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Enhanced direct electron transfer of redox protein based on Multiporous SnO_2 nanofiber-Carbon nanotube nanocomposite and its application in biosensing

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Abstract

A novel third generation H_2O_2 biosensor is fabricated using multiporous SnO_2 nanofiber/Carbon nanotubes (CNTs) composite as a matrix for the immobilization of redox protein onto glassy carbon electrode. The multiporous nanofiber (MPNFs) of SnO_2 is synthesized by electrospinning technique from the tin precursor. This nanofiber shows high surface area and good electrical conductivity. The SnO_2 nanofiber/CNT composite increases the efficiency of biomolecule loading due to its high surface area. The morphology of the nanofiber has been evaluated by scanning electron microscopy (SEM). Cyclic Voltammetry and amperometry technique are employed to study and optimize the performance of the fabricated electrode. A direct electron transfer between the protein's redox centre and the glassy carbon electrode is established after fabrication of the electrode. The fabricated electrode shows excellent electrocatalytic reduction to H_2O_2.The catalysis currents increases linearly to the H_2O_2 concentration in a wide range of 1.0 10^{-6} -1.4 × 10^{-4} M and the lowest detection limit was 30 nM (S/N=3). Moreover, the biosensor showed a rapid response to H_2O_2, a good stability and reproducibility.

Key Words: Multiporous SnO_2 nanofiber; Carbon Nanotube; Redox protein; Direct electrochemistry; Electrical contact; H_2O_2 sensing

1. Introduction

Various nanomaterials have been designed and applied in electrochemical biosensors preparation since they exhibit high electrical conductivity, biocompatibility, large surface area and chemical stability, etc. [1, 2]. The use of a nanomaterial as a carrier/host to immobilize the redox enzymes is promising in order to improve the electron transfer and the stability of enzymatic biosensors. It is obvious that a single nanomaterial is improbable to simultaneously offer all of these beneficial features which have led to recent developments and applications of composite nanomaterial [4]. It is possible to significantly promote catalytic activity and improve the optical, electrical, thermal, and mechanical properties by combining two or more nanomaterials in a single entity [5-6]. Carbon nanotubes (CNTs) have attracted great interest in the field of electrochemistry because of their unique physical & chemical features including electrical conductivity, large surface area for enzyme loading, electric-capacitance, mechanical and chemical stability [7]. The ability of carbon nanotubes (CNTs) to promote electron transfer between the biomolecules and the electrode surface has been studied extensively [7, 8]. The importance of the interactions between CNTs and other redox-active metal nanoparticles has been increasingly recognized and that is an area of interest to obtain nanotube/nanoparticles hybrid materials with useful properties [9]. Although carbon nanotube is a very good conductive nanomaterial for biosensor but the hybrid structure of CNTs with other nanoparticles/nanomaterials has more potential applications for the generation of electrical, optical and sensor devices [9, 10]. Recently, SnO_2 nanomaterials have drawn much attention to researchers because of its high electron mobility in solar cells [11, 12]. Additionally, a few studies on the use of SnO_2 in dye-sensitized solar cell (DSSC) and biosensor design have been observed [13-15]. For instance, multiporous SnO_2 nanofiber (MPNF) has shown an excellent electrocatalytic performance in Hb based H_2O_2 biosensor. Since,

Multiporous SnO_2 nanofiber provided 6–8 times higher surface area than the porous nanofibers (PNFs) and nanowires (NWs), it has a great potential for the development of a biosensor [15-16].

Redox enzymes/proteins are the most widely used biomolecules used in biosensor for the detection of different analytes [17]. It is important to obtain a high electroactivity of the enzyme or protein immobilized on the electrode surface of any biosensor [17]. The direct electron transfer is difficult to obtain due to the three-dimensional structure of redox enzymes/proteins which results the inaccessibility of the electroactive centers as well as its strong adsorption onto the electrode surface for subsequent passivation [18]. At the same time, unusual alignment of biomolecules on the surface of electrode can also be one of the reasons for inhibiting electron transfer between the electrode and enzymes/proteins [19]. As a result, a special electrode modification is needed for enhancing the electrons transfer rate of redox enzymes/proteins. Hemoglobin (Hb), a redox protein, has been used as a favorable biomolecules for the development of hydrogen peroxide (H_2O_2) biosensor based on the direct electron transfer (DET) reaction [17, 20-21]. It has already proved that the Hb shows greater stability than other commercially available redox heme proteins such as horseradish peroxidase, cytochrome C, and myoglobin, hemoglobin which is ideal for biosensor applications [22].

In this paper, for the first time, we report on a H_2O_2 biosensor based on the co-immobilization of Hb/CNTs /multiporous SnO_2 nanofibers onto glassy carbon electrode by using chitosan. The multiporous SnO_2 nanofibers were synthesized by electrospinning of tin precursor. Experimental results reveal that the immobilized Hb in nanocomposite established direct electron transfer between the Hb and a GCE. The amperometric response of this biosensor exhibited the excellent electrocatalytic reduction of H_2O_2.

2. Experimental

2.1. Reagents and materials

Multi-walled carbon nanotube (MWCNTs), Tin chloride pentahydrate ($SnCl_4.5H_2O$), polyvinylpyrrolidone (PVP) & dimethylformamide (DMF) were bought from Sigma. Hemoglobin (Hb), chitosan and stock solution of 30 wt % H_2O_2 were also obtained from Sigma and used as received and stored at 4 ^oC for further use. The distilled water (18 MΩ. cm) used to prepare all the solutions in this experiment were purified with the Nanopure□ water system. All other reagents were of analytical grade. 0.01 M Phosphate buffer of pH 7.0 was prepared freshly each time before use. The pH of Phosphate buffer was adjusted by using 1.0 M hydrochloric acid (HCl) or 1.0 M sodium hydroxide (NaOH) in order to achieve desired pH.

