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# The interface behavior of hemoglobin at carbon nanotube and the detection for $H_2O_2$

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**Abstract**

Direct electrochemistry of hemoglobin (Hb) is observed at carbon nanotube (CNT) interface. The adsorbing Hb can transfer electron directly at CNT interface compared with common carbon material. The heterogeneous electron transfer rate constant $k$ of Hb can be calculated as $0.062\,s^{-1}$, the transfer coefficient $\alpha$ is 0.21 and the average surface coverage of Hb on CNT surface is $3.58 \times 10^{-9} \pm 2.7 \times 10^{-10}$ mol/cm$^2$. It is found that the adsorbing Hb still keeps its catalytic activity to $H_2O_2$. This sensor was used to detect $H_2O_2$. The apparent Michaelis–Menten constant is calculated as $6.75 \times 10^{-4}$ mol L$^{-1}$.


*Keywords:* Hemoglobin; Carbon nanotube; Electrochemistry; Nanobiotechnique

## 1. Introduction

The study of the interaction and direct electron exchange between redox-proteins and electrode surface has attracted more and more researchers' attention [1–3]. These works are of great importance for not only studying the electron transfer between biomolecules in biological system, which can help us to understand the material metaboly and energy transform in the life process, but also investigating the novel enzyme biosensors [4].

Hemoglobin (Hb) is an important heme protein in the body. It is also an ideal model protein for studying the electron transfer of heme molecules [5,6]. But unfortunately, Hb has a big and complex structure (MW: 67,000), and the redox centers (heme groups) deeply immerse in body; so it is difficult for Hb to exchange electron with electrode surface directly [7–9]. Some mediators were used to impose the electron contact between the protein and electrodes [10,11]. However, the direct electron transfer is more attractive because it can help us not only to understand the intrinsic redox behaviors of protein, but also to develop the reagentless biosensors [12].

In order to achieve this goal, many methods were adopted, for example, biomembrance films [13], kieselgubr film [14], nano gold colloid particle [15] and organic solvent [16] were used in experiments, and the phenomena of direct electrochemistry were observed. More recently, Cai and Chen [17] used Nafion to immobilize Hb and CNT onto glass carbon electrode surface and study the direct electrochemistry of Hb. The stability of such methods is dissatisfied proverbially. From the point of view of reducing the complexity in sensor's fabrication and reagent expense, as well as rapid detection, the use of bare unmodified electrodes for the direct electrochemistry is undoubtedly the most attractive option. So, the better way to solve this problem is maybe to find new electrode material, which can improve the electron transfer between protein and electrode interface efficiently.

Carbon nanotube (CNT) is a novel carbon material discovered by Iijima in 1991 [18]. It has been found with excellent electrode performance [19]. More and more researchers have begun to pay close attention to CNTs; some significant results have been obtained [20,21]. In this paper, the direct electron

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transfer of Hb adsorbed onto carbon nanotube is reported. It is found that Hb has a reversible redox process at CNT surface. The interface behavior of Hb is also studied; it is found that the Hb at CNT surface still kept the catalytic activity to $H_2O_2$.

## 2. Experimental

### 2.1. Reagents

Carbon nanotubes were prepared by the catalytic chemical vapor deposition (CVD) of methane [22,23] A Co/MgO catalyst for the production of CNTs was prepared by a citric method [24]. For the CVD process, 100 mg of the catalyst was placed in a quartz tube mounted in a tube furnace. The whole tube was pumped down for 15 min and $N_2$ was let into the tube. $N_2$ flow was maintained as the furnace was heated to 850–1000 °C. The $N_2$ flow was then replaced by methane (99.5% pure) at a flow rate of 100–500 ml/min at atmospheric pressure. Subsequently, the methane flow lasted for 10–90 min, and was replaced by $N_2$ for 5 min, and then annealed at 900 °C for 1 h before being cooled down. The yield of CNTs was 100–300%/catalyst weight.

The catalyst was removed by dissolving in concentrated nitric acid, and then washed five times with DI water. The obtained CNTs may contain some –OH and –COOH groups on the surface [25]. To remove these groups, the CNTs were annealed at 900 °C for 3 h in a vacuum. The SEM and TEM images show that the CNTs are very pure, containing no amorphous particles [26]. The TEM image indicated that the CNTs were multiwalled CNTs.

