Talanta 80 (2010) 2177–2181

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Direct electrochemistry and electrocatalysis of hemoglobin on gold nanoparticle decorated carbon ionic liquid electrode

Wei Sun*, Peng Qin, Ruijun Zhao, Kui Jiao

Key Laboratory of Eco-Chemical Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, People's Republic of China

ARTICLE INFO

Article history:
Received 3 September 2009
Received in revised form 5 November 2009
Accepted 6 November 2009
Available online 14 November 2009

Keywords:
Ionic liquid
Hemoglobin
Gold nanoparticle
Direct electrochemistry
Electrocatalysis

ABSTRACT

In this paper a carbon ionic liquid electrode (CILE) was fabricated by using ionic liquid 1-ethyl-3-methylimidazolium ethylsulfate ([EMIM]EtOSO$_3$) as modifier and further gold nanoparticles were in situ electrodeposited on the surface of CILE. The fabricated Au/CILE was used as a new platform for the immobilization of hemoglobin (Hb) with the help of a Nafion film. Electrochemical experimental results indicated that direct electron transfer of Hb was realized on the surface of Au/CILE with a pair of well-defined quasi-reversible redox peaks appeared. The formal peak potential ($E^{0\prime}$) was obtained as −0.210 V (*vs.* SCE) in pH 7.0 phosphate buffer solution (PBS), which was the characteristic of Hb heme Fe(III)/Fe(II) redox couple. The fabricated Nafion/Hb/Au/CILE showed excellent electrocatalytic activity to the reduction of trichloroacetic acid (TCA) and the reduction peak current was in proportional to TCA concentration in the range from 0.2 to 18.0 mmol/L with the detection limit as 0.16 mmol/L (S/N = 3). The proposed electrode showed good stability and reproducibility, and it had the potential application as a new third-generation electrochemical biosensor.

1. Introduction

Direct electrochemistry between metalloproteins with electrodes has aroused great interests in the preparation of the biosensor and bioreactor [1]. But the direct electron transfer on the bare working electrode is difficult to be realized due to the deep burying of the electroactive center or the partly denaturation of the redox protein. Many methods have been proposed for the construction of protein film electrodes to achieve the direct electron transfer process between proteins and the working electrode. For this purpose various supporting films including insoluble surfactants, biopolymers, polyelectrolytes and hybrid films are used [2–4]. The presence of film on the electrode surface can provide a biocompatible microenvironment for proteins to retain their nature structure and enhance the direct electron transfer rate between the electroactive center and the underlying electrode. In recent years there are increasing interests on the direct electrochemistry of redox protein with ionic liquids (ILs) interface. ILs are green solvent with many specific physicochemical properties such as high ionic conductivity, wide electrochemical window, good stability and excellent biocompatibility [5]. Wei and Ivaska had reviewed the recent progresses of ILs in the field of electrochemical sensor [6]. ILs can be used as not only the supporting electrolyte but also the modifier for the chemically modified electrodes [7,8]. Xiong et al. studied the direct electrochemistry of hemoglobin (Hb) and its electrocatalytic activity towards hydrogen peroxide on the modified electrode with 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM]BF$_4$) as the electrolyte [9]. Maleki and co-workers fabricated a high performance carbon ionic liquid electrode (CILE) with *n*-octylpyridinium hexafluorophosphate ([OP]PF$_6$) as binder [10,11]. Our group also applied CILE to the investigation of protein electrochemistry with different nanomaterials [12–14].

Gold nanoparticle has been widely used for the immobilization of proteins, which act as the good conductive mediator to facilitate the electron transfer rate [15]. Many reports indicate that the gold nanomaterials with different appearances can be used as the immobilization matrix to realize the direct electrochemistry of redox protein. Yang et al. investigated the direct electrochemistry of Hb on a gold nanowire array [16]. Zhang et al. combined gold nanoparticle with polyvinyl alcohol and 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIM]PF$_6$) for the investigation on the electrochemical behaviors of Hb [17]. Hong and Dai constructed a novel amperometric biosensor for hydrogen peroxide and nitrite by immobilizing Hb on one-dimensional gold nanoparticle [18].

In this paper a gold nanoparticle decorated CILE was fabricated by in situ electrodeposition method. CILE has been elucidated with the advantages such as high conductivity, good stability and antifouling ability with a layer of IL also presented on the surface of CILE [5]. At the same time gold nanoparticles show good conductivity

* Corresponding author. Tel.: +86 532 4022681; fax: +86 532 4022681.
E-mail address: sunwei@qust.edu.cn (W. Sun).