2.2. Synthesis of multiporous SnO_2 nanofiber

Multiporous SnO_2 nanofiber was prepared according to the literature described in our previous work [11]. The synthesis process start with, a 3 g of PVP, dissolved in an equal volume ratio (1 : 1) of ethanol and DMF. Various concentrations of the tin precursor such as 5.5 mM, 7 mM, 8.5 mM, 10 mM, 11.5 mM were dissolved to this solution at room temperature until the solution became transparent. The used concentrations were labeled as C0, C1, C2, C3 and C4, respectively. The viscosity of the solution for electrospinning was measured with a rheometer (LVDV III Ultra, Brookeld Co., USA) and the solutions were then transferred to a plastic syringe with steel needles for electrospinning process, and the procedure was operated by the

following parameters : (i) applied voltage, 25 kV, (ii) the injection rate, 0.6 mL h^{-1}, (iii) the distance between the collector and the tip of the needle, 20 cm, (iv) humidity, 40–45%, and (v) 1200 rpm speed of the rotating collector. Solid nanofibers were then collected from the collector and annealed at temperature 600 °C for 3 hour at heating rate 0.5 °C min^{-1}.

2.3. Fabrication of electrode

Fabrication of electrode with multiporous SnO_2 nanofiber, CNTs and hemoglobin was executed by following protocol. A glassy carbon electrode (GCE) (3 mm of diameter) has been used as a base electrode to immobilize (MPNFs) of SnO_2, Hb and CNT. Prior to modification, the glassy carbon electrode was polished with 0.05 μm alumina slurries and was then sonicated, rinsed with water, acetone, and doubly-distilled water. Stock solution of 10 mg of (MPNFs) of SnO_2 was made in 100 μl phosphate buffer and 10 mg of CNTs was made in 100 μl phosphate buffer. The stock solution of 2 mg/ml of Hb was made in phosphate buffer (pH 7.0). Flowingly, 5 μL of (MPNFs) of SnO_2, 5 μL of CNTs, 5 μL of Hb from their stock solution and 2 μL (2 mg/ml) of chitosan were mixed and casted on the GCE surface and allowed to dry for overnight. The modified electrode was rinsed with water prior to measure the electrochemical properties. The final electrode is denoted as SnO_2-NF/CNTs/Hb/Ch/GCE electrode throughout the paper. Moreover, we have also fabricated other electrodes denoted as CNTs/Hb/Ch/GCE and SnO_2-NF/Hb/Ch/GCE electrodes for a comparison.

2.4. Surface characterization and electrochemical measurement

The morphology and composition of the synthesized multiporous SnO_2 nanofiber was characterized by scanning electron microscope (SEM) (JEOL JSM 5900 LV) at an acceleration voltage of 13 kV. Electrochemical measurements were carried out with a Potentiostat PARSTAT 2273 and a three-electrode configuration was employed in this study. The fabricated SnO_2-NF/CNTs/Hb/Ch/GCE biosensor was employed as the working electrodes. A platinum wire electrode was used as the counter electrode, and a KCl saturated Ag/AgCl electrode was used as the reference electrode. For cyclic voltammetry (CV) the working electrode was cycled in a 0.1M phosphate buffer by applying voltage in the range between 0.1 V and -0.7 versus Ag/AgCl. Amperometric measurements were carried out in a stirred cell by applying a potential of −0.4 V to the working electrode. A magnetic stirrer was used for this experiment. All electrolytes were deoxygenated by bubbling ultra-pure nitrogen at least for 15 min prior to the electrochemical measurements. All the measurements were performed at room temperature.

3. Results & Discussion

3.1. Surface characterization of multiporous SnO_2 nanofiber

Scanning electron microscopy (SEM) was conducted to characterize the morphologies of the multiporous nanofiber of SnO_2. The SEM image (Fig. 1a) represents the morphology of multiporous SnO_2 nanofiber. According to the image, the obtained multiporous SnO_2 nanofiber showed a fibrous structure in all areas with some fiber-fiber interconnections. We have already reported in our previous work about the MPNFs which were constructed with grains size of 5–15

nm [16]. And the MPNFs were smaller than the grain size of the PNFs (15–25 nm) [1]. Crystal like structure is observed in MPNFs due to the lower grain size. And relatively higher polycrystallinity of MPNFs is also a reason for that structure. The smaller grains and many channels in MPNFs are therefore the source of its high surface area which allow for a high density proteins/enzyme loading during the development of any biosensor. The elemental analysis of synthesized SnO_2 nanoporous was further done by using energy dispersive spectrometer (EDS). As shown in figure 1 b, emission peaks of Sn and O observed in the EDS spectrum represents the presence of tin and oxygen elements, confirming that the formed MPNFs of SnO_2.

3.2. Direct Electrochemistry of the modified electrodes

Fig. 2 illustrates the cyclic voltammograms (CVs) of the (i) bare, (ii) Hb/CNT/Ch/GCE, (iii) SnO_2-NF/Hb/Ch/GCE and (iv) SnO_2-NF/CNTs/Hb/Ch/GCE electrodes recorded in a 0.1M phosphate buffer (pH 7.0) at a scan rate of 60 mV/s. As expected, neither oxidation nor reduction peak is observed in the CV of the bare electrode. In contrast, a small pair of oxidation peak appears in the CV of the CNTs/Hb/Ch/GCE electrode and in the CV of the SnO_2-NF/Hb/Ch/GCE. The result indicates that the presence of CNT or SnO_2 increases the electrical activity of Hb present in the electrode. However, a well-defined larger redox peak appears in the CV of the SnO_2-NF/CNTs/Hb/Ch/GCE electrode. The anodic and cathodic peak potentials are located at -0.3 V and -0.365 V respectively. Both the anodic and cathodic current is higher than the SnO_2-NF/Hb/Ch/GCE electrode. The formal potential $E_n \mid E_n = (E_{pa} + E_{pc})/2$ was calculated to be -0.325 V.

