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Direct electrochemistry and electrocatalytic activity of catalase immobilized onto electrodeposited nano-scale islands of nickel oxide

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Abstract

Cyclic voltammetry was used for simultaneous formation and immobilization of nickel oxide nano-scale islands and catalase on glassy carbon electrode. Electrodeposited nickel oxide may be a promising material for enzyme immobilization owing to its high biocompatibility and large surface. The catalase films assembled on nickel oxide exhibited a pair of well defined, stable and nearly reversible CV peaks at about −0.05 V vs. SCE at pH 7, characteristic of the heme Fe (III)/Fe (II) redox couple. The formal potential of catalase in nickel oxide film were linearly varied in the range 1–12 with slope of 58.426 mV/pH, indicating that the electron transfer is accompanied by single proton transportation. The electron transfer between catalase and electrode surface, (k_s) of 3.7(±0.1) s^{-1} was greatly facilitated in the microenvironment of nickel oxide film. The electrocatalytic reduction of hydrogen peroxide at glassy carbon electrode modified with nickel oxide nano-scale islands and catalase enzyme has been studied. The embedded catalase in NiO nanoparticles showed excellent electrocatalytic activity toward hydrogen peroxide reduction. Also the modified rotating disk electrode shows good analytical performance for amperometric determination of hydrogen peroxide. The resultant catalase/nickel oxide modified glassy carbon electrodes exhibited fast amperometric response (within 2 s) to hydrogen peroxide reduction (with a linear range from 1 μM to 1 mM), excellent stability, long term life and good reproducibility. The apparent Michaelis–Menten constant is calculated to be 0.96(±0.05)mM, which shows a large catalytic activity of catalase in the nickel oxide film toward hydrogen peroxide. The excellent electrochemical reversibility of redox couple, high stability, technical simplicity, lake of need for mediators and short preparations times are advantages of this electrode. Finally the activity of biosensor for nitrite reduction was also investigated.

Keywords: Catalase; Electrodeposition; Nickel oxide; Nano-scale islands; Hydrogen peroxide; Nitrite

1. Introduction

The electrical contacting of redox enzymes with electrodes is a subject of extensive research over the last decade, with important implications for developing biosensing enzyme electrodes, biofuel cells and bioelectronic systems [1,2]. Furthermore, direct electrochemistry of proteins and enzymes can provide a good model for mechanistic studies of their electron transfer activity in biological systems [3]. In addition, achieving direct electron exchange between redox proteins or enzymes and electrodes simplifies these devices by removing the requirement of chemical mediators, and thus has a great significant in preparing the third generation biosensors, enzymatic bioreactors and biomedical devices [4–6].

Catalase (Cat) (EC 1.11.1.6) is a heme protein belonging to the class of oxidoreductases with ferriprotoporphyrin-IX at the redox center. It catalyses the disproportionation of hydrogen peroxide into oxygen and water without the formation of free radicals [7]. In addition catalase is a redox enzyme that is present in all aerobic organisms [8]. Therefore, direct electrochemistry of catalase provides a model for investigating mechanisms of redox transformations between enzyme molecules in biocatalysts and metabolic processes involving electron transportation in biological systems.

It is difficult for enzymes and proteins to exchange electrons with electrodes surfaces directly, because they usually have

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large and complex structure, where the redox centers are deeply immersed in the bodies [9,10], and three dimensional structures hinder interaction with the electrode, the adsorptive denaturation of proteins onto electrodes and the unfavorable orientations at the electrode. These inhibitions can be overcome by modifying electrodes with some mediators and promoters [11,12] or incorporating enzymes and proteins into various modified films. It has been generally considered that the introduction of mediators and promoters is likely to favor the orientation of protein molecules over the electrode surface or connect the electrode communication between electron and protein molecules. Furthermore, the enzymes and proteins immobilized in films on electrode surfaces should exhibit well defined and direct electrochemical responses, retain their native structure and bioactivity and demonstrate good stability.

Many methods have been developed to immobilize catalase onto electrode surfaces to improve direct electrochemistry and enzymatic reactivity of catalase. The incorporation, direct electrochemistry and electrocatalytic activity of catalase in lipid films [13], didodecyl-dimethylammonium bromide liquid crystal film [14], modified glassy carbon paste electrodes [15], polyacrylamide hydrogel films [16], gold electrode modified with single wall carbon nanotubes [17] and polyamidoamine dendrimer [18] have been reported. Although the phenomena direct electrochemistry of catalase was obtained for modified electrodes, few reversible electrochemical behavior and low catalytic activity were observed. Hence, it is pertinent to explore and develop a new and suitable matrix for entrapment of biological molecules on electrode surfaces.

