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Full Length Research Paper

Direct electron transfer of hemoglobin on nickel oxide nanoparticles modified graphite electrode

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Direct electron transfer of hemoglobin, immobilized on a nickel oxide nanoparticles modified graphite electrode, was studied. Nickel oxide nanoparticles synthesized by electrochemical methods. The prepared nanoparticles were characterized by scanning electron microscope (SEM) and transmission electron microscope (TEM). The resulting electrode displayed an excellent redox behavior for the hemoglobin. The hemoglobin showed a quasi-reversible electrochemical redox behavior with a formal potential of -48±5 mV (versus Ag/AgCl) in 50 mM potassium phosphate buffer solution at pH 7.0 and temperature 25°C. The cathodic transfer coefficient was 0.45 and electron transfer rate constant was evaluated to be 1.95 s^{-1}. Furthermore, the modified electrode was used as a biosensor and exhibited a satisfactory stability and sensitivity to H_2O_2. The linear range of this biosensor for H_2O_2 determination was from 15 to 650 µM while standard deviation in 40 µM H_2O_2 concentration was 2.8% for 4 repetitions.

Key words: Electron transfer, hemoglobin, nickel oxide, nanoparticles, biosensor.

INTRODUCTION

The alteration of electrode surface with the use of nanostructure materials as a mediator, in a word nanofabrication, is advantageous for the achievement of direct electron transfer (ET) between the biomolecule and the electrode (Sanz et al., 2005). The direct ET can be difficult to attain, because the prosthetic groups of the biomolecules are burried deeply in the biomolecules (Campuzano et al., 2003). Nanomaterials are commensurate in size to proteins and the multivalent functionalization on their surfaces holds great promise for controlling the biomolecular recognition (Salimi et al., 2006).

Direct ET has extended beyond the field of bioelectrochemistry. It is a new interdisciplinary area, which combines biotechnology with electrochemical science and focuses on the structural organization and electron transfer functions of biointerfaces on electrode surfaces (Giovanelli et al., 2003). Bioelectrochemistry has proven to be both a useful way to understand the electrochemical properties and principles of biomolecules as well as a powerful method for the exploitation of these biomolecules at the biointerfaces of biosensors and bioelectronics (Dempsey et al., 2004; Gopel and Heiduschka, 1995).

Since that time, electrochemical devices have opened up new possibilities for studying the redox process of heme proteins (Gopel and Heiduschka, 1995; Turner et al., 1987). Many reports have described the electrochemistry of heme proteins in terms of modifier-electrode and modifier-protein interactions (Campuzano et al., 2003; Turner et al., 1987). Many promoters, like some small organic compounds, amino acids together with some derived molecules, small peptides and conductive

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polymers, have been found to promote the direct electrochemistry of heme proteins on the electrode surface. Recently, the matrices used for the heme proteins immobilization are a series of inorganic porous and nanomaterials (Dempsey et al., 2004; Salimi et al., 2006). The study of direct electron transfer between protein and electrode represents not only a basic feature for the application of biocatalysts in chemical sensors and other electrochemical devices (Dempsey et al., 2004; Campuzano et al., 2003), but may also provide a model for the investigation of electron transfer processes in biological systems (Salimi et al., 2006; Turner et al., 1987).

In our previous studies, we had demonstrated that the nanosilver-modified graphite electrode could be used in the electrochemical ligand binding investigations of the hemoglobin. We had also revealed that the nanosilver facilitates electron transfer between hemoglobin and the surface of graphite electrode (Rezaei-Zarchi et al., 2007).

Here, we investigate the electrochemical behavior of hemoglobin in the presence of nickel oxide nanoparticle-modified graphite electrode and the design of a biosensor for the determination of hydrogen peroxide by Hb-immobilization onto the surface of modified graphite electrode.