We further studied the effect of the scan rates on the response of SnO_2-NF/CNTs/Hb/Ch/GCE electrode. Our results exhibit (Fig 3a) the linear enhancement of both the oxidation and reduction peak current with the increase of scan rates from 50 to 140 mV/s. The peak-to-peak separation also increases evenly with the increases of scan rates as shown in fig.3b. All the results established that the redox peak arises from the electrochemical reaction of the immobilized Hb and direct electron transfer occurs between the immobilized Hb and the SnO_2-NF/CNTs/Ch/GCE electrode. These results also suggests that all the electroactive heme proteins at the SnO_2-NF/CNTs/Hb/Ch/GCE electrode had been reduced on the forward cathodic scan [21] and the SnO_2/CNTs composite plays the key role in enhancing the electron transfer. The nature of redox process in the electrode was surface controlled process [24]. Also the SnO_2/CNTs provide a good matrix for immobilization of redox protein and biosensor fabrication.

3.3. Electrochemical response of the SnO_2-NF/CNTs/Hb/Ch/GCE biosensor to H_2O_2

Fig. 4 shows the typical CVs of the SnO_2-NF/CNTs/Hb/Ch/GCE electrode recorded in the absence and presence of 1.0×10^{-5} M H_2O_2 in 0.1M Phosphate buffer at pH 7.0 at a scan rate of 50 mV/s. The SnO_2-NF/CNTs/Hb/Ch/GCE electrode gives its diffusional redox wave in the absent of H_2O_2 in electrolyte (solid line), whereas, with the addition of hydrogen peroxide, a sharp enhancement of cathodic current is appeared with a simultaneous decrease in the corresponding anodic peak. And this change indicates that the enhanced reduction current in the presence of H_2O_2 is due to the presence of heme redox active center of immobilized Hb on SnO_2/CNTs/Ch composite [25]. The reaction of redox group of Hb with H_2O_2 can be illustrated with the following possible reaction mechanism [23]:

$$\text{Hb Fe(III)} + H_2O_2 \rightarrow \text{Compound I Fe}\{(IV)=O)\} + H_2O \quad \text{..... (2)}$$

$$\text{Compound I } (Fe^{4+}=O) + e^- + H^+ \rightarrow \text{Compound II} \quad \text{..... (3)}$$

$$\text{Compound II} + e^- + H^+ \rightarrow \text{HB Fe(III)} + H_2O \quad \text{..... (4)}$$

The oxidation form of the heme group (Hb Fe(III)) of Hb in the propinquity of the electrode is increased in the presence of H_2O_2 in electrolyte. That results the enhancement of reduction current and the decrease of the oxidation current

3.4. Optimization of experimental parameters

The impact of the applied electrode potential and pH of the phosphate buffer on the amperometric response of the SnO_2-NF/CNTs/Hb/Ch/GCE electrode to H_2O_2 was investigated. Fig. 5a exhibits the influence of applied potential on the current response to 1.5×10^{-5} M H_2O_2 in 0.1M Phosphate buffer saline at pH 7.0 on the applied potential varied from 0.1 to -0.5V. The reduction current increased when the applied potential was decreased from 0 to −0.4 V. Further decreasing the applied potential from -0.4V, the amperometric response started to decrease. The maximum reduction current was obtained at -0.4V and therefore, the potential −0.4 V was chosen as the working potential for this biosensor.

It is known that the proper pH value of electrolytes is also essential to achieve a high performance of the biosensor. The effect of pH on the amperometric response of the SnO_2-NF/CNTs/Hb/Ch/GCE electrode was investigated in the presence of 1.5×10^{-5} M H_2O_2 in 0.1M

Phosphate buffer while the pH was varied from 4.0 to 10.0. Fig. 5b shows the current increases with the increasing of the pH from 4.0 to 7.0. However, a decrease of reduction current is noticed when the pH is further increased from 8.0 to 10.0. The current response is very low at the lower pH values (<4.0) of electrolytes and the reason might be the denaturation of biomolecules [26]. The maximum reduction current was achieved at a pH of 7.0. Therefore, the pH 7.0 was chosen as the optimal pH value of electrolyte, which agrees with the concept that the pH range of 6.0–7.5 provides the optimum conditions for enzymatic mediated electrochemical reactions [27].

3.5. Amperometric response and stability of the biosensor

The amperometric response of the SnO_2-NF/CNTs/Hb/Ch/GCE electrode has been investigated by successively increasing the H_2O_2 concentration in a stirred cell containing 0.1M phosphate buffer under the optimized pH and potential in order to further study the performance of the electrode. Fig. 6a shows the typical amperometric response of the SnO_2-NF/CNTs/Hb/Ch/GCE electrode for successive additions of H_2O_2. The corresponding calibration plot for the amperometric response curve is shown in Fig. 6b. The response time for this fabricated electrode was very fast, reaching 95% of its maximum response in about 5 s. The biosensor shows a linear response ranging from 1 μM to 140 μM concentrations of H_2O_2 with a correlation coefficient of 0.996. The detection limit was determined to be 30 nM (based on S/N=3).

3.6. Stability and repeatability of the biosensor

The long-term stability of this fabricated biosensor was investigated by storing the electrode in phosphate buffer (pH 7.0) at 4^0 C for 35 days. The electrode retained 90% of its initial response. The repeatability of the biosensor for current response was also studied and the relative standard

deviation (R.S.D.) was 3.5% for 10 successive assays using the same enzyme electrode. The performance of this fabricated biosensor was compared with other recent H_2O_2 biosensors (Table 1). These results indicate that the proposed biosensor offers good stability and acceptable reproducibility with low detection limit.