The combination of biological molecules and novel nanomaterials components is of great importance in the processes of developing new nanoscale devices for future biological, medical and electronic applications [19]. A large number of nanomaterials such as carbon nanotubes [20–22], clay nanoparticles [23] and nanometer-sized gold colloid particles [24] also have been shown to be suitable for the incorporation of enzymes and proteins. Metal oxide particles and nanoparticles such as manganeous oxide [25], zirconium oxide [26], titanium oxide [27,28], tungsten oxide [29], iridium oxide [30] and iron oxide [31] have been used successfully for immobilization and direct electrochemistry of enzymes and proteins. Due to structure stability and small size of inorganic nanoparticles, they provided a favorable microenvironment for redox proteins and enzymes to transfer electrons with underlying electrodes and their application for electrochemical catalysis of various substances. Electrochemical co-deposition of enzymes and a matrix is a convenient single step method, which is fast and well controlled [32]. In the past years the research on the synthesis of nanosized porous nickel oxide materials and its applications in catalytic industry and electrochromic devices has been rather intense [33–35]. A stable nickel hydroxide film was electrodeposited anodically at the electrode surfaces, in alkaline solution containing nickel salts [36]. However, formation of nickel hydroxide in acidic or natural solutions is not possible, and the anodic oxidation of nickel electrodes or nickel coated electrodes leads to the formation of a passive and electrochemically inert nickel oxide layer [37]. Recently our group studied the immobilization, direct electrochemistry and electrocatalytic activity of biomolecules [38,39]. In the present report nickel oxide nano-scale islands are first electrochemically deposited onto a glassy carbon electrode and then their biocompatibility for immobilization of catalase is investigated. Finally, direct voltammetry and bioelectrocatalytic activity of catalase immobilized on NiO nano-scale islands were investigated by various spectroscopic and electrochemical techniques. The ability of biosensor for electrocatalytic reduction of hydrogen peroxide and nitrite were also evaluated.

2. Experimental

2.1. Chemicals and reagents

Catalase (EC 1.11.1.6) from bovine liver was purchased from Sigma and used without further purification. 5 mg ml^{-1} catalase solutions (pH 7) were stored at 4 °C. The phosphate buffer solutions (PBS) (0.05 M) were prepared form H_3PO_4, KH_2PO_4 and K_2HPO_4 and the pH was adjusted with HCl and KOH solutions. H_2O_2 (30% w/w) was from Merck, its diluted solution was prepared daily. $Ni(NO_3)_2{\cdot}H_2O$, $NaNO_2$ and other reagents used were of analytical reagent grade. All solutions were prepared with double distillated water. Pure N_2 was passed through the solution to avoid possible oxidation during the experiments. Ultraviolet and visible (UV–Vis) absorption spectra were recorded with a Carry 1A UV–vis spectrometer (Perkin-Elmer instruments) on an ITO glass electrode. The morphologies of the surface were observed with a Vega-Tesacn electron microscope. Electrochemical experiments were performed with a computer controlled μ-Autolab modular electrochemical system (Eco Chemie Ultecht, The Netherlands), driven with GPES software (Eco Chemie). A conventional three-electrode cell was used with a Ag/AgCl (sat KCl) as reference electrode, a Pt wire as counter electrode and a glassy carbon disk as working electrode. Voltammetry on electrodes coated with catalase–nickel oxide was done in buffers containing no catalase. All experiments were carried out at ambient temperature of 20 ± 1 °C.

2.2. Preparation of nickel oxide modified glassy carbon and indium tin oxide electrodes

The glassy carbon electrode (2 mm diameter) was carefully polished with alumina on polishing cloth. The electrode was placed in ethanol and sonicated to remove adsorbed particles. Nickel was initially electrodeposited (−1.0 V, 5 min deposition time) on a glassy carbon electrode from a 1 mM nickel nitrate pH 4 acetate buffer solution. The potential was repetitively cycled (30 scans) from 1.5 to −1.0 V at scan rate 100 mV s^{-1} in fresh phosphate solution for electrodissolution and passivation of a nickel oxide layer at a GC electrode [36,37]. The effective area of the electrode modified with nickel oxide nanoscale islands was determined as 0.033 cm^2 from cyclic voltammograms of 10 mM $K_4[Fe(CN)_6]$ in buffer solution at pH 7. The same procedure was used for electrodeposition of nickel oxide nanoparticles onto an indium thin oxide(ITO) glass electrode.

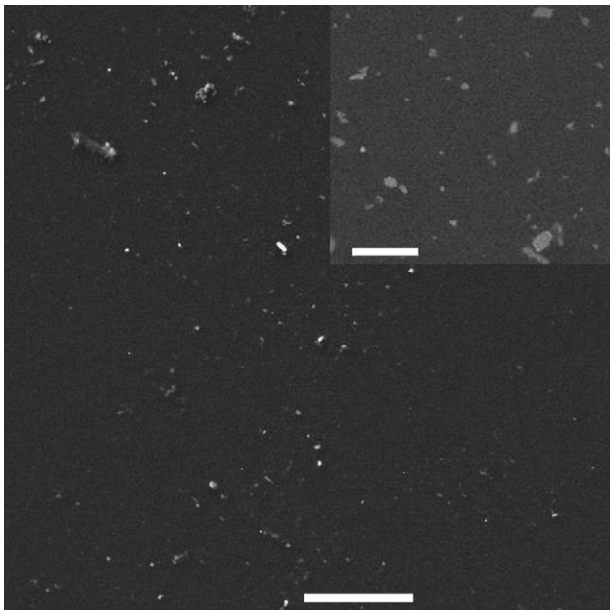

Fig. 1. SEM image of the electrodeposited nickel oxide on glassy carbon, scale bare 20 μm. Inset is the SEM image with higher magnification for the same sample, scale bar is 2 μm.