Hemoglobin was purchased from Sigma. All other chemicals were purchased from Aldrich. Millipore water was used for preparing all of the solutions.

### 2.2. Fabrication of CNTPME

The CNT powder microelectrodes electrodes (CNTPMEs) were fabricated according to the reported paper [20]; in brief, a 100 μm diameter Pt micro electrode was first chemically etched to form a cavity of tens of μm deep, and then ground the etched tip on a flat plate (such as glass slide) with CNTs until the micro cavity was filled with CNTs. The graphite powder microelectrodes (GPMEs) were fabricated by the same way.

Ten mg CNT powder was mixed with 100 μl 0.1 M HAc-NaAc (pH 5.4) solution containing 50 mg/ml Hb and 0.1 M KCl, and the mixture was dried at 1 °C with $N_2$ flow. The modified CNT powder was used to fabricate Hb-modified CNTPME (Hb-CNTPME) according to the same method. The compared electrode, Hb-modified graphite powder microelectrodes electrodes (Hb-GPMEs), were fabricated by the same way.

### 2.3. Electrochemistry measurement

A three-electrode cell was used in the measurement, with GPME, CNTPME, Hb-CNTPME or Hb-GPME as working electrode, and a saturated calomel reference electrode (SCE) and a platinum wire counter electrode. Electrochemistry measurements were performed with CHI 660A Electrochemistry System (CH Instruments, Inc.). The solution consisted of 0.1 M HAc-NaAc (pH 5.4) and 0.1 M KCl, which was thoroughly deaerated with high purity nitrogen. Experiments were conducted at 25 °C.

## 3. Results and discussion

Just same as the results obtained by other groups [7–9], the electrochemical responses of Hb are poor at conventional electrodes, no well-defined redox peaks appear at Hb-GPME (Fig. 1, curve b) compared with bare GPME (curve a). However, a pair of redox peaks appears between −0.4 V −0.2 V at Hb-CNTPME (curve d) compared with unmodified CNTPME (curve c). The formal potential $E^{\circ'}$ is around −0.278 V, the peak potential separation $\Delta E_p$ is 119 mV ($E_{pa}$ = −0.219 V, $E_{pc}$ = −0.338 V for scan rate of 20 mV $s^{-1}$), indicating that the redox is nearly quasi-reversible. This electrochemical response can be attributed to the redox of $Fe^{3+}/Fe^{2+}$ couple in Hb. These results show that Hb has a distinguished direct electrochemistry at CNT surface, the electron transport between HB and CNT surface is greatly improved.

The CV curves for the redox of Hb at various scan rates are shown as Fig. 2. It is found that the peak current increases along with the rising of scan rate, while the $\Delta E_p$ expands slowly. At low scan rate, the cathodic peak current rises linearly with the scan rate, $\nu$ (curve a), not with $\nu^{1/2}$(curve b), as shown in Fig. 3, indicating that the redox reaction is a surface process. The slope of the straight line ($r$ = 0.999) of $I_{pc}$ versus $\nu$ is $3.69 \times 10^{-6} \pm 6.14 \times 10^{-8}$ A $V^{-1}$ $s^{-1}$.

For a surface process, surface coverage concentration ($\Gamma$) and the number of electron transport ($n$) can be obtained according to Laviron's equation [27]

$$I_p = \frac{n^2 F^2 \nu A \Gamma}{4RT} = \frac{nFQ\nu}{4RT}$$

where $Q$ is the amount of charge; $A$ is the electrode area and $I_p$, $F$, $R$ and $T$ have their usual meanings. From the curves in Fig. 2, $Q$ can be calculated to $3.85 \times 10^{-7} \pm 2.89 \times 10^{-8}$ C; A is $1.12 \times 10^{-3}$ $cm^2$ from our previous work [20]. So, from the slope of the Fig. 3(a), $n$ is 0.98 ± 0.07, indicating that a single electron reaction happened at CNT interface. The average surface coverage of Hb on CNT surface, $\Gamma$ can be also obtained, and it is $3.58 \times 10^{-9} \pm 2.7 \times 10^{-10}$ mol/$cm^2$.