4. Conclusions

In summary, we have demonstrated a sensitive and selective H_2O_2 biosensor with multiporous SnO_2 nanofiber, CNTs and Hb immobilized onto glassy carbon electrode. The morphological characteristics of synthesized SnO_2 nanofiber were studied firstly with the SEM observation. The unique properties of Hb/SnO_2/CNTs composite facilitate the direct electron transfer of Hb and the fabricated electrode. The amperometric response of the biosensor has long range linearity to the concentration of H_2O_2. Additionally the developed biosensor exhibits fast response and low detection limit. The designed biosensor also showed high sensitivity, long-time stability and repeatability. Overall performance of the fabricated biosensor suggests that the biosensor can be favorable in sensitive detection of H_2O_2 in industry level and also the multiporous SnO_2 nanofiber can be a potential nanomaterial for fabricating biosensor incorporation of other nanomaterials.

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Table and Figures Captions

Table 1 :Comparison of the performance of different H_2O_2 biosensors,

Fig. 1. a) SEM image of the MPNFs of SnO_2 b) EDS spectrum.

Fig. 2. Cyclic voltammograms response of (i) bare GCE electrode; (ii) CNTs/Hb/Ch/GCE electrode; (iii) SnO_2-NF/Hb/Ch/GCE electrode and (iv) SnO_2-NF/CNTs/Hb/Ch/GCE electrode in 0.1 M phosphate buffer at pH 7.0 at a scan rate of 60 mV/s.

Fig. 3. Cyclic voltammograms response of resulting SnO_2-NF/CNTs/Hb/Ch/GCE electrode at scan rate of 50, 60, 80, 90, 100, 110, 120 and 140 mV/s (from inside to outside) in 0.1 M phosphate buffer at pH 7.0.

Fig. 4. Cyclic voltammograms response of the SnO_2-NF/CNTs/Hb/Ch/GCE electrode in the absence and presence (dashed line) of 1.0×10^{-5} M H_2O_2 in 0.1M phosphate buffer at pH 7.0 at a scan rate of 50 mV/s.

Fig. 5. (a) Dependence of catalytic current of the SnO_2-NF/CNTs/Hb/Ch/GCE electrode at different applied potentials measured in phosphate buffer at pH 7.0 containing 1.5×10^{-5} M H_2O_2. (b) influence of pH on catalytic current of the SnO_2-NF/CNTs/Hb/Ch/GCE electrode in phosphate buffer containing 1.5×10^{-5} M H_2O_2 at potential of -0.4 V.

Fig. 6. (a) Amperometric response of SnO_2-NF/CNTs/Hb/Ch/GCE electrode with total addition of 2.0×10^{-4} M of H_2O_2 in phosphate buffer at pH 7.0 at potential of –0.4 V. (b) Calibration plots for the proposed SnO_2-NF/CNTs/Hb/Ch/GCE electrode.

Table 1

Comparison of the performance of different H_2O_2 biosensors (HRP = Horseradish peroxidase; Hb = Hemoglobin; LOD = Limit of detection)

Electrode	LOD	stability	references
Hb in CNTs with Pd@Fe3O4	63 nM	83% (14 days)	[28]
Hb in CNTs with Silver sulphde nanosphere	40 uM	87% (100 cycles)	[29]
Hb in CNTs with collagen	910 nM	90% (21 days)	[30]
Hb in CNTs with PSMA-*g*-3ABA	320 nM	90% (14 days)	[31]
HRP in CNTs with Co3O4 nanoparticles	740 nM	90% (8 days)	[32]
HRP in CNTs with sol-gel film	14000 nM	95% (60 days)	[33]
HRP in CNTs with PVB	167 nM	91.6% (50 days)	[34]
HRP in CNTs with modified carbon fiber & ionic liquid	130 nM	Not specified	[35]
HRP in screen printed CNTs	850 nM	95% (30 days)	[36]
Proposed biosensor	30 nM	90% (35 days)	This work

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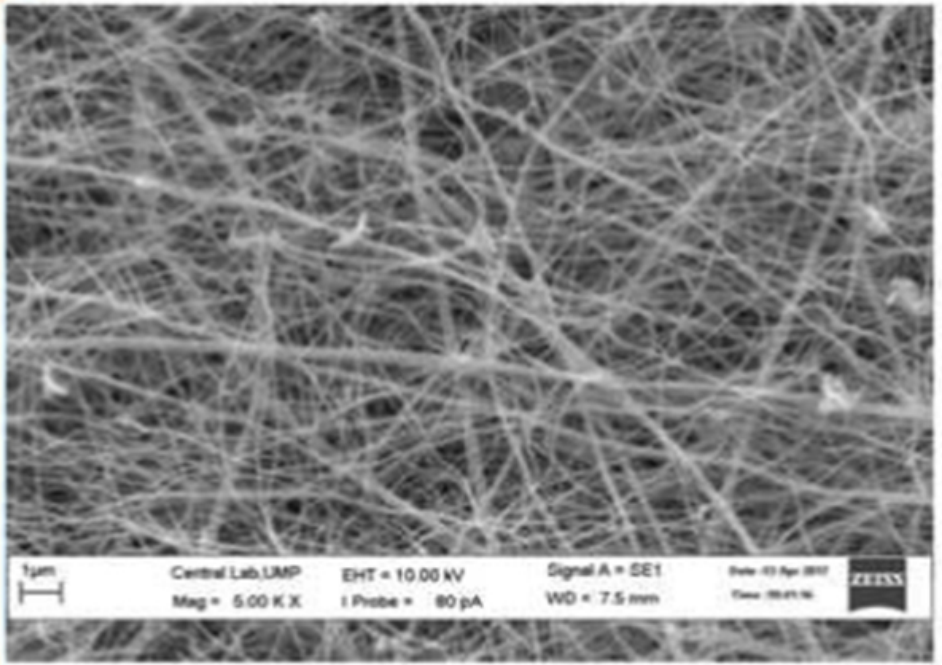
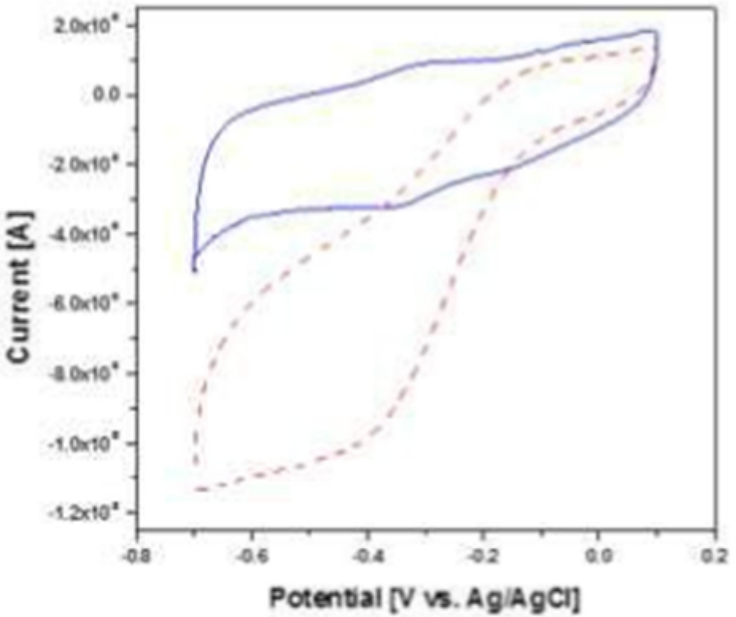
Highlight of the work