2.3. Immobilization of catalase onto a GC and ITO electrodes modified with nano-scale islands of nickel oxide

Cyclic voltammetry has been used for immobilization of catalase onto nickel oxide nanoparticles with a one step method. After the deposition of metallic nickel onto GC electrode, the electrode was immersed in fresh phosphate solution containing 5 mg ml^{-1} catalase and the potential was repetitively cycled (30 scans) from 1 to −0.5 V at scan rate 100 mV s^{-1} for electrodissolution and passivation of a nickel oxide layer and immobilization of catalase. This procedure also was employed for immobilization of catalase and nickel oxide nanoparticles on an ITO electrode. Finally, the modified electrode was removed from catalase solution, washed with double distilled water and stored in pH 7 buffer solution at refrigerator temperature (4 °C) before being used in experiments.

3. Results and discussions

3.1. Characterization of nano-scale islands of nickel oxide

In order to investigate the formation and growth of the nickel oxide particles, scanning electron microscopy (SEM) images of an electrodeposited NiO film on glassy carbon electrodes were obtained. Fig. 1 shows the SEM image for the surface of a modified electrode. As we can see low concentration of small particles are grown by electrodeposition on the amorphous glassy carbon surface. In addition to small submicron particles distributed on the surface, large agglomerated particles are also observed on the image (top left). Electron dispersion spectroscopy (EDS) analysis (not shown) proves that these particles are NiO. The inset of this figure shows SEM image with higher magnification for the same sample (the scale bar is about 2 μm). This image shows that the NiO particle size varies from under 100 nm to slightly less than 700 nm (for the large particles on the bottom right). The formation of nickel oxide layer on the electrode surface was checked by recording cyclic voltammograms of the modified electrode in alkaline solution (not shown). The cyclic voltammogram exhibits a broad oxidation peak started at 0.3 V vs. reference electrode, which can be attributed to the dissolution of nickel and formation of nickel oxide. The anodic peak current increased up to the 200th cycle and then a current plateau and stable voltammetric response is obtained. After nickel dissolution and oxide formation in alkaline solution, the anodic peak at 0.4 V is due to the oxidation of the $Ni(OH)_2$ phase to NiO(OH) while the corresponding cathodic peak at 0.35 V represents the reduction of NiO(OH) to $Ni(OH)_2$ [36].

3.2. UV–visible spectroscopic analysis

It is well known that the shape and position of the sorbent absorption bands can provide information about possible denaturation of heme proteins [40], so UV–visible spectra was employed to investigate the interaction between catalase and nickel oxide nanoparticles. Fig. 2 shows the spectra of catalase in pH 7.0 phosphate buffer solutions and Cat–nickel oxide film electrodeposited on ITO glass electrode. The catalase–nickel oxide film (Curve b) showed a soret absorption band at 402 nm similar to that of catalase in pH 6 phosphate buffer solution (Curve a), indicating no observable denaturation of catalase on the NiO film. The same absorption band has been observed for catalase incorporated in polyacrylamide hydrogel film [16].

3.3. Electrocatalytic properties and charge transfer kinetics of catalase incorporated on nano-scale islands of nickel oxide

Fig. 3 shows the cyclic voltammograms of glassy carbon electrode, glassy carbon electrode modified with catalase, glassy carbon electrode modified with nickel oxide nanoparticles and glassy carbon electrode modified with nickel oxide nano-scale islands and catalase in phosphate buffer pH 7 at scan rate 20 mV s^{-1}. Neither the bare GC electrode nor nickel oxide

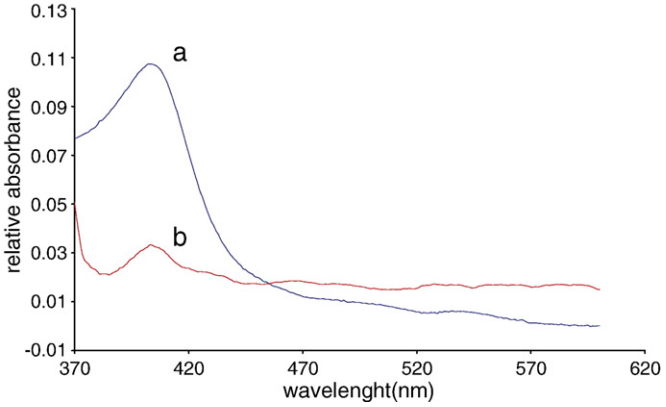

Fig. 2. UV–visible spectra of catalase in PBS (pH 7)phosphate buffer solution (curve a) and Cat–NiO film on ITO electrode(curve b).