EXPERIMENTAL

Reagents

Hemoglobin (Hb) and finer alumina powder was purchased from Sigma, St. Louis, USA. The phosphate buffer solution (PBS) consisted of a potassium phosphate solution (KH_2PO_4 and K_2HPO_4 from Merck, 0.05 mol L^{-1} total phosphate) at pH 7.0. An acetate buffer solution (CH_3COONa and CH_3COOH from Merck, 0.10 mol L^{-1}) was freshly prepared. $Ni(NO_3)_2.H_2O$ and the other reagents were reagent grade materials from Merck. Deionized water was used to prepare all solutions and to rinse the electrodes double distilled water was used to prepare all solutions and to rinse the electrodes. Stock solutions were stored at 4°C.

Apparatus and measurements

Electrochemical measurements were carried out with a potentiostat/ galvanostat (Model 263A, EG&G, USA) using a single compartment voltammetric cell, equipped with a platinum rod auxiliary electrode, an Ag/AgCl reference electrode (Metrohm & Co., Leinfelden-Echterdingen, Germany) and a nickel oxide nanoparticle-modified graphite electrode with a disk diameter of 2.0 mm as the working electrode (Azar Electrode Co., Orumiah, Iran). A three-electrode cell was also used, employing a graphite electrode or modified-graphite electrodes, acting as the working electrodes. A platinum wire was applied as the counter electrode. All potentials were reported with respect to this reference. All experiments were performed at 25±1°C. The experimental solutions were de-aerated using high purity nitrogen for 30 min and a nitrogen atmosphere was kept over the solutions during the measurements. All the electrochemical measurements were carried out in 0.05 M PBS, pH 7.0, at 25 ± 1°C.

Scanning electron microscopic images were recorded using a ZEISS DSM 960, while atomic force microscopic studies were performed with the help of a DME (controller, Dual Scope C-21) and a scanner (DS 95-50).

Preparation of Hb/nickel oxide NP/graphite electrode

Prior to the Hb immobilization, the surfaces of graphite electrode (1 mm in diameter) and alumina slurry (1.0, 0.3 and 0.05 µm) were polished, formerly to a mirror-finish with fine emery papers and then with a polishing cloth, thoroughly rinsed with de-ionized water. The electrode was then successively sonicated in ethanol and double distilled water to remove the adsorbed particles. Then, cyclic scans were carried out in PBS (0.05 mol L^{-1}, pH 7.0) in the potential range from -0.50 to 1.0 V, until the repetitive cyclic voltammograms (CVs) were obtained. The solution, in which the nickel deposition was conducted, typically consisted of 15 ml acetate buffer (pH 4.0). The nickel was initially electrodeposited (-0.80 V, 5 min. deposition time) on a graphite electrode from a 1 m ml^{-1} nickel nitrate pH 4.0 acetate buffer solution. Afterwards, the Ni– graphite electrode was placed into a fresh PBS (pH 7.0) and electrochemically passivated with the potential cycling method (protocol for the NiO NPs/ graphite electrode) (Gopel and Heiduschka, 1995; Laviron, 1979).

The NiO NPs/ graphite electrode was placed into a fresh PBS including 5 mg ml^{-1} Hb (pH 7.0, 3 to 5°C) for 8 h. At the end, the modified electrode was washed in deionized water and placed in PBS (PH 7.0) at a refrigerator (3 to 5°C), before being employed in the electrochemical measurements as the working electrode.

RESULTS AND DISCUSSION

Figure 1a shows the SEM image of graphite electrode surface before the construction of nickel oxide nanoparticles. Figure 1b illustrates the SEM image of nanometer-scale nickel oxide particles, generated onto the graphite electrode surface in various sizes. Figures 1c and d shows the TEM images of nickel oxide nanoparticles after being scraped from the electrode surface. Because the surface-to-volume ratio increases with the decreasing size, the smaller NPs are able to play a very important role during the immobilization process.

