Int. J. Electrochem. Sci., 7 **(2012)** 5185 - 5194

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

A Biosensor for Determination of H_2O_2 by Use of HRP Enzyme and Modified CPE With Zno Nps

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Received: 29 April 2012 / *Accepted:* 19 May 2012 / *Published:* 1 June 2012

A novel method for the immobilization of Horseradish peroxidase (HRP) on carbon paste electrode was described. In this research, we used of zinc oxide nanoparticles as modifier electrochemical behavior of carbon paste electrode. Based on the direct electrochemistry of this study, a new biosensor for H_2O_2 ranging from 20 to 350 μM was constructed. The designed biosensor displays rapid response, expanded linear response range, and excellent repeatability. The simple and fast fabrication of the sensor makes it superior to other techniques. The mentioned biosensor also shows high selectivity towards H_2O_2, which opens up its practical applications.

Keywords: biosensor, HRP enzyme, hydrogen peroxide, Zno nanoparticles

1. INTRODUCTION

The area of electrochemical sensors continues to broaden and blend with many other topics, including some for which other fundamental reviews are being written [1]. For instance, electrochemical principles for the detection of analytes are highly relevant in microfluidics and the broader field of separation science for the purpose of injection, pumping, valving, and detection. The moving of droplets by the electrowetting effect is based on electrochemical principles. In recent years, the use of electrochemical methods in studying protein and enzyme electron transfer reaction kinetics, thermodynamics, and mechanisms directly with electrodes has gained increasing attention [2]. Having

redox enzymes directly connected to electrode made it possible to exploit the naturally high efficiency of these biological systems for electrochemical studies, biosensors, and bio-electro-analytical devices that do not use any mediators [3]. Direct electron transfer of some proteins and enzymes for biosensors has received considerable attention in recent years[4].

The studies on direct electron transfer process between proteins or enzymes and electrodes cannot only provide us the information to elucidate their metabolic processes in the biological system, but also establish a foundation for constructing the third generation of electrochemical biosensors and a new kind of bioreactors[5]. Metal and semiconductor nanoparticles have received considerable attention during recent years. They have unique chemical, electrical and optical properties due to their size-dependent characteristics and quantum-sized effect[6]. They prove to be promising for practical applications in the diverse fields such as; electronic nanodevices, molecular catalysts, multi-functional reagents and biosensors [7]. Nanoparticles are very different from their bulk materials in their electronic, optical and catalytic properties originating from their quantum-scale dimensions [8]. Zinc oxide (ZnO), a versatile semiconductor material, has been attracting attention because of the commercial demand for optoelectronic devices operating at blue and ultraviolet regions [4]. ZnO is a wurtzite-type semiconductor with band gap energy of 3.37 eV and it has very large excitation binding energy (60 meV) at room temperature [9]. Recently, special attention has been devoted to the morphology, as ZnO can form different nanostructures [6-8]. Thermal stability, irradiation resistance and flexibility to form different nanostructures are the advantages that expedite its potential wide applications in photodetectors, surface acoustic wave devices, ultravioletnanolaser, varistors, solar cells, gas sensors, biosensors, ceramics, field emission, and nanogenerator [10-12]. Carbon paste electrodes (CPEs) belong to promising electrochemical or bioelectrochemical sensors of wide applicability. Also we used of carbon paste electrode as worker electrode in this research. Here, we have investigated the electrochemical behaviour of HRP in the presence of zinc oxide nanoparticles-modified carbon paste electrode and designed a biosensor for the determination of hydrogen peroxide by immobilization of HRP onto the surface of zinc oxide nanoparticles-modified carbon paste electrode.

2. EXPERIMENTAL

2.1. Materials

Horseradish peroxidase (HRP) was purchased from sigma and other chemicals used were purchased from Merck Company. Phosphate buffer solutions (PBS, 0.2M) with various pH values were prepared by mixing stock standard solutions of Na_2HPO_4 and NaH_2PO_4 and adjusting the pH with H_3PO_4 or NaOH. All other chemicals were of analytical grade and all the solutions were prepared with doubly distilled water.