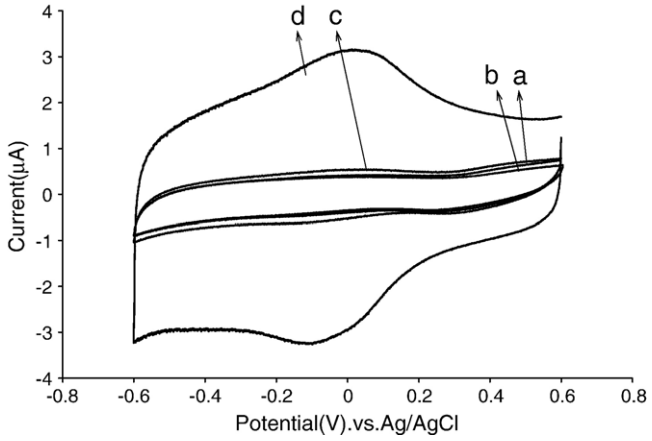

Fig. 3. CVs of glassy carbon electrode in PBS pH 7, scan rate 100 mV s^{-1} (a), glassy carbon electrode modified with catalase (b), glassy carbon electrode modified with nickel oxide nanoparticles (c), glassy carbon electrode modified with nickel oxide nanoparticles and catalase (d).

modified glassy carbon electrodes showed any electrochemical response, however, Cat/NiO modified glassy carbon electrode in PBS displayed a pair of well formed redox peaks at about (E_{pc}=−0.09 V, E_{pa}=−0.02 V) and the ratio of anodic to cathodic peak currents is about one. This indicates that catalase undergoes a quasi-reversible redox process (Fe^{III}/Fe^{II} redox couple) at the glassy carbon electrode modified with nickel oxide film. This indicated nickel oxide nano-scale islands have an effect on the kinetics of the electrode reaction for catalase, and provide a favorable microenvironment for electron transfer of the proteins with the glassy carbon electrode. The formal potential ($E^{0'}$), estimated as the mid-point of the reduction and oxidation potentials was −0.055 V. This formal potential is 200–400 mV more positive than the catalase formal potential at the surface of other modified electrodes presented in the recent works, such as; dimyristophosphatidycholine (DMPC) lipid film −0.426 V [13], polyamidoamine dendrimer −0.469 V [18], chitosan −0. 458 V [41], collagen film −0.41 V [42], poly acryl amide hydrogel film −0.459 V [16], didodecyldimethyl ammonium bromide −0.162 V [14], and gold electrode modified with single wall carbon nanotubes −0.414 V [20]. For catalase immobilized on silica sol–gel and cysteine modified gold electrode [43] the formal potential was 0.08 V, that is about 130 mV more positive than catalase immobilized on nickel oxide islands, but peak potential separation is about 194 mV in comparison to 70 mV in our study. The high surface activation of the modified electrode decreases the formal potential of catalase at the nickel oxide film in comparison to the above mentioned film electrodes presented in the literature. Potential shifts are due to the different film components, which may interact with catalase or affect the electric double layer of the electrode. To obtain the kinetic parameters of catalase at NiO film the effect of scan rate was examined in 0.1 M phosphate buffer solution in the absence of oxygen. Fig. 4 shows recorded cyclic voltammograms at different scan rates. The peak currents vs. scan rates plot shown in inset exhibits a linear relationship (R^2=0.9989). Moreover, Ep remains virtually constant within the range of scan rates studied. It is clear that catalase adsorbed onto the surface undergoes a quasi-reversible electron transfer with the nickel oxide nano-scale islands. The peak to peak separation is about 70 mV at scan rates below 100 mV s^{-1}, suggesting facile charge transfer kinetics over this range of sweep rates. On the other hand it is found that at a scan rate greater than 2 V s^{-1}, ΔEp increases by increasing the scan rate. The values of peak to peak potential separations are proportional to logarithm of the scan rate for scan rate higher than 2000 mV s^{-1} (inset of Fig. 4). The heterogenous electron transfer rate constant (k_s) of the catalase immobilized on NiO/GC electrode was estimated by the method of Laviron for a typical thin layer electrochemical system[44,45]. The transfer coefficient and heterogeneous electron transfer rate constant of catalase are about 0.45 and 3.7±0.1 s^{-1} respectively. This value shows that the electron transfer of catalase on nickel oxide film

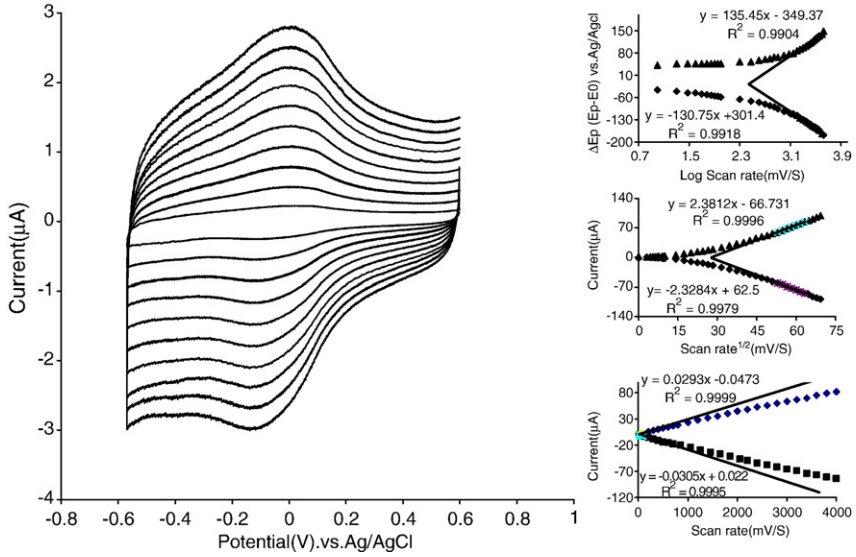

Fig. 4. CVs of Cat/NiO modified GC electrode at various scan rate in pH 7 PBS, from inner to outer, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mV s^{-1}, Insets are plots of anodic and cathodic peak currents vs. scan rate and square root of scan rate and peak potential separations vs. log (scan rates).