In order to obtain the kinetic parameters of Hb redox at CNT, the scan rate was increased. It is found when scan rate is above 0.07 V $s^{-1}$, $\Delta E_p$ is more than 200 mV. Fig. 4 shows

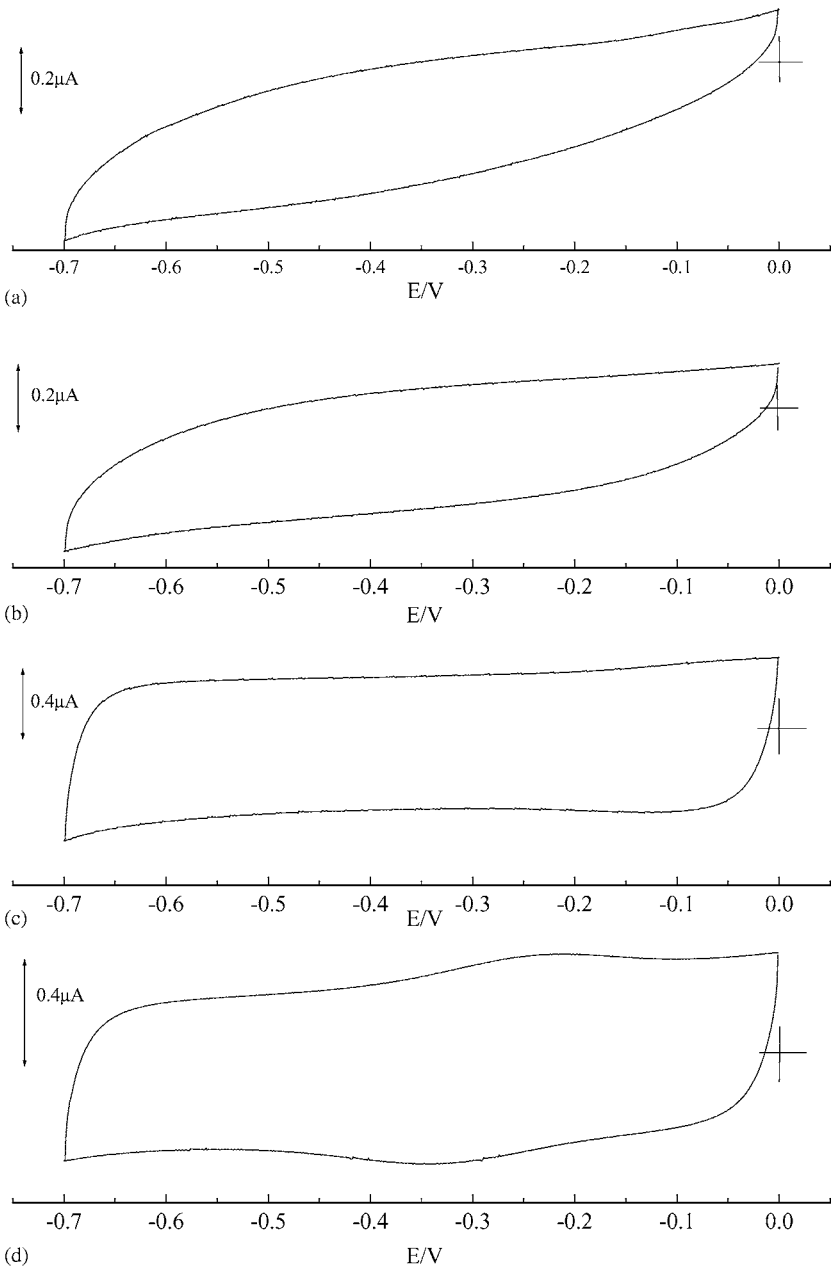


Fig. 1. CV curves at bare GC (a), Hb-GC (b), CNTPME (c) and Hb-CNTPME (d) in 0.1 M HAc-NaAc (pH 5.4) containing 0.1 M KCl at a scan rate of 20 mV $s^{-1}$.

the $E_p$ versus the logarithm of scan rate, it is clear that the peak potential depends linearly with the log $\nu$ at high scan rate.