The electron transfer of the proteins, at the bare electrodes, is very slow so that the redox peak of proteins can usually not be observed (Dempsey et al., 2004; Gopel and Heiduschka, 1995). Figure 2a shows a typical cyclic voltammogram (CV) of the NiO-NPs/ graphite electrode. Figure 2b shows a cyclic voltammogram of a Hb/NiO-NPs/graphite electrode in 50 mM phosphate buffer at pH 7.0. The Hb showed quasi-reversible electro-chemical behavior with a formal potential of -48 ± 5 mV (vs Ag/AgCl), cathodic and anodic peaks were not observed using the bare graphite electrode. This shows that NiO-NPs acts as a facilitator of electron transfer from the redox species of Hb to the electrode surface and vice versa. These results are in line with the previous work that explains the behavior of nanoparticles as the facilitators of electron transfer (Rezaei-Zarchi et al., 2007).

To further investigate the Hb characteristics at the Hb/NiO-NPs/graphite electrode, the effect of scan rates on the Hb voltammetric behavior was studied in detail. The baseline subtraction procedure, for the cyclic

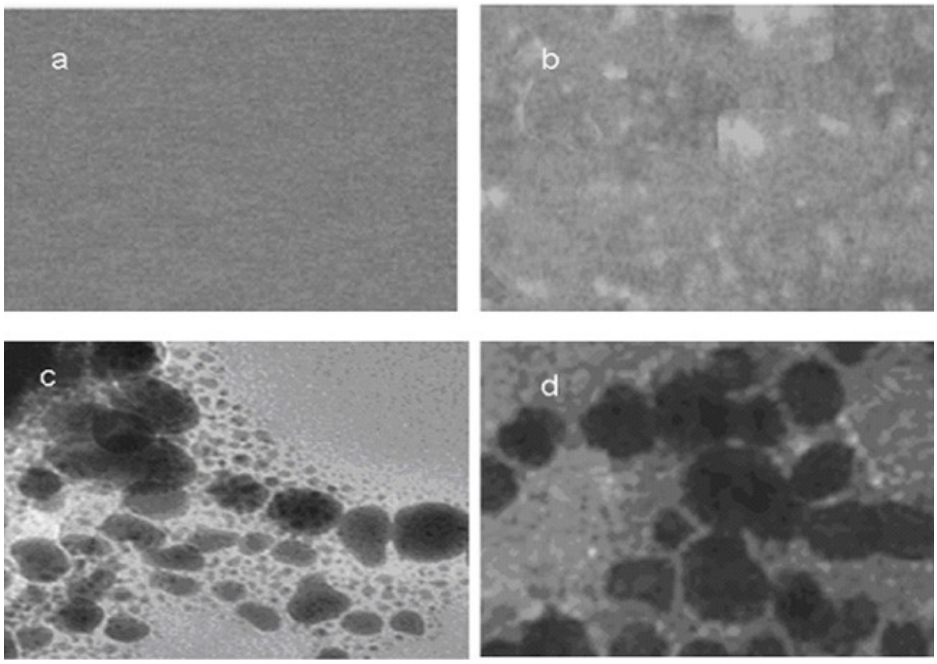

Figure 1. (a) SEM image of the graphite electrode surface before the construction of the NiO NPs. (b) The SEM image of electrodeposited NiO NPs on the graphite electrode surface, (c and d) TEM images of NiO NPs after being scraped from the electrode surface.