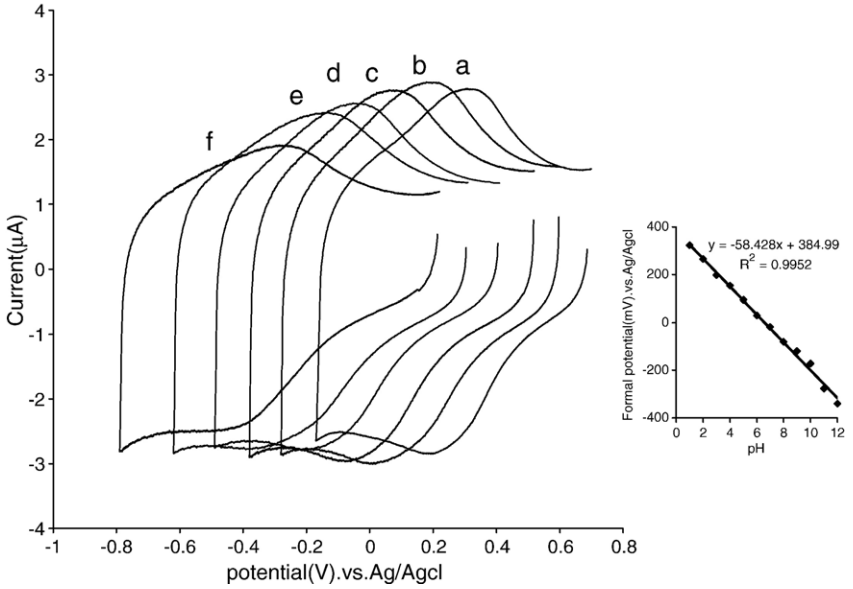

Fig. 5. CVs of Cat/NiO modified GC electrode in different pH solutions, (a) 2 (b) 4 (c) 6 (d) 8 (e) 10 and (f) 12, scan rate 100 mV s^{-1}. Inset plot of formal potential vs. pH values.

is facile. It is assumed that the nano-scale islands of nickeloxide increase the effective surface area, active point for adsorbing catalase and also make the film more porous for facilitating electron transfer. In addition, the nanostructure of nickel oxide may act as molecular wire enhancing the direct electron transfer of catalase. In fact, nickel oxide nano-scale islands could play the role of an efficient electron conducting tunnel and have a very high ratio of surface to volume [46]. According to the slope of the Ip–v curve and the following equation the surface concentration (Γ_c) of catalase on the surface of nickel oxide modified GC electrode was estimated [47].

$$\mathrm{Ip} = n^2F^2vA\Gamma_c/4RT \quad (1)$$

Where v is the sweep rate, A is the effective surface area (0.033 cm^2) of the modified electrode and the other symbols have their usual meaning. From the slope of cathodic peak currents vs. scan rate the calculated surface concentration of catalase is 1.15×10^{-10} mol cm^{-2}, indicating a sub-monolayer of catalase on the nickel oxide/GC electrode. We have investigated the influence of nickel oxide islands concentration on electrode surfaces (by increasing nickel nitrate concentration to 5 mM in Section 2.2) on the fraction of electroactive protein. Results show that with increasing nickel oxide islands on the electrode surface, the surface concentration of electroactive catalase in the islands film decreased (not shown) suggesting that only catalase in the inner layers closest to the electrode surface can exchange electron with the electrode. Furthermore, the biocatalytic activity of the biosensor for hydrogen peroxide reduction was decreased with increasing population of nickel oxide nano-scale islands on electrode surfaces.

3.4. pH effect and stability of catalase–nickel oxide modified film

The effect of pH on the potential of the catalase immobilized on nickel oxide modified glassy carbon electrode was checked in different buffer solutions(pH 2–12) in the absence of oxygen. Cyclic voltammograms of catalase–nickel oxide films showed a strong dependence on pH of buffers (Fig. 5). The peak currents decreased with increasing pH values. Both reduction and oxidation peak potentials of the Fe^{III}/Fe^{II} redox couple of catalase–nickel oxide-GC electrode shifted negatively with an increase in pH. The pH dependences of the peak potentials are expressed as follows:

$$E^{0=}-58.428\ \mathrm{pH} + 384.99\ (R^2 = 0.9956) \quad (2)$$

This slope is reasonably close to the theoretical value of −59 mV pH^{-1} at 25 °C for a one proton coupled reversible single electron transfer.