- We have used our newly fabricated Multiporous SnO_2 with Carbon Nanotube to prepare a new type of matrix to immobilize redox protein.

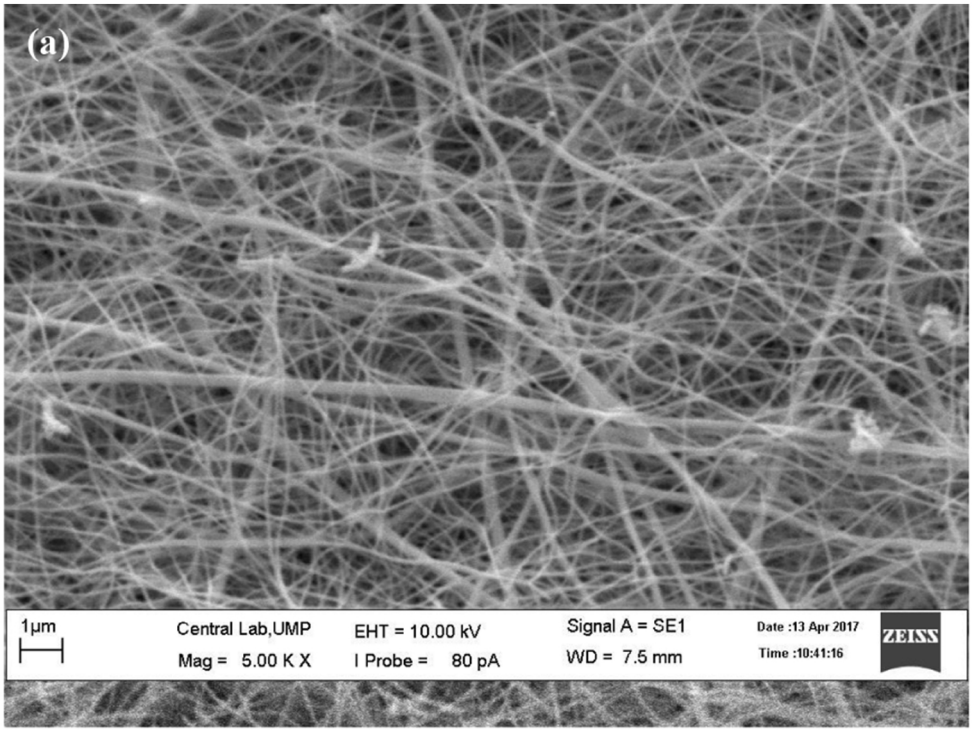
- Our electrochemical results reveal that the biocompatible characteristics of SnO_2/CNT composite furnish a favorable microenvironment for sustaining the activities of immobilized Hb, and enables direct and rapid electron transfer between the proteins and electrode.

- The fabricated electrode has a high electrocatalytic property for sensing hydrogen peroxide (H_2O_2). A longer linear range, low LOD and very quick response time in the sensing are really noteworthy. The key innovation is that this leads to a general new concept in sensing and that the matrices that are produced are made cheaply and reliably in a short time. We believe that the paper opens up new avenues in the field of enzyme based biosensors/bioelectronics.





Graphics Abstract


(a)
1µm
Central Lab,UMP
Mag = 5.00 K X
EHT = 10.00 kV
I Probe = 80 pA
Signal A = SE1
WD = 7.5 mm
Date :13 Apr 2017
Time :10:41:16
ZEISS

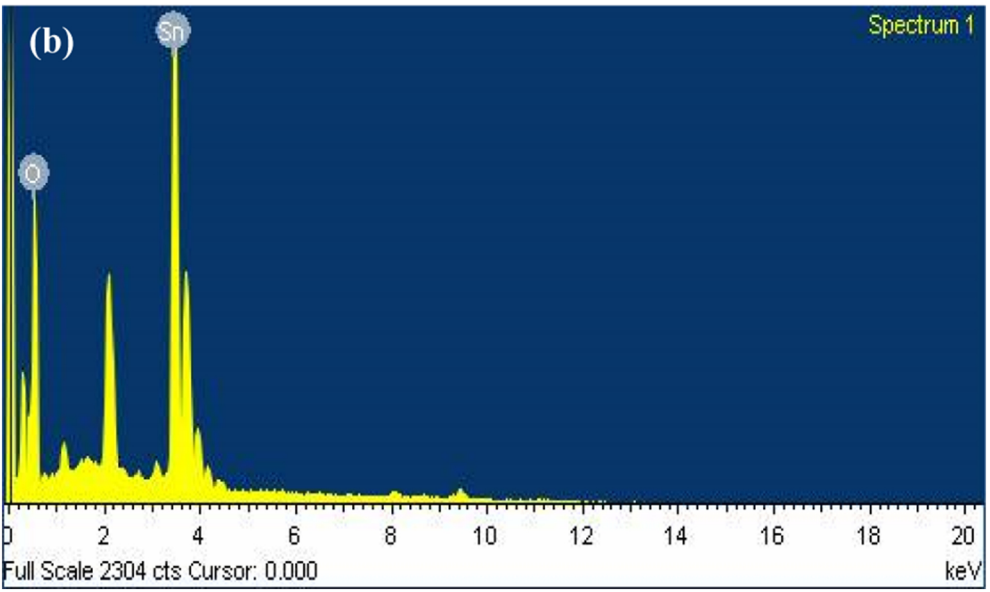

(b)
Spectrum 1
Sn
O
0
2
4
6
8
10
12
14
16
18
20
Full Scale 2304 cts Cursor: 0.000
keV