2.2. Apparatus

Cyclic voltammetric experiments were performed with a model EA-201 Electro Analyzer (chemilink systems), equipped with a personal computer was used for electrochemical measurement and treating of data. A conventional three electrode cell was employed throughout the experiments, with bare or Zno nanoparticles modified carbon paste electrode (4.0 mm diameter) as a working electrode, an Ag/AgCl as a reference electrode and a platinum electrode as a counter electrode. The phase characterization was performed by means of X-ray diffraction (XRD) using a D/Max-RA diffractometer with CuKα radiation. The experimental solutions were de-aerated using highly pure nitrogen for 30 min. and a nitrogen atmosphere was kept over the solutions during the measurements. All the electrochemical measurements were carried out in 0.1 M PBS, pH 7.0 at 25 ± 1 ^{o}C.

2.3. Preparation of Zno nanoparticles

To prepare of ZnO NPs, in a typical experiment, a 0.45M aqueous solution of zinc nitrate (Zn $(NO_3)_2 \cdot 4H_2O$) and 0.9M aqueous solution of sodium hydroxide (NaOH) were prepared in distilled water. Then, the beaker containing NaOH solution was heated at the temperature of about 55◦C. The Zn $(NO_3)_2$ solution was added drop wise (slowly for 1 h) to the above-heated solution under high-speed stirring. The beaker was sealed at this condition for 2 h. The precipitated ZnO NPs were cleaned with deionized water and ethanol then dried in air atmosphere at about 60◦C.

2.4. Preparation of Zno nanoparticles modified carbon paste electrode

The Zno nanoparticles modified carbon paste electrode was prepared by hand mixing of carbon powder, binder and 10 mg Zno nanoparticle with silicon oil in an agate mortar to produce a homogenous carbon paste. Other steps of produced modified carbon paste electrode were similar to preparation of bare carbon paste electrode. A conventional three electrode cell was employed throughout the experiments, with bare or ZnO nanoparticles modified carbon paste electrode (4.0 mm diameter) as a working electrode, an Ag/AgCl as a reference electrode and a platinum electrode as a counter electrode.

2.5. Preparation of HRP-Zno nanoparticles modified carbon paste electrode

The modified electrode that produced in previous section was used for production of HRP-Zno nanoparticles modified carbon paste electrode. In this section, the HRP was immobilized by dropping 2 μl of 10 mg/ml of the protein solution onto the Zno nanoparticles modified carbon paste electrode and dried for about 30 min. at room temperature. The electrode was then gently washed with de-ionized double distilled water and put at 4 ºC when not in use.

3. RESULTS AND DISCUSSION

3.1. X-Ray diffraction of Zno nanoparticles

The x-ray diffraction data were recorded by using Cu Kα radiation (1.5406 A^o). The intensity data were collected over a 2θ range of 20-80^o. The average grain size of the samples was estimated with the help of Scherrer equation using the diffraction intensity of (101) peak. x-ray diffraction studies confirmed that the synthesized materials were ZnO with wurtzite phase and all the diffraction peaks agreed with the reported JCPDS data and no characteristic peaks were observed other than ZnO. The mean grain size (D) of the particles was determined from the XRD line broadening measurement using Scherrer equation[13]:

$$D=0.89\lambda / (\beta Cos\theta) \qquad (1)$$

Where λ is the wavelength (Cu Kα), β is the full width at the half- maximum (FWHM) of the ZnO (101) line and θ is the diffraction angle. A definite line broadening of the diffraction peaks is an indication that the synthesized materials are in nanometer range. The lattice parameters calculated were also in agreement with the reported values. The reaction temperature greatly influences the particle morphology of as-prepared ZnO powders. Figure 1 (a &b) shows the XRD patterns of ZnO nanoparticles.