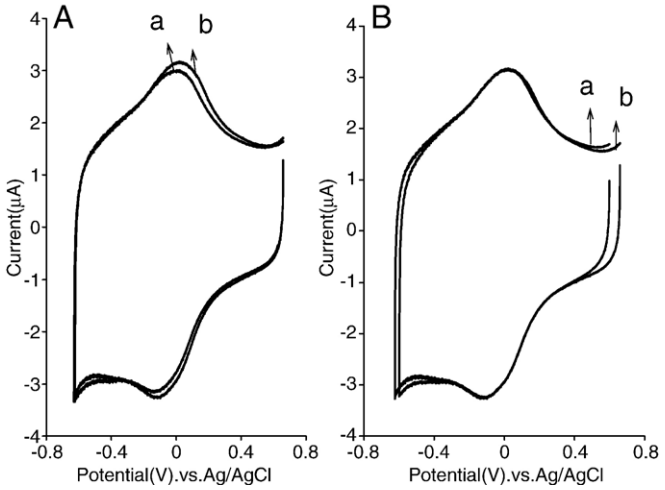

Fig. 6. (A) The 1st (a) and 200th (b) recorded cyclic voltammograms of modified catalase-NiO–GC electrode. (B) Recorded cyclic voltammograms of the fresh modified electrode (a) and after it kept at refrigerator for 10 days (b), electrolyte was PBS pH 7 and scan rate was 100 mV s^{-1}.

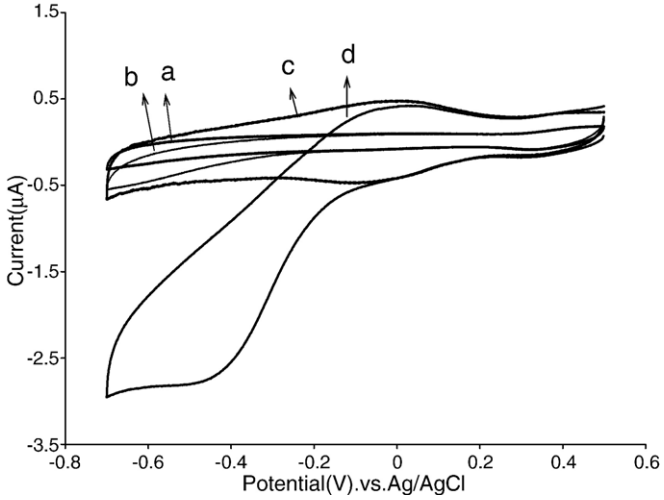

Fig. 7. CVs at 20 mV s^{-1} in pH 7 PBS for (a) GC-NiO film in buffer containing no H_2O_2 (b) as (a) in the presence 1.0 mM of H_2O_2, (c) and (d) as (a) and (b) for Cat/NiO modified glassy carbon electrode.

Long term stability is one of the most important properties for sensors, biosensors and bioreactors. The stability of Cat–nickel oxide film electrodes was investigated by cyclic voltammetry. The working stability of the modified electrode was verified by monitoring the remaining amount of active substance after successive sweeps of cyclic voltammograms. The peak height and peak potential of the surface immobilized film formed by cycling the electrode potential over the range of 0.6 to −0.6 V remained nearly unchanged and the amount of catalase remaining on the electrode surface was almost 96% of its initial value after 200 cycles in electrolyte solution with scan rate of 100 mV s^{-1} (Fig. 6A). In addition no significant decrease can be seen after replacing the electrolyte that has been used for 200 repetitive cycles with fresh solution. The stability of modified film was investigated by recording cyclic voltammogram of Cat–NiO electrode in buffer solution (pH 7), after storing it in refrigerator (4 °C) for 10 days. The CV peak potentials remain at the same positions and the reduction peak currents decreased by only about 2% (Fig. 6B). Thus, high stability of modified electrode is related to the chemical stability of nickel oxide film, the interaction between catalase and nickel oxide and strong adsorption of catalase on nickel oxide nano-scale islands. Therefore, the Cat–nickel oxide modified glassy carbon electrode, can be used as a biosensor due to its long term stability and excellent electron transfer rate constant.

3.5. Electrocatalytic reduction of H_2O_2 on Cat–NiO-GC electrode

It was reported that enzymes and proteins containing heme groups are able to reduce hydrogen peroxide electrocatalytically. In order to verify whether the catalase immobilized on nickel oxide is denatured or not, the electrochemical experiments in the presence of hydrogen peroxide was carried out. Fig. 7 shows cyclic voltammograms of modified electrode in the absence and presence of H_2O_2. As shown in Fig. 7 for bare NiO modified glassy carbon electrode, no redox response of H_2O_2 can be seen in the potential range from 0.6 to −0.7 V. However, at the Cat–NiO modified GC electrode, the reduction current of enzyme islands film was greatly increased due to catalytic reduction of hydrogen peroxide, while the oxidation peak has largely disappeared. The decreased overvoltage and increased peak current of hydrogen peroxide reduction confirm that catalase has high catalytic ability for H_2O_2 reduction. Therefore, Cat/NiO nano-scale islands are suitable for use as third generation, mediatorless biosensors. In order to evaluate the activity of catalase immobilized on NiO film, the cyclic voltammograms of the modified electrode in the presence of different concentrations of hydrogen peroxide were recorded. Fig. 8. shows the cyclic voltammograms for the reduction of hydrogen peroxide on Cat–NiO-GC electrode at different concentration range. The catalytic peak currents were proportional to the concentration of H_2O_2 with a linear range from 0.1 to 5 mM. The linear regression equation for the concentration range from