For the redox monolayer modified electrode, the peak potentials can be represented [28,29]

$$E_{pc} = E^{\circ\prime} - \frac{2.3RT}{\alpha n F}\log\frac{\alpha}{m}$$

$$E_{pa} = E^{\circ\prime} + \frac{2.3RT}{(1-\alpha)nF}\log\frac{1-\alpha}{m}$$

$$m = \frac{RT}{F}\frac{k}{n\nu}$$

The transfer coefficient, $\alpha$, can be calculated according to the slopes of anodic process (curve a) and cathodic process (curve b), and the result is 0.21. According to the above equations, it can be obtained

$$\Delta E_p = \frac{2.3RT}{(1-\alpha)\alpha n F}\left[\alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nF} - \log k\right] + \frac{2.3RT}{(1-\alpha)\alpha n F}\log\nu$$

According to $\Delta E_p \sim \log\nu$ (as shown the insert in Fig. 4), the heterogeneous electron transfer rate constant $k$ of Hb can be calculated as 0.062 $s^{-1}$.

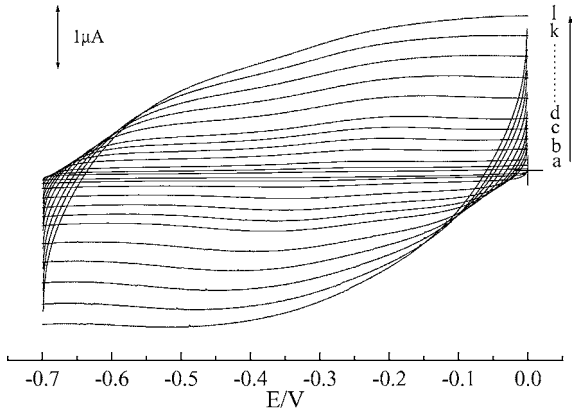


Fig. 2. The CV curves for Hb-CNTPME at various scan rate (mV/s): (a) 2; (b) 5; (c) 10; (d) 20; (e) 30; (f) 40; (g) 50; (h) 70; (i) 90; (j) 110; (k) 130; (l) 150. The other conditions are as in Fig. 1.

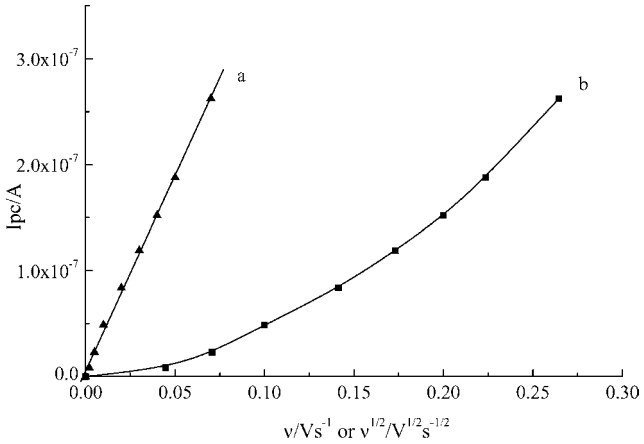


Fig. 3. Plots of $I_{pc}$ $\nu$ (a) and $I_{pc}$ $\nu^{1/2}$ (b) at Hb-CNTPME.

The differential pulse amperometry experiments were also used to study the electron transport at CNT interface. The results are shown in Fig. 5. It can be found that the peak appears only at CNT surface (curve d, Hb-CNTPME) compared with