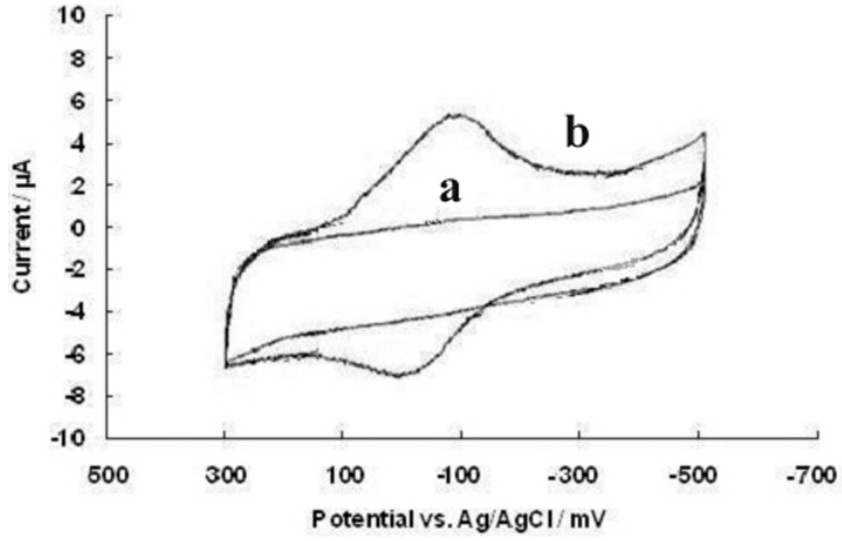

Figure 2. Cyclic voltammograms, using (a) the NiO NPs graphite electrode in 50 mM phosphate buffer and (b) Hb/ NiO -NPs/graphite electrode in 50 mM phosphate buffer (scan rate: 100 mV) s^{-1}).

voltammograms, was obtained in accordance with the method reported by Bard and Faulkner (2001). A linear dependence of anodic and cathodic peak currents on the scan rates is shown in Figures 3a and b. It can be seen that the redox peak currents increased linearly with the scan rate, the correlation coefficients were 0.993 and 0.984, respectively and high value of slopes were obtained. This phenomenon suggested that the redox process was an adsorption-controlled one and the immobilized Hb was stable and was highly efficient. Also no decrease in the peak current was observed after repeated cycles of this experiment.

These findings indicate that Hb is strongly adsorbed onto the surface of modified electrode. As could be seen,