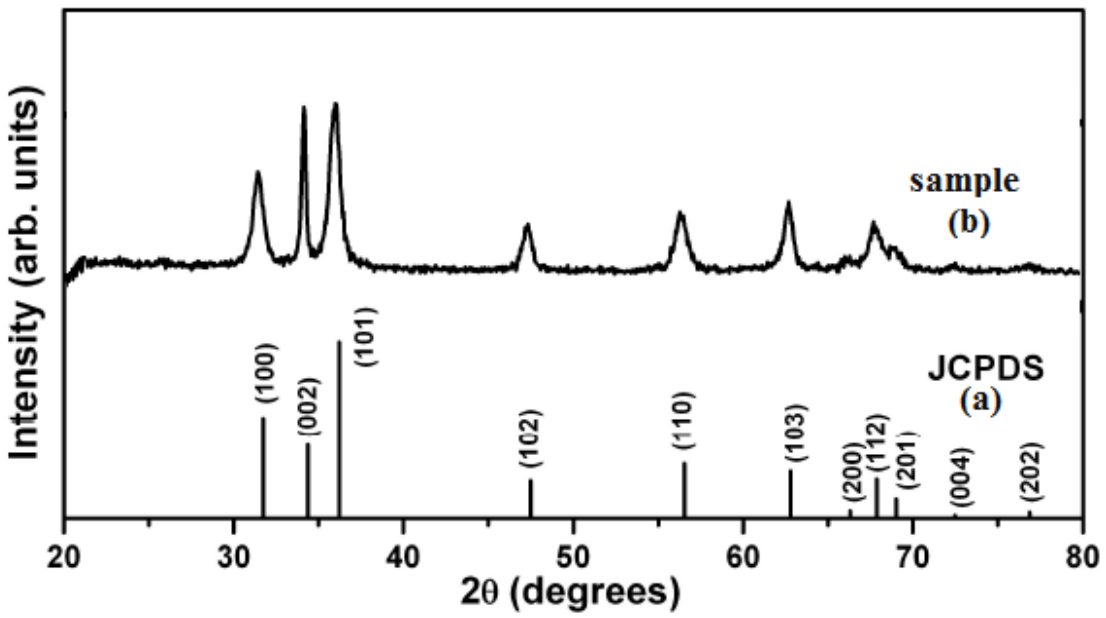

Figure 1. XRD patterns of ZnO nanoparticles. (a) Indicate standard XRD pattern and (b) indicate sample XRD pattern.

3.2. Effect of zinc oxide nanoparticles on the electron transfer from HRP to the surface of electrode

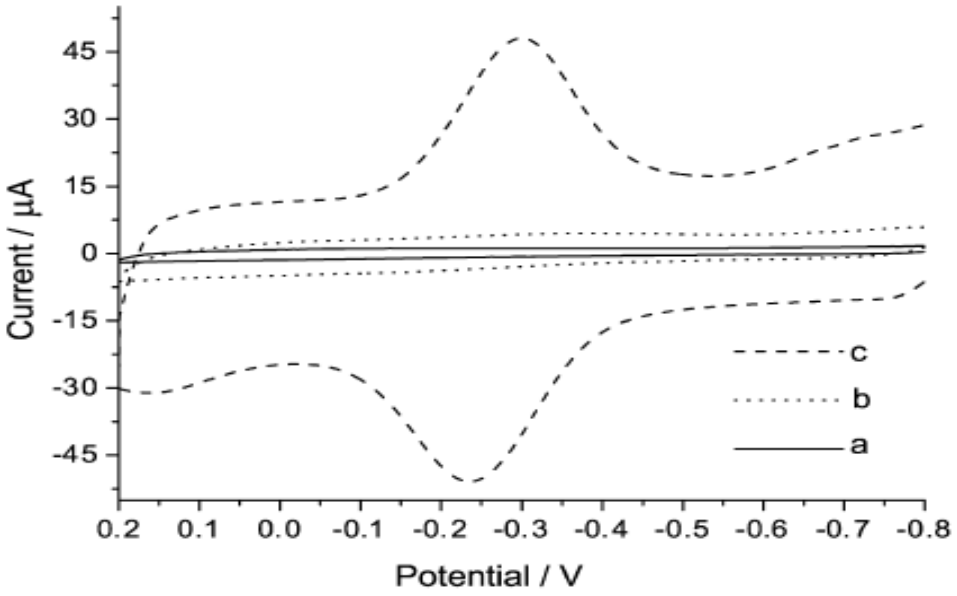

Figure 2. Cyclic voltammograms using a) the bare carbon paste electrode in 0.1 M PBS, b) zinc oxide nanoparticles modified carbon paste electrode in 0.1 M PBS and c) zinc oxide nanoparticles modified carbon paste electrode in the presence of 30 µM HRP in 0.1 M PBS (scan rate: 100 mV s^{-1}).