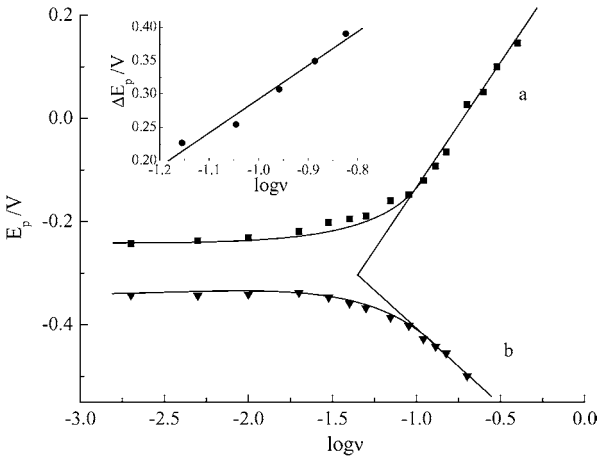


Fig. 4. Plots of anodic peak potential (a) and cathodic peak potential (b) against the logarithm of scan rate. Insert: plot of $\Delta E_p$ against log $\nu$.

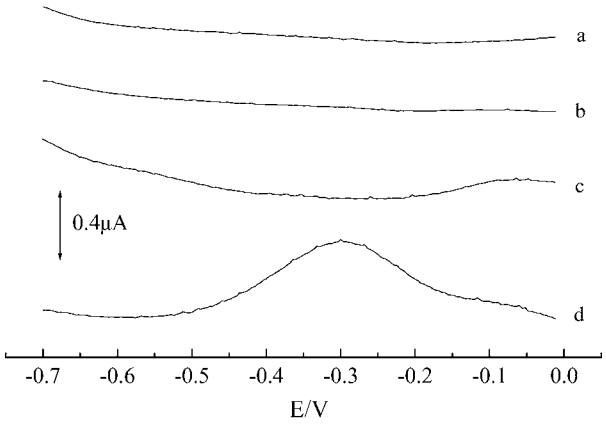


Fig. 5. Differential pulse voltammetry curves at bare GC (a), Hb-GC (b), CNTPME (c) and Hb-CNTPME (d). Initial potential, 0 V; final potential, −0.7 V; pulse width, 60 ms; amplitude, 50 mV. The other conditions are as in Fig. 1.

conventional graphite surface (curve b, Hb-GPME), where curve c and a are the bare CNT and graphite, respectively. It can be seen that the electron transport process is greatly improved at CNT interface.

The stability of Hb-CNTPME was also tested. It is found that the CV peak currents of Hb remain unchanged after Hb-CNTPME has been stored in air for 25 days, indicating that Hb-CNTPME is very stable.

It is well known that Hb contains four subunits, each of them has one redox iron heme, one proton is concerned with the electron transport process, and this process can be concluded as the below equation

$$\mathrm{Hb-Fe(III)} + \mathrm{H^+} \Leftrightarrow \mathrm{Hb-Fe(II)}$$

So the influence of pH was also studied, and the results are shown as Fig. 6. It can be seen that the pH of the solution has a strong influence on the electrochemical behavior of Hb at CNT surface. The redox peaks' potential shift negatively with the increase of pH from 3.6 to 7.2, while the peaks' current

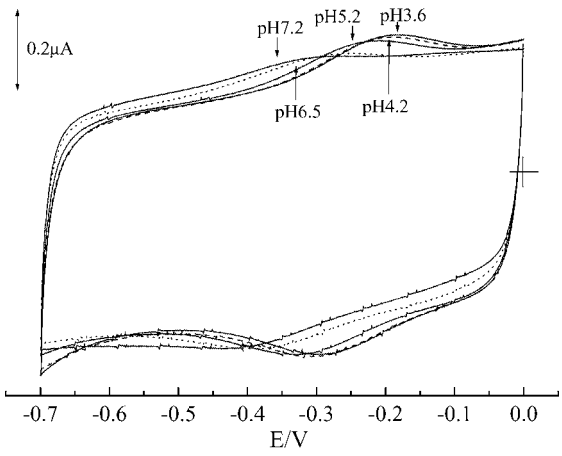


Fig. 6. CV curves for Hb-CNTPME in different pH buffer.