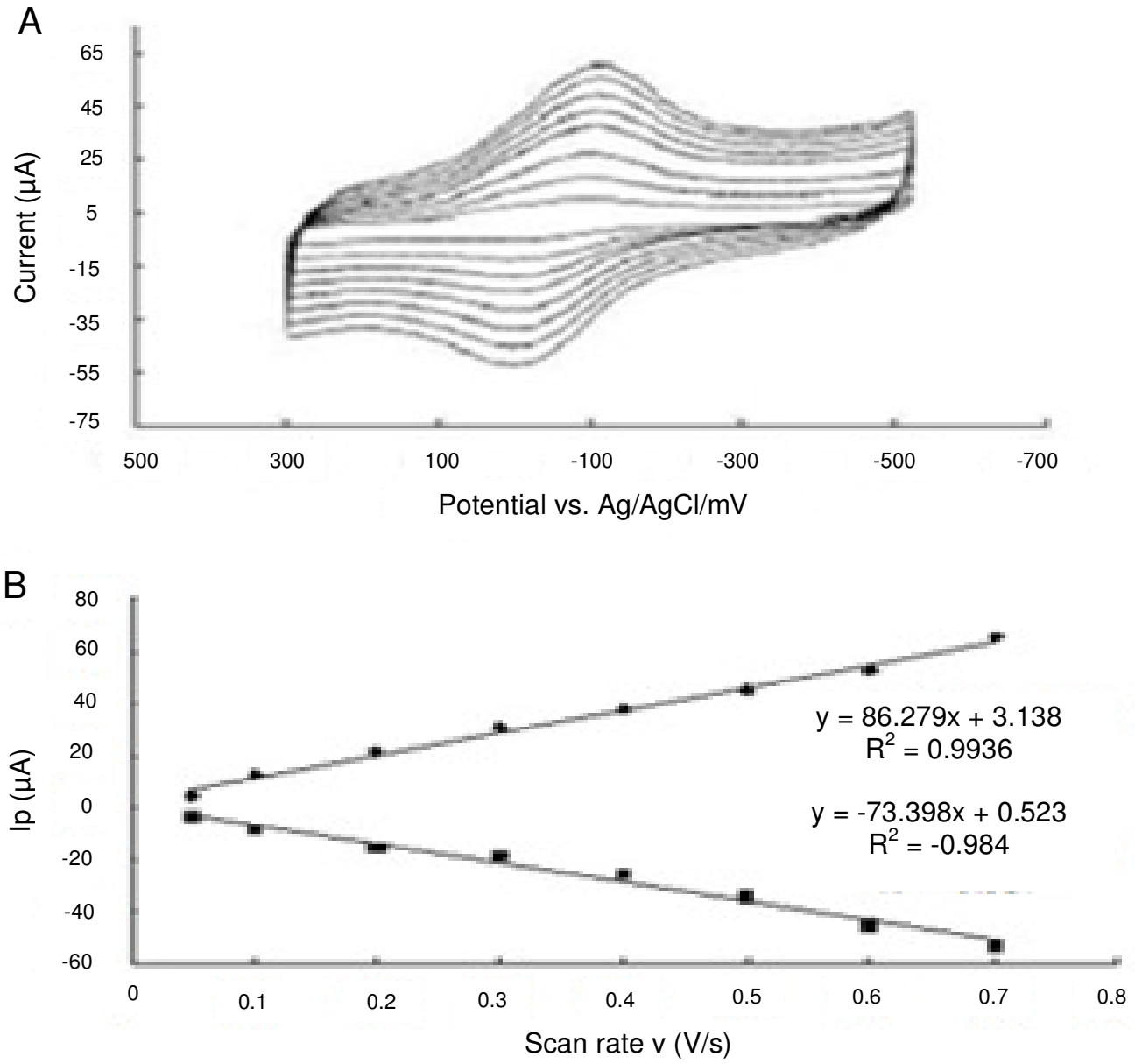

Figure 3. (a) Typical cyclic voltammograms of Hb/NiO-NPs/ graphite electrode at different scan rates. The voltammograms (from inner to outer) designate scan rates of 50, 100, 200, 300, 400, 500, 600 and 700 mV s^{-1}, respectively. (b) Dependence of the anodic and cathodic peak currents on the scan rates. All the data were obtained at pH 7.0 and in 50 mM phosphate buffer solution.

in the range from 400 to 1000 mV s^{-1}, the cathodic peak potential (*E*pc) changed linearly versus ln *v* with a linear regression equation of y Z= 0.0424x + 0.3063 , *r* = 0.957. According to the following equation (Laviron, 1979):

$$E_p = E^{0'} + \frac{RT}{\alpha n F} - \frac{RT}{\alpha n F} \ln v'$$

Where *α* is the cathodic electron transfer coefficient, *n* the number of electrons, *R*, *T* and *F* are gas, temperature and Faraday constant, respectively (*R* = 8.314 J mol^{-1} K^{-1}, *F* = 96493 C/mol, *T* = 298 K), and *αn* is calculated to be 0.45. Given 0.3 < *α* <0.7 in general (Ma et al., 2000), it could be concluded that *n* = 1 and *α* = 0.45. From the width of the peak at mid height and low scan rate, we can also obtain *n* = 1 (Ma et al., 2000). So, the redox reaction between Hb and modified graphite electrode is a single electron transfer process. In order to calculate the value of apparent heterogeneous electron transfer rate constant (*k*s), the following equation was used (Laviron, 1979):

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log(\frac{RT}{nFv}) - \alpha(1-\alpha)\frac{nF\Delta E_P}{2.3RT}$$