0039-9140/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2009.11.026

and biocompatibility [19]. So a layer of gold nanoparticle was electrodeposited on the surface of CILE to get a novel platform for protein electrochemistry, which combined the advantages of CILE and gold nanoparticle. Then Hb was selected as the model redox protein and it was immobilized on the surface of Au/CILE with Nafion film. Nafion is a sulfonated tetrafluoroethylene based fluoropolymer–copolymer with many remarkable characteristics such as highly conductivity to cations, excellent film-forming ability, thermal and chemical stability as well as the selectively and highly permeable to water, which has been widely used in the fabrication of electrochemical biosensors [20]. The direct electrochemistry of Hb was further investigated carefully with a pair of well-defined redox peaks appeared on the cyclic voltammogram. The experimental results indicated that the direct electron transfer process was realized on the modified electrode, which proved the advantages of the prepared gold nanoparticle decorated electrode. The electrocatalytic ability of Hb on the modified electrode to the reduction of trichloroacetic acid (TCA) was further investigated.

2. Experimental

2.1. Reagents and apparatus

1-Ethyl-3-methylimidazolium ethylsulfate ([EMIM]$EtOSO_3$, Hangzhou Chemer Chemical Co. Ltd., China), bovine hemoglobin (Hb, MW 64500, Tianjin Chuanye Biochemical Co. Ltd., China), graphite powder (average particle size 30 μm, Shanghai Colloid Chemical Plant, China), chloroauric acid ($HAuCl_4$, Shanghai Chemical Plant, China) and trichloroacetic acid (TCA, Tianjin Kermel Chemical Reagent Co. Ltd., China) were used as received. 0.1 mol/L phosphate buffer solutions (PBS) were used as the supporting electrolyte. All the solutions were purged with highly purified nitrogen for 30 min and an inert nitrogen environment was maintained in the electrochemical cell during the electrodeposition and electrochemical measurements. Other chemicals used were of analytical reagent grade and the solutions were prepared with doubly distilled water.

A LK 2005 electrochemical workstation (Tianjing Lanlike Chemistry & Electron High Technology Co. Ltd., China) was employed for the electrodeposition and electrochemical measurements. Electrochemical impedance spectroscopy (EIS) was achieved on a CHI 750B electrochemical workstation (Shanghai CH Instruments, China). A traditional three-electrode system, which was consisted of a self-made Hb modified electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as counter electrode, was used throughout. Scanning electron microscopy (SEM) was performed on a JSM-6700F scanning electron microscope (Japan Electron Company, Japan).

2.2. Electrode preparation

CILE was prepared according to the following procedure: 200 μL of [EMIM]$EtOSO_3$, 800 μL of liquid paraffin and 3.2 g of graphite powder were hand-mixed in an agate mortar and ground carefully. A portion of resulting homogeneous paste was packed firmly into a glass tube (Ø = 4 mm) and the electrical contact was established by a copper wire to the end of the paste in the inner hole of the tube.

Gold nanoparticle modified CILE (Au/CILE) was prepared based on a reported procedure [21]. In general gold nanoparticle was electrodeposited onto the newly prepared CILE with a solution of 5.0 mmol/L $HAuCl_4$ and 0.5 mol/L KNO_3 by controlled potential at −0.4 V for 300 s. The Au/CILE was rinsed with doubly distilled water and dried in air for further modification. Hb modified electrode was fabricated by casting 8.0 μL of 10.0 mg/mL Hb aqueous solution on the surface of Au/CILE and staying silent to allow the water evaporated gradually. Then 8.0 μL of 0.5% Nafion solution was cast on the electrode surface, which could form a stable film and fixed Hb on the surface of electrode tightly. During these procedures a small bottle was covered over the electrode so that the solvent could evaporate slowly to get a uniform film. The modified electrode was stored at 4 °C when not in use and denoted as Nafion/Hb/Au/CILE. Other modified electrodes were prepared by the similar procedure for the comparison.

3. Results and discussion

3.1. Characterization of gold nanoparticle modified carbon ionic liquid electrode

Fig. 1 showed the SEM images of different modified electrodes. On bare CPE (Fig. 1A) a layer of irregularly flasks of graphite powder was isolated. On the CILE (Fig. 1B) a more uniform surface was observed without the separated carbon layer. The results were in accordance with the previous reported results [12,13]. After electrodeposition of gold nanoparticle on the CILE surface, the interface appeared with highly regular and uniform (Fig. 1C). The average diameter of the formed gold nanoparticle was about 150 nm, indicating the successfully deposition of gold nanoparticle on the CILE. The resulted electrode was further used for the protein immobilization with an increased surface area and a three-dimensional structure.