Figure 1

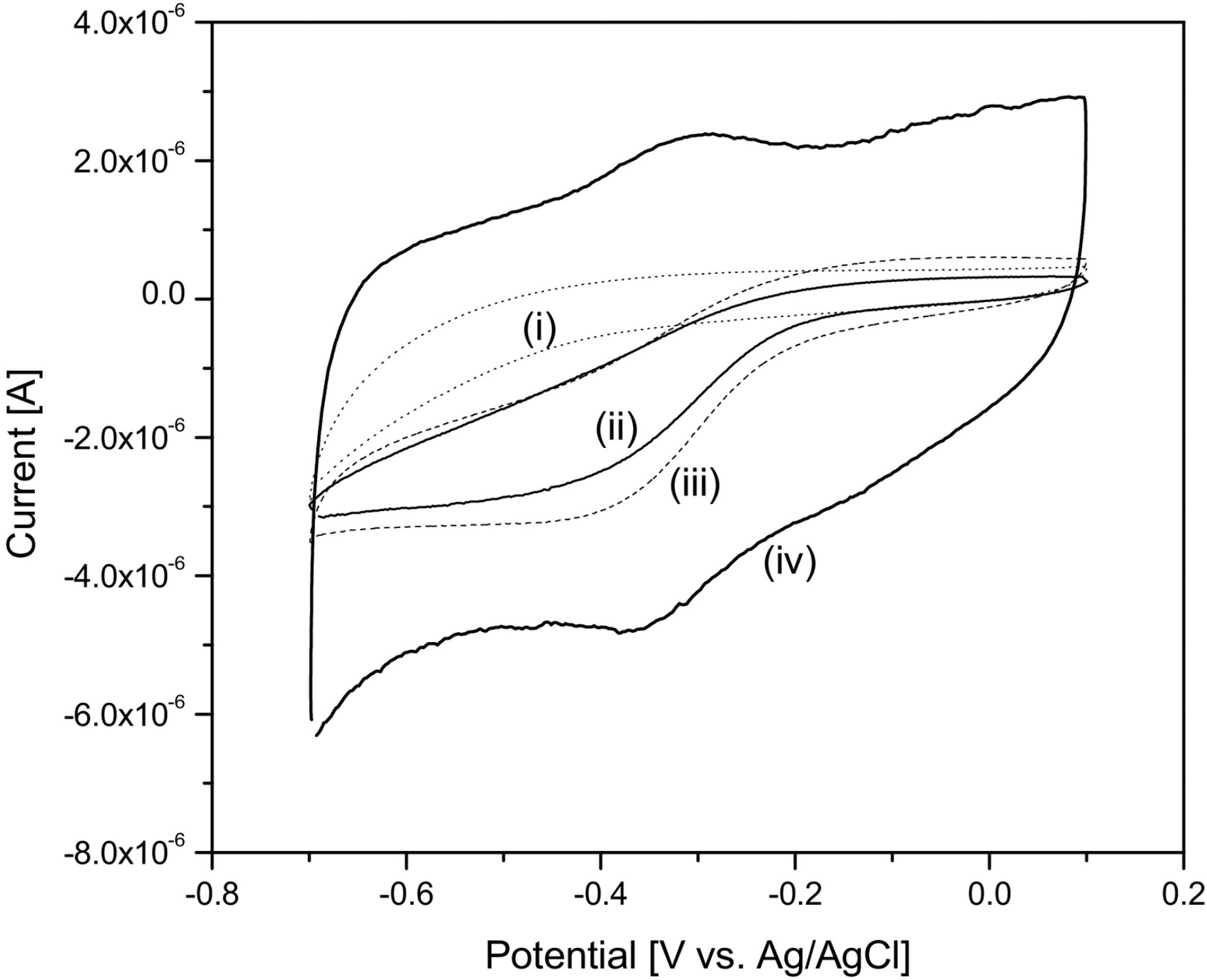

4.0x10-6
2.0x10-6
0.0
-2.0x10-6
-4.0x10-6
-6.0x10-6
-8.0x10-6
Current [A]
-0.8
-0.6
-0.4
-0.2
0.0
0.2
Potential [V vs. Ag/AgCl]
(i)
(ii)
(iii)
(iv)