Figure 2 (a) shows a typical cyclic voltammogram (CV) of the bare carbon paste electrode. Figure 2 (b) shows a cyclic voltammogram of a zinc oxide nanoparticles modified carbon paste electrode in a solution of 0. 1 M phosphate buffer solution, while the Figure 2 (c) shows a cyclic voltammogram of a zinc oxide nanoparticle modified carbon paste electrode in a solution of 30 µM HRP in 0.1 M PBS buffer at pH 7.0. The cathodic and anodic peaks are at −0.300 V and −0.236 V vs. Ag/AgCl, respectively. From these findings, the formal potential was calculated as (Ec+Ea)/2 = −0.268 V vs. Ag/AgCl. As shown in the figure, we can observe fine Quasi-reversible redox peaks of HRP using the zinc oxide nanoparticles modified carbon paste electrode, while no redox peaks were observed using bare carbon paste electrode. The results show that the zinc oxide nanoparticles acts as a facilitator of electron transfer from the redox species of HRP to the electrode surface and vice versa. These results are in line with some previous works that explain the behaviour of nanoparticles as the facilitator of electron transfer [14-16].

3.3. Application of zinc oxide nanoparticles modified carbon paste electrode for the concentration determination of HRP

Figure 3 (a) demonstrates that the increased HRP concentration, to the solution, caused increased cathodic and anodic peak currents at different scan rates. Figure 3 (b) represents the relationships between both cathodic and anodic peak currents and the concentration of HRP in 0.1 M

PBS solution. The correlation coefficient was 0.9996 and 0.9995 for catodic and anodic peaks respectively and detection limit is 5μM. This observation can be used for the concentration determination of HRP in the solution.