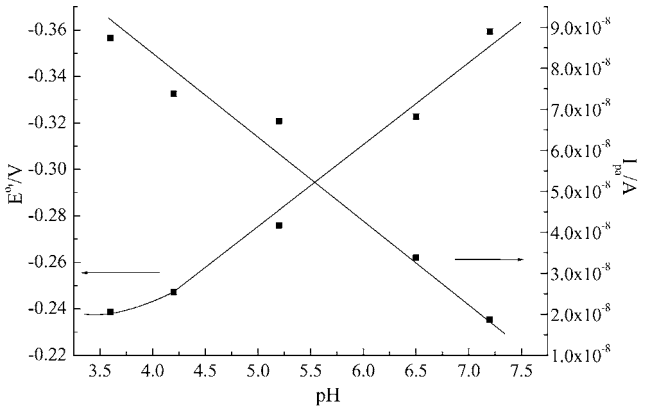


Fig. 7. Influence of pH on formal potential and peak current for Hb-CNTPME in different pH buffer.

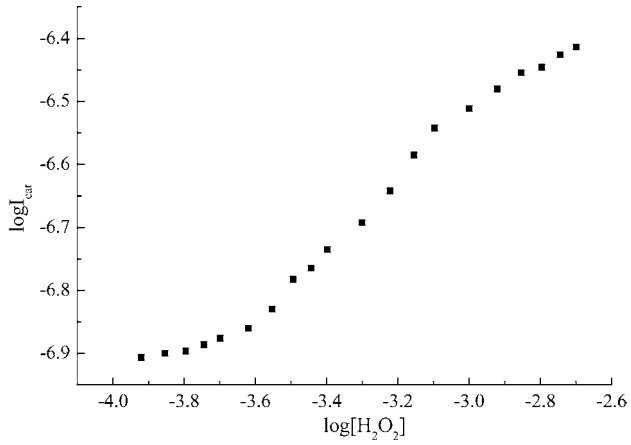


Fig. 9. Plot of the logarithm of catalysis current to the logarithm of concentration of $H_2O_2$. The other conditions are as in Fig. 1.

also decreases at the same time. The pH dependence of the formal potential $E^{\circ'}$ and $I_{pa}$ are shown in Fig. 7. It is found that formal potential $E^{\circ'}$ has a linear relationship with pH from 4.2 to 7.2, and the peak current $I_{pa}$ is also linear with pH ($r$ = 0.9859). The slope of $E^{\circ'}$ versus pH is $-0.03895\,V\,pH^{-1}$ ($r$ = 0.9969), which is close to the other group's result (Wang [13], $-46.4\,mV\,pH^{-1}$). As the other researchers, this result is different from the theoretical value of $-58\,mV\,pH^{-1}$ for a reversible one proton and one electron transfer reaction; the clear explanation is unknown [30].

In order to investigate the catalytic activity of the surface Hb to hydrogen peroxide, $H_2O_2$ was added in the solution. It is found that Hb kept its activity, as shown in Fig. 8. It can be seen that, after the addition of $H_2O_2$, a well-defined cathodic peak appears between $-0.5\,V \sim -0.8\,V$ (curve c) compared with the former CV curve of Hb-CNTPME (curve a). However, at bare CNTPME (curve b), no voltammatry response is observed at the same potential in the same condition. These results indicate that the adsorption of Hb at CNT interface does not damage its native structure; the Hb keeps its native structure.

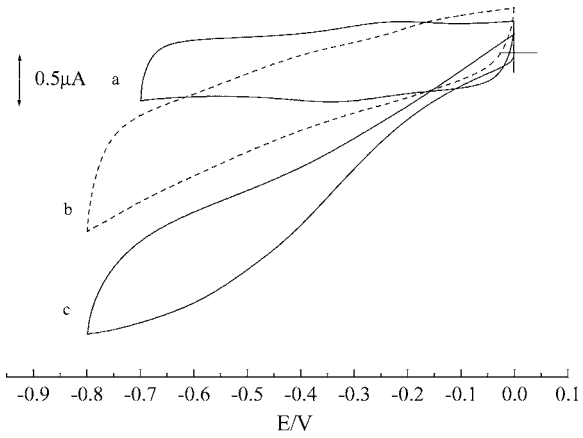


Fig. 8. The voltammogram curves at Hb-CNTPME in buffer (curve a), and at CNTPME (curve b), Hb-CNTPME (curve c) in buffer containing 20 mM $H_2O_2$. The other conditions are as in Fig. 1.