Figure 2

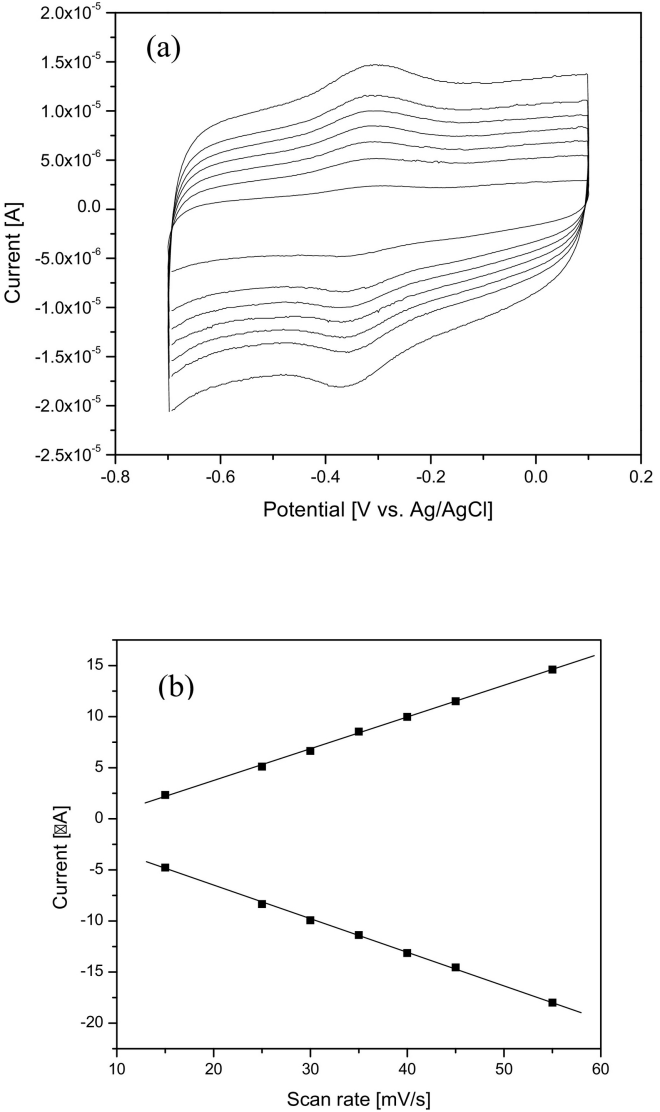

(a)
2.0x10-5
1.5x10-5
1.0x10-5
5.0x10-6
0.0
-5.0x10-6
-1.0x10-5
-1.5x10-5
-2.0x10-5
-2.5x10-5
Current [A]
-0.8
-0.6
-0.4
-0.2
0.0
0.2
Potential [V vs. Ag/AgCl]
(b)
15
10
5
0
-5
-10
-15
-20
Current [⊠A]
10
20
30
40
50
60
Scan rate [mV/s]

Figure 3

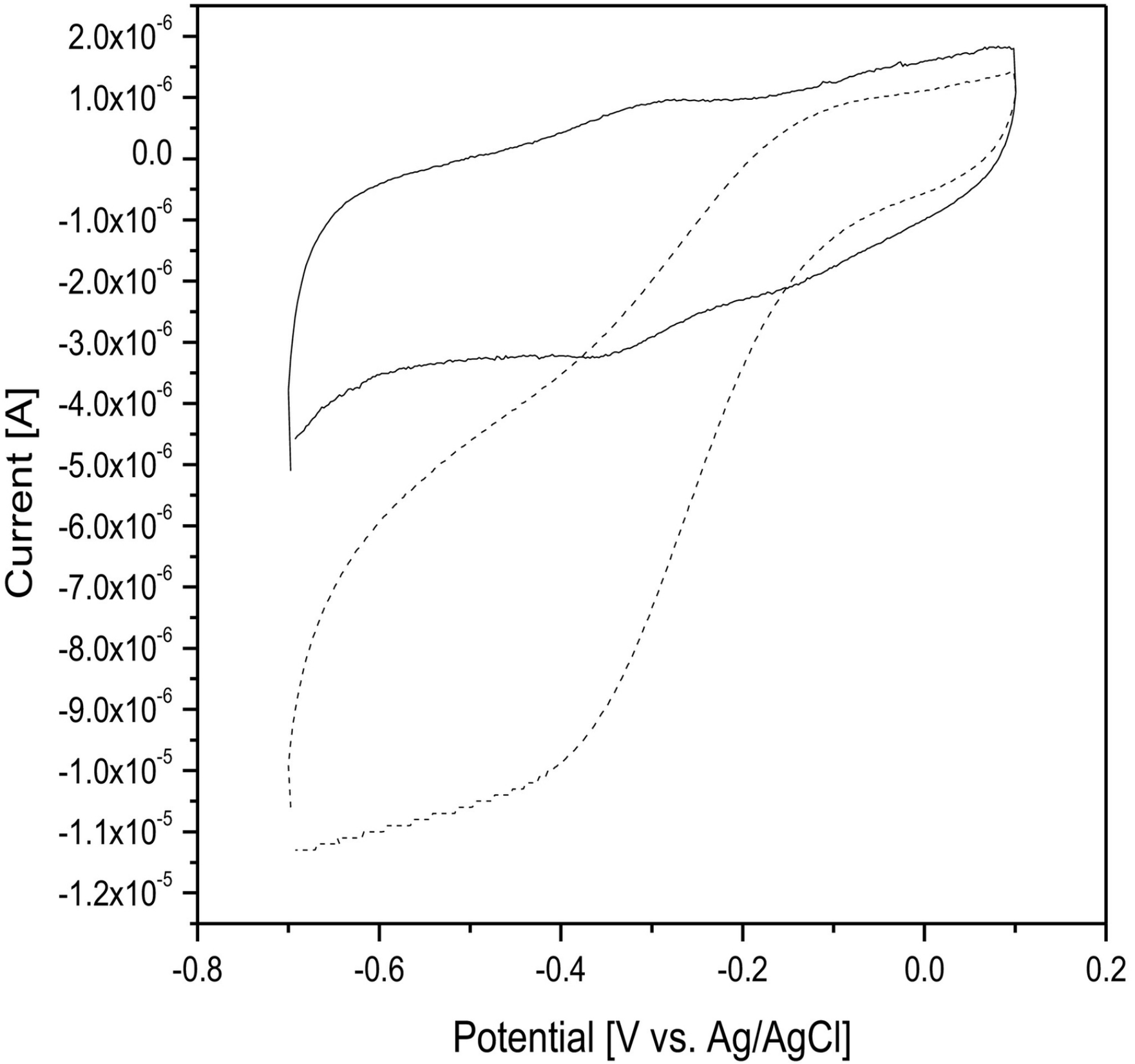
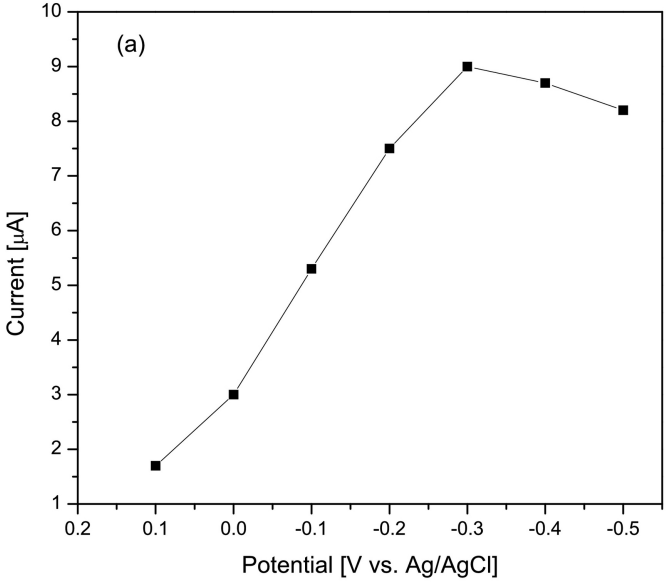