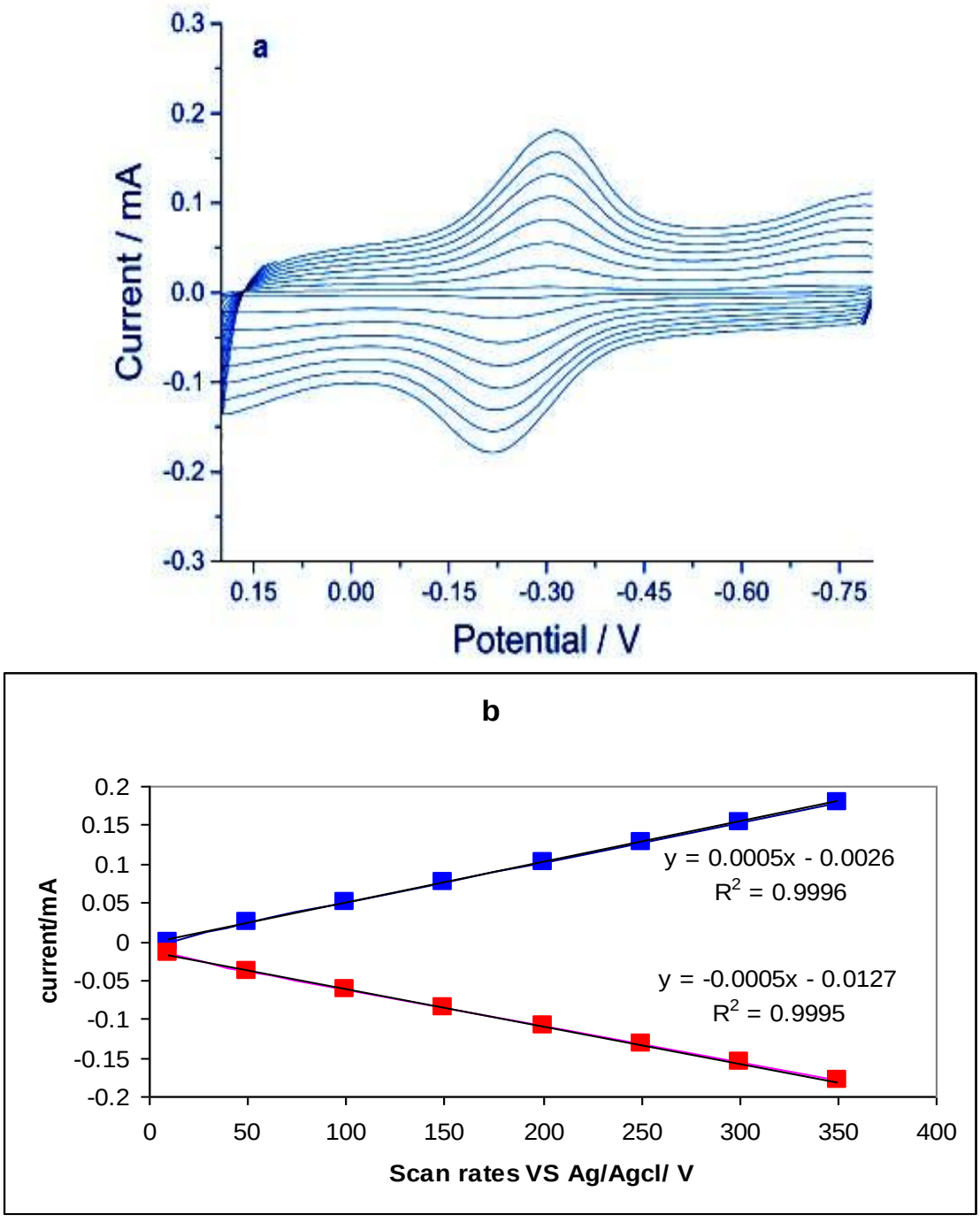

Figure 3. (a) Typical cyclic voltammograms of HRP/ZnO-NPs/ carbon paste electrode at different scan rates. The voltammograms (from inner to outer) designate scan rates of 10, 50, 100, 150, 200, 250, 300 and 350 mV s^{-1}, respectively. (b) Dependence of the anodic and cathodic peak currents on the scan rates. All the data were obtained at pH 7.0 and in 0.1M phosphate buffer solution.

3.4. Effect of pH on the formal potential of HRP- zinc oxide nanoparticles modified carbon paste electrode

Figure 4 shows the formal potential of HRP, immobilized onto the zinc oxide nanoparticles modified carbon paste electrode; in PBS has a strong dependence on the pH of solution.

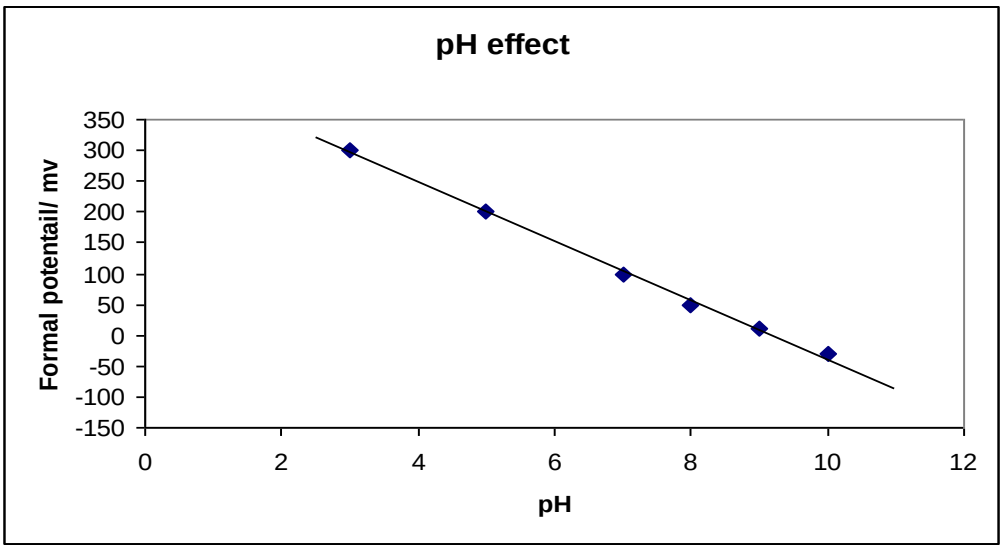

Figure 4. Effect of pH on the formal potential of HRP- zinc oxide nanoparticles modified carbon paste electrode.