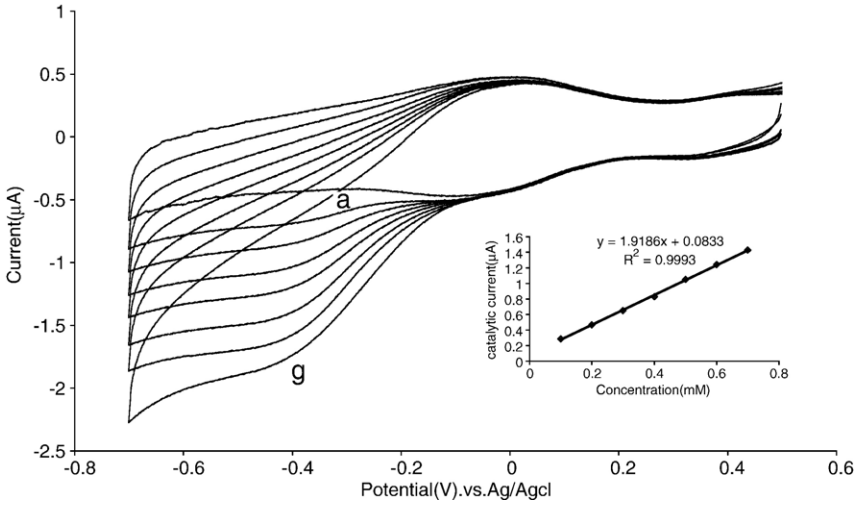

Fig. 8. CVs of Cat/NiO modified GC electrode in the presence different concentration of H_2O_2 in PBS (pH 7) at 20 mV s^{-1}, from inner to outer 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mM. Inset the catalytic response vs. hydrogen peroxide concentrations.

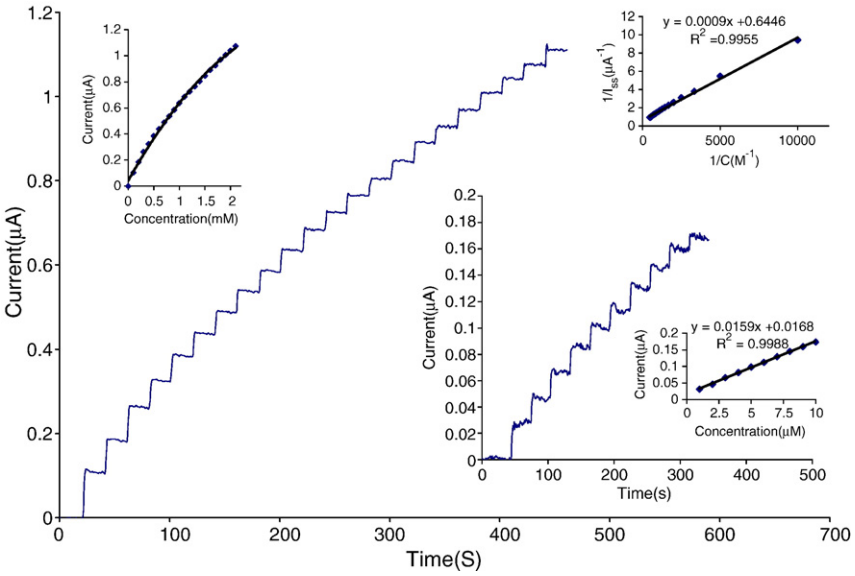

Fig. 9. Amperometric response of rotating Cat/NiO modified GC electrode to H_2O_2, conditions −0.3 V constant potential, pH 7.0 and rotation speed is 2000 rpm, (A) successive addition of 100 μM and (B) 1 μM: insets plot of chronoamperometric current vs, H_2O_2 concentration and linear calibration curve for determination of K_M.

0.1–0.8 mM was I (μA)=1.9186 C (mM)+0.0833, r=0.9993. The detection limit is estimated to be 10 μM when the signal to noise ratio is 3. At higher hydrogen peroxide concentration, the CV response showed a leveling-off tendency, including a typical Michaelis–Menten process [48]. The electrocatalytic reduction of hydrogen peroxide at Cat–NiO film glassy carbon electrode was also studied by hydrodynamic amperometry, which is one of the most widely employed techniques for biosensors.