2.0x10-6
1.0x10-6
0.0
-1.0x10-6
-2.0x10-6
-3.0x10-6
-4.0x10-6
-5.0x10-6
-6.0x10-6
-7.0x10-6
-8.0x10-6
-9.0x10-6
-1.0x10-5
-1.1x10-5
-1.2x10-5
Current [A]
-0.8
-0.6
-0.4
-0.2
0.0
0.2
Potential [V vs. Ag/AgCl]

Figure 4


(a)
Current [μA]
Potential [V vs. Ag/AgCl]
10
9
8
7
6
5
4
3
2
1
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5

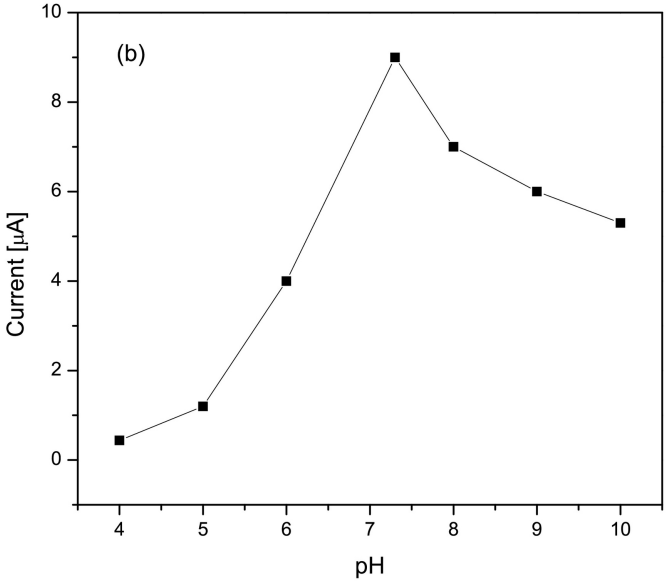
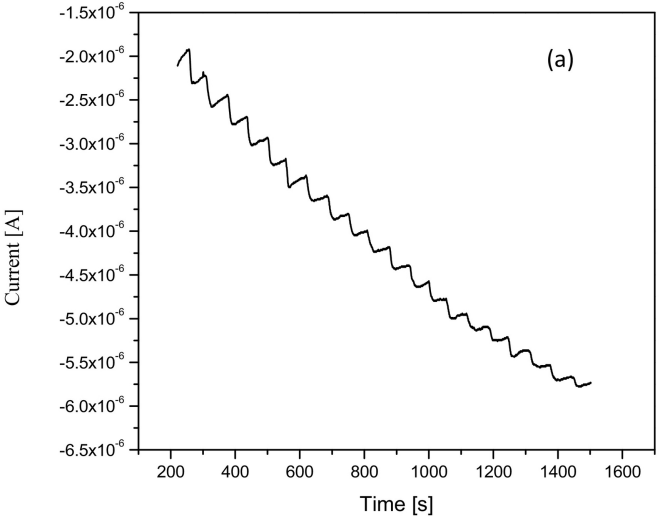

(b)
Current [μA]
pH
10
8
6
4
2
0
4
5
6
7
8
9
10

Figure 5


(a)
Current [A]
-1.5x10-6
-2.0x10-6
-2.5x10-6
-3.0x10-6
-3.5x10-6
-4.0x10-6
-4.5x10-6
-5.0x10-6
-5.5x10-6
-6.0x10-6
-6.5x10-6
200
400
600
800
1000
1200
1400
1600
Time [s]

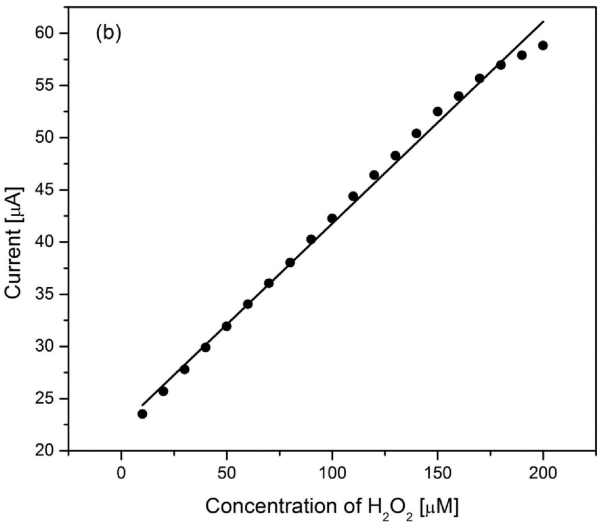

(b)
Current [μA]
60
55
50
45
40
35
30
25
20
0
50
100
150
200
Concentration of H2O2 [μM]

Figure 6