All the changes in the peak potentials and currents with solution pH were reversible in the pH range from 3 to 10. An increase in the solution pH caused a negative shift in both cathodic and anodic peak potentials. Plot of the formal potential versus pH (from 3 to 10) showed a line with the slope of -47 mV pH^{-1}, which was close to the expected value of -57 mV pH^{-1} for a reversible proton-coupled single electron transfer at 291.15 K, indicating that one proton participated in the electron transfer process [17].

3.5. Application of HRP- zinc oxide nanoparticles modified carbon paste electrode for H_2O_2 concentration determination

The cyclic voltammograms of the HRP- zinc oxide nanoparticles modified carbon paste electrode in PBS, at pH 7.0, containing different concentrations of H_2O_2 are shown in Figure 5a. Upon the addition of H_2O_2 to the electrochemical cell, the reduction peak current of the immobilized HRP increased, indicating a typical electro-catalytic behavior to the reduction of H_2O_2. The electro-catalytic process could be expressed as follows [18].

$$H_2O_2 + HRP_{red} \rightarrow HRPox + H_2O \qquad (1)$$

$$HRPox + 2\ e^- \rightarrow HRP_{red}$$

The calibration curve (Figure 5b) shows the linear dependence of the cathodic peak current on the H_2O_2 concentration in the range of 20-350 μM. The relative standard deviation was 2.8% for 4 successive determinations at 35 μM and the detection limit was 8 μM. The recent experiment has introduced a new biosensor for the sensitive determination of H_2O_2 in the solution. It is reported that the cathodic reduction of H_2O_2 can be possible in acidic electrolyte on Ag electrodes at an over-voltage of about - 1.0 V [19].