Hb-CNTPME was used to detect $H_2O_2$ by amperometric experiment at $-0.8\,V$. It is found that the steady state current ($I_{Hb}$) rise with the addition of $H_2O_2$ and quickly reaches the stable value (less than 7 s), indicating that the Hb-CNTPME has a short response time for $H_2O_2$. The same experiment was also carried out at CNTPME, and the steady state current ($I_{CNT}$) was obtained too. At Hb-CNTPME, $H_2O_2$ can also exchange electron with CNT interface directly, so $I_{Hb}$ should include the $I_{CNT}$. Then the catalysis current should be defined as: $I_{cat} = I_{Hb} - I_{CNT}$.

The dependence of catalysis current on the concentration of $H_2O_2$ is shown in Fig. 9. It can be seen that log $I_{cat}$ of $H_2O_2$ at Hb-CNTPME increases linearly with log $C_{H_2O_2}$. The current is linear with the $H_2O_2$ concentration from $2.1 \times 10^{-4} \sim 9 \times 10^{-4}\,mol\,L^{-1}$ with a correlation coefficient of 0.9967. The detection limit is $9 \times 10^{-6}\,mol\,L^{-1}$ with the signal to noise ratio of 3.

The apparent Michaelis–Menten constant ($K_m$) is a very important parameter to evaluate the kinetics of enzyme and substrate, it can be calculated according to Lineweaver–Burk equation [31]

$$\frac{1}{I_{cat}} = \frac{1}{I_{max}} + \frac{K_m}{I_{max}}\frac{1}{c}$$

where $c$ is the concentration of the substrate, $I_{max}$ is the maximum current (according to the curve fitting for Fig. 9, it can be obtained as $5.07 \times 10^{-7}$ A). So, the dependence of $1/I_{cat}$ on $1/c$ is shown in Fig. 10. It is found that $1/I_{cat}$ has a linear relationship with $1/c$ with a correlation coefficient of 0.9959. From the slope of $1330.6\,mol\,A^{-1}\,L^{-1}$, the $K_m$ can be calculated as $6.75 \times 10^{-4}\,mol\,L^{-1}$, which is close to the results obtained by other groups (Li [14], 975 $\mu mol\,L^{-1}$; Chen [15], 120 $\mu mol\,L^{-1}$).

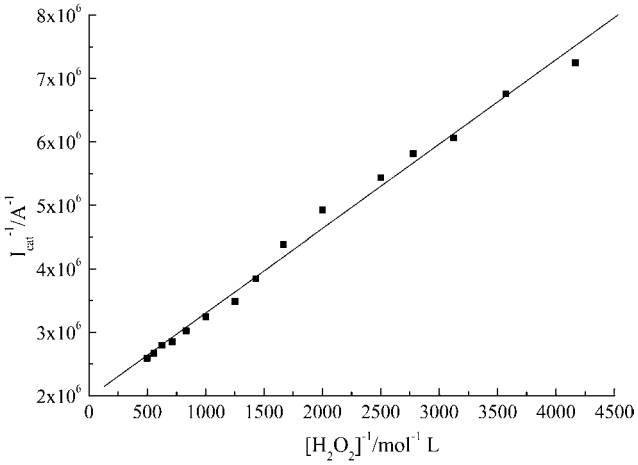


Fig. 10. Double reciprocal plot of the catalysis current to the concentration of $H_2O_2$. The other conditions are as in Fig. 1.

## 4. Conclusion

Hb is found with direct electrochemistry at CNT interface, the kinetic parameters are calculated, $k$ of Hb can be calculated as $0.062\,s^{-1}$ and the transfer coefficient $\alpha$ is 0.21. It is also found that Hb keeps its native structure, and still has its catalytic activity to $H_2O_2$, the apparent Michaelis–Menten constant is also calculated as $6.75 \times 10^{-4}\,mol\,L^{-1}$. The Hb-CNTPME is used to determinate $H_2O_2$. It is found that further work is needed to improve the detection ability. Although the detection limit is not good enough, these results will help us understand the intrinsic redox behaviors of enzyme.

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