The constant potential of the rotating modified electrode (rotation speed 2000 rpm) was set at −0.3 V vs. SCE after optimization, and the catalytic reduction current was monitored while aliquots of hydrogen peroxide were added. The stepped increase of H_2O_2 concentration in buffers caused the corresponding growth of catalytic reduction currents. As shown in Fig. 9 during the successive addition of 1 μM hydrogen peroxide, a well defined response is observed. The plot of response current vs. H_2O_2 concentration is linear over the wide concentration range from 1 μM to 1 mM. The calibration plot over the concentration range 1–10 μM (10 points) has a slope of 15.9 nA/μM (sensitivity), a correlation coefficient of 0.9988 and the detection limit of 0.60 μM. An extremely attractive feature of the Cat/NiO modified GC electrode, is its fast response time (i.e.<2 s) and its highly stable amperometric response toward hydrogen peroxide. When the concentration of hydrogen peroxide was higher than 2 mM, a response plateau was observed, showing the characteristics of the Michaelis–Menten kinetic mechanism. The apparent Michaelis–Menten constant (K_M), which gives an indication of the enzyme–substrate kinetics, can be obtained from the Lineweaver–Burk equation [49]:

$$1/I_{ss} = 1/I_{max} + K_M/I_{max}.1/C \quad (3)$$

Here, I_{ss} is the steady-state current after the addition of substrate, C is the bulk concentration of substrate and I_{max} is the maximum current measured under saturated substrate solution. K_M can be obtained by the analysis of slope and intercept of the plot of the reciprocals of the steady-state current versus H_2O_2 concentration. The Michaelis–Menten constant of the system (K_M) in this work was found to be 0.96(±0.05) mM, implying that the Cat/NiO modified glassy carbon electrode exhibits a higher affinity for hydrogen peroxide. As is well known the smaller K_M shows the higher catalytic ability. The value of K_M for Cat in this work is smaller than that obtained at horseradish peroxidase(HRP)-based H_2O_2 sensor [49]. Therefore it clearly shows that the peroxidase activity of entrapped catalase is greatly enhanced, which is comparable to or even higher than that of the native peroxidase, HRP. The stability of this H_2O_2 biosensor has been examined as well. After being exposed to air in the refrigerator for 5 days, signals decreased by less than 4% and after two weeks by less than 7%.

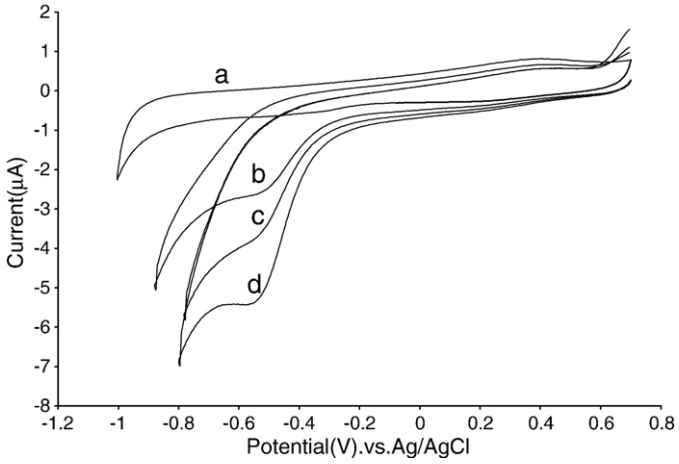

Fig. 10. CVs at 20 mV s^{-1} in pH 5.5 PBS for (a) Cat–NIO modified GC electrode in buffer containing no NO_2^- (b, c and d) as (a) in the presence 10, 20 and 30 mM of NO_2^-.

3.6. Electrocatalytic activity of Cat–NiO-GC electrode toward nitrite reduction

Shen, et al. studied electrocatalytic reduction of nitrite with heme proteins immobilized onto polyamidoamine and polyacrylamide hydrogel films [18,50]. In this study catalytic reduction of nitrite was studied at Cat–NiO nanoparticles glassy carbon electrode. As shown in Fig. 10 a new reduction peak appeared at about −0.55 V when NO_2^- was added into pH 5.5 acetate buffer, and the peak current increased with a further addition of nitrite. Noticeably, no corresponding electrochemical signal is observable either at a bare GC electrode or GC electrode modified with nickel oxide nanoparticles (free of catalase) in the same nitrite solution until −1.1 V. Therefore the catalytic process comes from the specific enzyme catalytic reaction between catalase and nitrite, which indicates a large decrease in activation energy for the reduction of nitrite in the presence of catalase.

4. Conclusion

A one step electrochemical method can be used for electrodeposition of nickel oxide nano-scale islands and immobilization of catalase onto glassy carbon electrode. The properties of catalase entrapped in NiO film was characterized by both spectroscopic and electrochemical techniques. This method is advantageous when compared to other immobilization procedures because of the short immobilization time and no need for an electron transfer mediator or specific reagent. Nickel oxide nanoparticles provide a favorable microenvironment for direct electron transfer of catalase. The immobilized catalase retained their biological activity and showed a good electrocatalytic response to hydrogen peroxide and nitrite reduction. Immobilization of catalase onto nickel oxide film could combine advantages such as high biocompatibility of nickel oxide nanoscale islands, large specific surface area for enzyme loading, promoting effect for electron transfer between electrode and catalase, and excellent biocatalytic activity toward hydrogen peroxide and nitrite reduction. Finally the new matrix is strongly recommended for immobilization of many other enzymes or proteins in fabricating third generation biosensors.

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