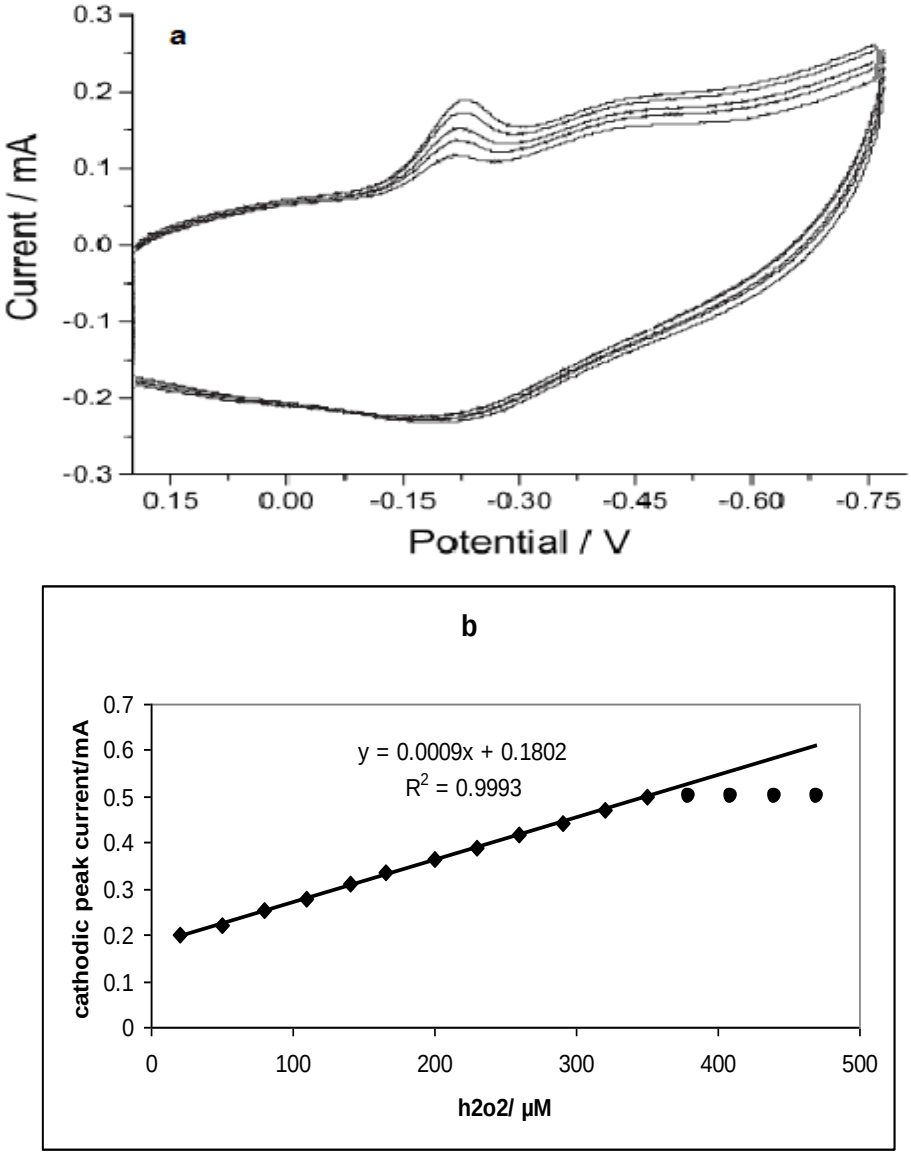

Figure 5. a) Cyclic voltammograms obtained at an HRP- zinc oxide nanoparticles modified carbon paste electrode in 0.1 M PBS (pH 7.0) for 15.0, 20.0, 25.0, 30.0, 35.0 μM concentrations of H_2O_2 and b) the relationship between cathodic peak current of HRP and different concentrations of H_2O_2 (scan rate: 100 mVs^{-1}).

Decreasing the redox potential and the use of wide pH range are the main purposes during the designing process of a sensor. So, to fulfill the above requirements, HRP-modified electrodes are being used, by different workers, for H_2O_2 determination [20, 21, 22]. The present work, zinc oxide nanoparticles became immobilized after its deposition on the surface of carbon paste electrode, without using any other immobilizer. Therefore, it is shown that the carbon paste electrode does not need any immobilizer of other materials for helping it in attaching to the surface of electrode. In comparison to the previous works, our work shows the facilitated electron-transfer of HRP to the surface of electrode and vice versa, and the preparation of the modified electrode is easier and faster than that stated in these previous studies. Moreover, graphite powder and paraffin for produce of carbon paste electrode is much cheaper and less rare than the other electrodes.

3.6. Stability of the H_2O_2 biosensor

The stability of HRP/ Zno NPs/ CPE electrode biosensor has been checked by carrying out experiments at the regular interval of a week and it has been found that HRP/ Zno NPs/ CPE electrode based optical biosensor retains its 95% activity after 21 days. The loss in the activity of biosensor is not due to the denaturation of Horseradish peroxidase but it is due to the poor adhesion of Zinc oxide Nanoparticles on the carbon paste electrode. For a result, interface materials have not high effect on operation of this biosensor. Undoubtedly, nanotechnology in combination with bioelectrochemistry can extremely influence the development rate of these scientific fields. However, a number of challenges remain to be faced, which are related to the processing of the electrode modifications in a more controlled method. The charge transport mechanism in the nanostructured biointerfaces presents a great interest, requiring further investigation. Nevertheless, the recent advances have been important for the comprehension of the nanostructured biointerfaces. As a result, it will be possible to study the modern material sciences, including bioelectronics, biocatalysis and biosensing.

4. CONCLUSIONS

In this study we investigated electrochemical behavior of HRP enzyme by using of zinc oxide nanoparticles and carbon paste electrode. Finally results of our research work lead to design a new biosensor for determination of hydrogen peroxide. The detection of hydrogen peroxide is very important in many industrial, biological and medical works and especially in food production factories. Zinc oxide nanoparticles were used as electron transfer facilitator in this work. The designed biosensor have very good stability, low detection limit and are very cheap for trade productions.

ACKNOWLEDGEMENT
We gratefully acknowledge of educational helps of Professor Dr. Saeed Rezaei-zarchi from payame Noor University, Yazd province, I.R. of IRAN.

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