Electrochemical behaviors of different modified electrodes in a 5.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl solution were investigated with the cyclic voltammetric results as shown in Fig. 2. A pair of quasi-reversible redox peaks was observed on the CPE (curve a), and the redox peak currents were much smaller than that of CILE (curve b). The result was attributed to the presence of the high conductive IL on the electrode surface. After the electrodeposition of gold nanoparticle on the surface of CILE (curve c), a remarkable enhance of redox peak currents occurred, indicating the high conductive metal of gold on the electrode surface. According to the Randles–Sevcik equation [22]: $I_{P,c} = (2.69 \times 10^5)n^{3/2}AD^{1/2}C^*v^{1/2}$, the effective surface area of different modified electrodes can be estimated. Where $I_{P,c}$ is the reduction peak current (A), n is the electron transfer number, A is the effective surface area (cm^2), D is the

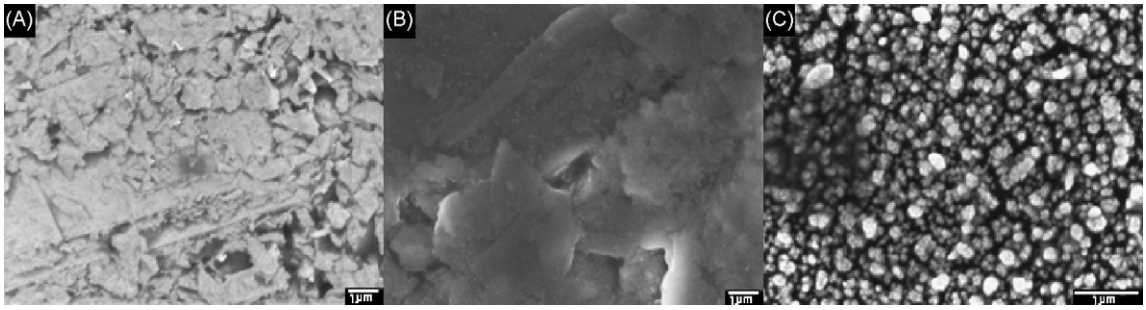

Fig. 1. SEM images of (A) CPE, (B) CILE and (C) Au/CILE.

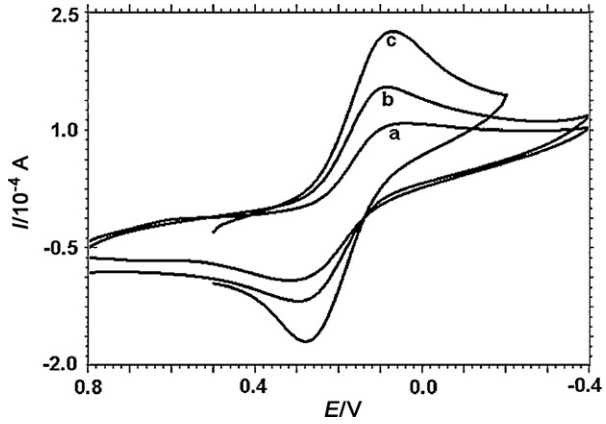

Fig. 2. Cyclic voltammograms of (a) CPE, (b) CILE and (c) Au/CILE in a 5.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl solution with the scan rate of 100 mV/s.

confusion coefficient of $K_3[Fe(CN)_6]$ in the solution (cm^2/s), C^* is the concentration of $K_3[Fe(CN)_6]$ (mol/cm^3) and v is the scan rate (V/s), Based on this method, the average effective surface area of CPE, CILE and Au/CILE were calculated as 0.109, 0.146 and 0.197 cm^2, respectively. So the effective surface area of Au/CILE was increased for 1.35 time than that of CILE, which was due to the presence of nanostructure gold particle on the electrode surface.

3.2. Electrochemical impedance spectroscopy of different electrodes

EIS is a commonly used method to probe the interface information of the impedance changes during the stepwise-assemble process of the surface modified electrode. In EIS the semicircular part at higher frequencies corresponds to the electron transfer limited process and the linear part at lower frequencies corresponds to the diffusion process. The value of electron transfer resistance (R_{et}) is estimated according to the diameter of the semicircle of the Nyquist plots at the high frequency region, which controls the electron transfer kinetics of redox probe at the electrode surface and reflects the interfacial electron transfer ability. The EIS results of different electrodes in a solution of 10.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl with the frequencies swept from 10^5 to 0.1 Hz were shown in Fig. 3. Here Z' and Z'' are the real variable and the negative value of the imaginary variable of impedance. Then the Randles circuit (inset) is further chosen to fit the impedance data obtained in the experiments. On the bare CILE a straight line appeared (curve a), which was due to the high ionic conductivity of ionic liquid present in the carbon paste electrode. On the Au/CILE, the R_{et} value was also close to zero (curve b), indicating that the high conductive gold nanoparticle present on the CILE facilitated the electron transfer. When Nafion was cast on the Au/CILE, the R_{et} value was estimated to be 480 Ω (curve c), which was due to the presence of Nafion film on the electrode surface hindered the electron transfer of $[Fe(CN)_6]^{3-/4-}$. After Hb was fixed in the Nafion film, the R_{et} value was further increased to 691 Ω (curve d), which indicated that Hb molecules were successfully immobilized on the surface of Nafion/Au/CILE and further hindered the electron transfer of electrochemical probe.