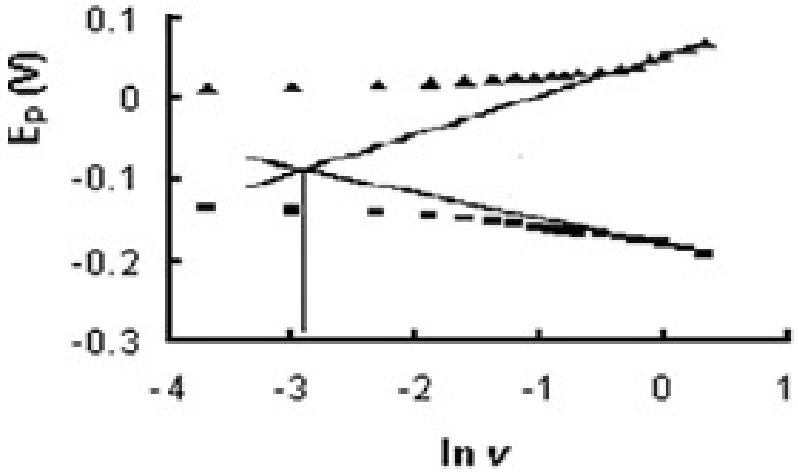

Figure 4. Relationship between the peak potential (Ep) and the natural logarithm of scan rate (ln v) for Hb/NiO-NPs/ graphite electrode in 50mM PBS (pH 7.0).

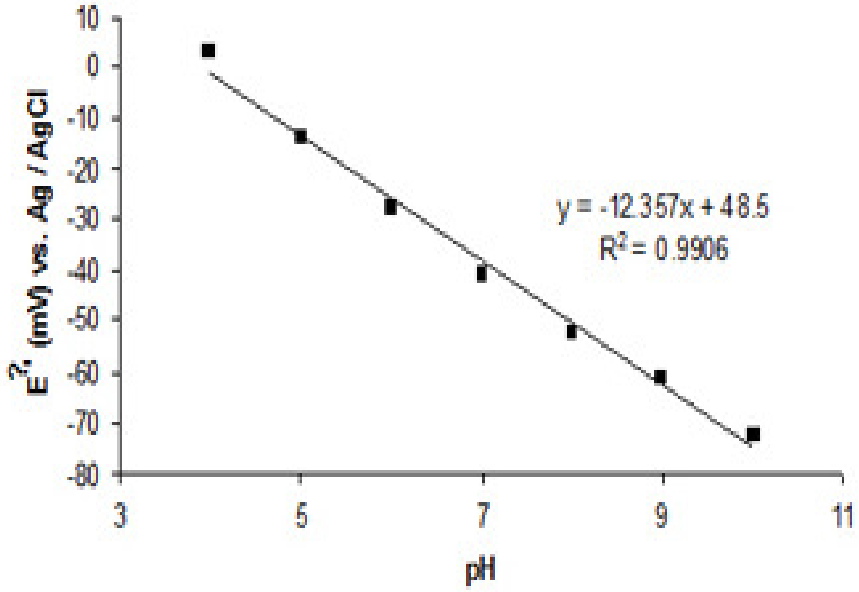

Figure 5. Effect of pH on the formal potential of Hb/NiO-NPs/graphite electrode.

According to Figure 4, in a range from 400 to 1000mV/s, the anodic peak potential (*E*pa) is also linear to ln *v* with a linear regression equation of $y = 0.0728x + 0.2411$, $r = 0.973$. Figure 4 also indicates that $nE\text{p} > 200$ mV so at cross point A, ln *v*c = ln*v*a = –4.21, and *k*s was calculated to be 1.95 s^{-1}. This value is higher than the *k*s value (1.13 s^{-1}) reported for redox proteins in DNA film at graphite electrode (Cheng et al., 2000).

Figure 5 shows the formal potential of Hb, immobilized onto the NiO-NPs/ graphite electrode, in PBS has a strong dependence on the pH of solution. All the changes in the peak potentials and currents with solution pH were reversible in the pH range from 4 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 4 to 10) showed a line with the slope of -44 mV pH-1, which was close to the expected value of -57.8 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 (Shang et al., 2003).

