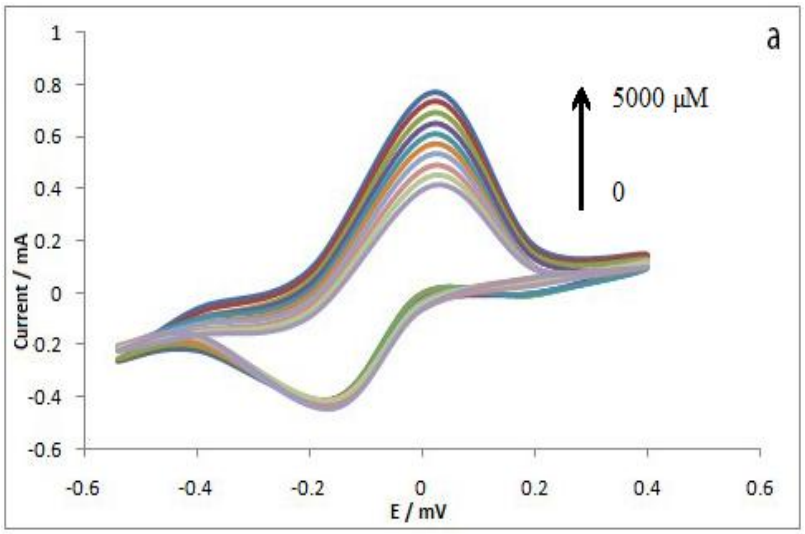


Figure 7. Effect of pH on formal potential of Hb/CuO/CPE in 0.1M PBS (pH=7) at 700 mV s^{-1} scan rate.

3.6. H_2O_2 detection based on electrocatalysis of Hemoglobin/CuO/Carbon Paste Electrode(Hb/CuO/CPE)

Hemoglobin immobilized on the carbon paste electrode modified with copper oxide nanoparticles, exhibited an acceptable electrochemical activity for H_2O_2 reduction. In Fig. 8 (a) cyclic voltammograms of the electrode in absence and presence of H_2O_2 were evaluated. When H_2O_2 was added to 0.1 M PBS (pH = 7) solution, reduction peak of the modified electrode was increased and at the same time the oxidation peak partially was decreased. In Fig. 8 (b), linear dependence of cathodic peak currents with concentration of H_2O_2 in the range of 0 - 4500 µM was showed. Detection limit of biosensor was 0.7 µM at signal to noise ratio of 3.



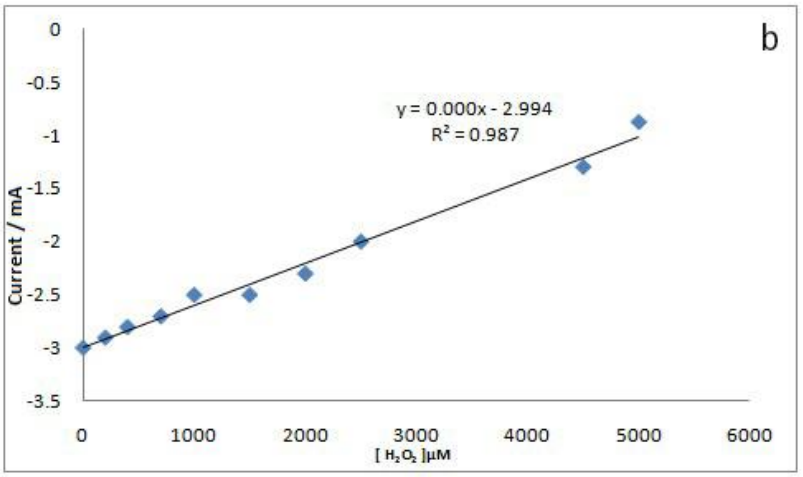


Figure 8. (a) CVs of Hb/CuO/CPE in 0.1M PBS (pH=7) at different concentrations of H_2O_2 (0 to 5000). (b) Plot of cathodic peak currents vs scan rate H_2O_2 concentrations from 0 to 4500 (at 100 mV $s^{-1)}$

The detection limit of the biosensor was compared with other H_2O_2 biosensor systems using Hb. As shown in Table 1 it is even lower. The designed biosensor exhibited higher efficiency than previous H_2O_2 sensors [47,48,49]. In this study a new biosensor for determining the concentrations of H_2O_2 in the solutions was designed.

Table 1. The biosensors based on hemoglobin for H_2O_2 detection.

Electrode material	Type of tranducer/technique	Detection limit	Reference
Magnetic chitosan microsphere/glassy carbon	Amperometric	21 µM	41
Carbonized TiO_2 nanotubes/ glassy carbon	Amperometric	0.92 µM	42
AuNPs/ZnO/Gr modified glassy carbon electrode	Amperometric	0.8 µM	43
Graphene modified carbon fiber microelectrode	Amperometric	2 µM	44
AuNPs-carbon aerogel (Au-CA)	Amperometric	2 µM	45
Chitosan-ferrocene/ grapheme/ GCE	Amperometric	3.8 µM	46

When the designed biosensor was kept in 0.1 M PBS (pH=7) at 4º C, maintained 94% of its initial activity after 25 days and exhibited acceptable stability. Biosensor degeneration due to denaturation of hemoglobin, when copper oxide nanoparticles were used for modification surface of carbon paste electrode, was much less. Furthermore in the subsequent investigations it was found that the interfering factors had less effects on activity of the designed biosensor. For determining 50 μM of H_2O_2, preparation of five electrodes showed admissible reproducibility with the relative standard deviation of 4.8%. The Hb/CuO/CPE has good renewability because the relative standard deviation of six successive determinations of 50 μM H_2O_2 was 5%. The only challenge was under control modification of the electrode by copper oxide nanoparticles.

4. CONCLUSIONS

Direct electrochemistry of hemoglobin on carbon paste electrode and a pair of reversible peaks with formal potential (E°) of - 70 mV were easily achieved. The modified electrode prepared in this study has a good ability to reduce H_2O_2. The biosensor designed in this work has high performance and is a new sample of the third generation biosensors which can be used in medicine and industry.

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