The cyclic voltammograms of the Hb/ NiO-NPs/ graphite electrode, in PBS, at pH 7.0, containing different concentrations of H_2O_2 are shown in Figure 6. Upon the

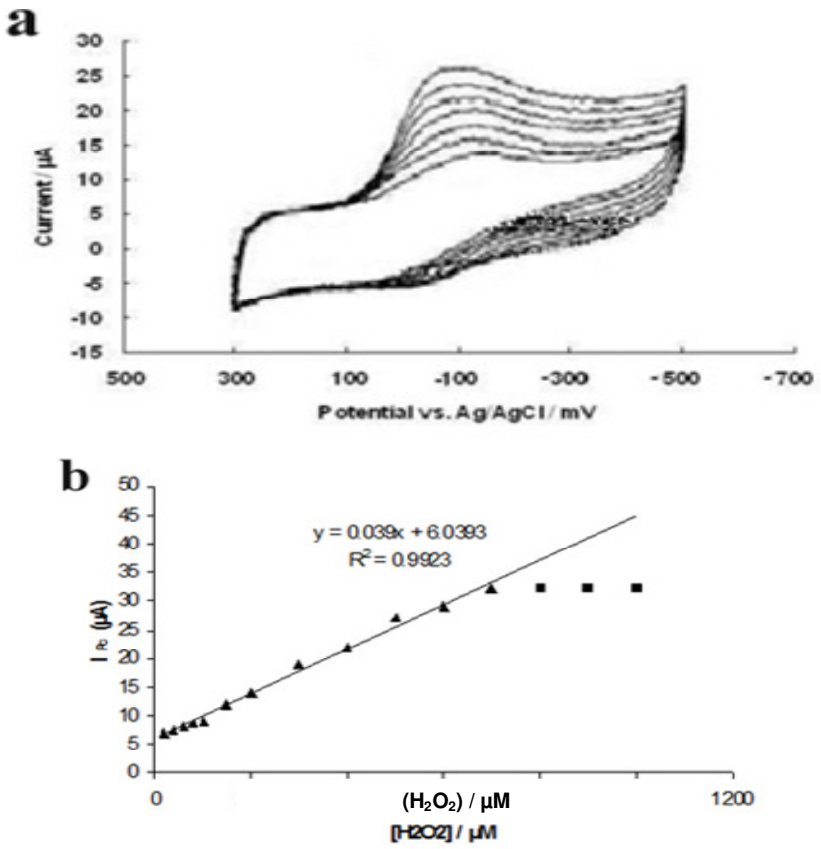

Figure 6. (a) Cyclic voltammograms obtained at an Hb/NiO-NPs/graphite electrode in 50 mM phosphate buffer solution (pH 7.0) for different concentrations of and (b) the relationship between cathodic peak current of Hb and different concentrations of H_2O_2 (scan rate: 100 mVs-)

addition of H_2O_2 to the electrochemical cell, the reduction peak current of the immobilized Hb increased, indicating a typical electro-catalytic behavior to the reduction of H_2O_2. The electro-catalytic process could be expressed as follows: (Turner, 2007) the calibration curve (Figure 6b) shows the linear dependence of the cathodic peak current on the H_2O_2 concentration in the range of 15 to 650 µM. In Figure 6 at higher concentration of H_2O_2, the cathodic peak current decreased and remains constant. This implies electrocatalytic property of electrode (Chaplin and Bucke, 1990). Thus, this experiment has introduced a new biosensor for the sensitive determination of H_2O_2 in solution.

The direct electron transfer of Hb at a bare graphite electrode is so slow that the redox peak could not be observed. The NiO-NPs significantly promotes the electron-transfer rate of Hb. It was suggested that when NiO-NPs acts as a promoter between the Hb and graphite electrode, a biocompatible microenvironment is offered. The proportionality of peak currents to sweep rate and the constancy of integration of reduction peaks at different scan rates represents the characteristics of diffusion less electro chemical behavior. When the prepared enzyme-electrode was used as a third generation biosensor, it showed a satisfactory sensitivity and reproducibility for H_2O_2.

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