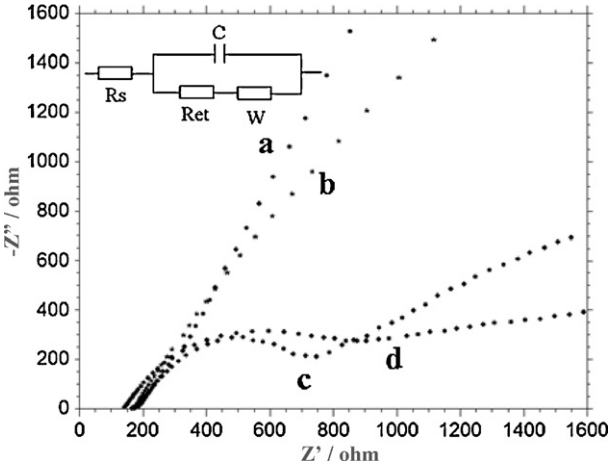

Fig. 3. Electrochemical impedance spectroscopy for (a) bare CILE, (b) Au/CILE, (c) Nafion/Au/CILE and (d) Nafion/Hb/Au/CILE in the solution of 10.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl with the frequencies swept from 10^5 to 0.1 Hz. Inset is the Randles circuit model for the modified electrodes in the cell.

3.3. Direct electrochemistry of Hb

Fig. 4A showed the cyclic voltammograms of different electrodes in 0.1 mol/L PBS at the scan rate (v) of 100 mV/s. No redox peak appeared on CILE (curve a) and Au/CILE (curve b) but with increased background current, which also proven the existence of gold nanoparticle on the CILE surface. While on Nafion/Hb/CILE (curve c) a pair of small redox peaks appeared, indicating that the direct electron transfer rate of Hb with CILE was very slow. While on the Nafion/Hb/Au/CILE (curve d) a remarkable enhanced electrochemical response was observed with a pair of well-defined

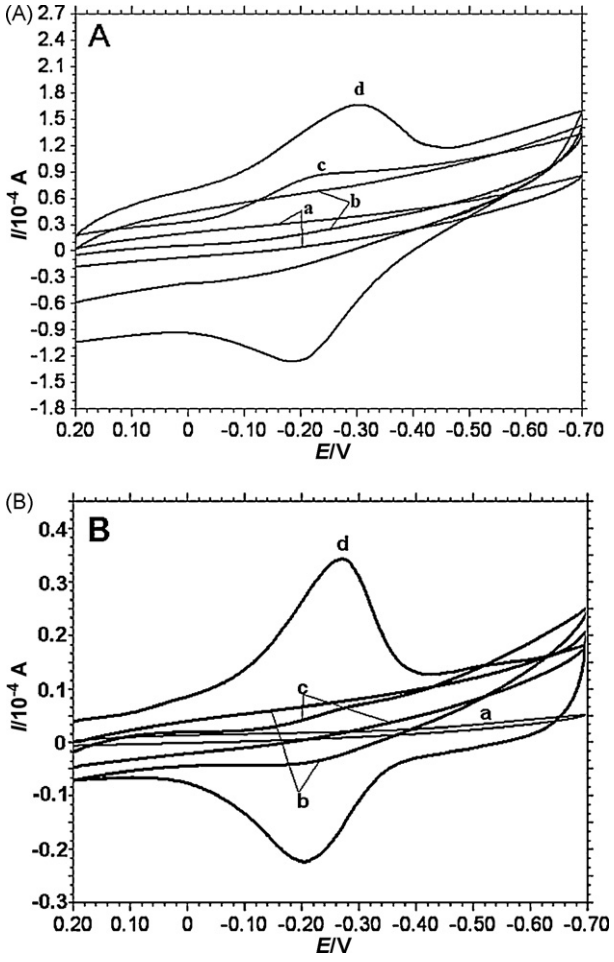

Fig. 4. (A) Cyclic voltammograms of (a) bare CILE, (b) Au/CILE, (c) Nafion/Hb/CILE and (d) Nafion/Hb/Au/CILE at the scan rate of 100 mV/s in pH 7.0 PBS. (B) Cyclic voltammograms of (a) bare CPE, (b) Au/CPE, (c) Nafion/Hb/CPE and (d) Nafion/Hb/Au/CPE at the scan rate of 100 mV/s in pH 7.0 PBS.

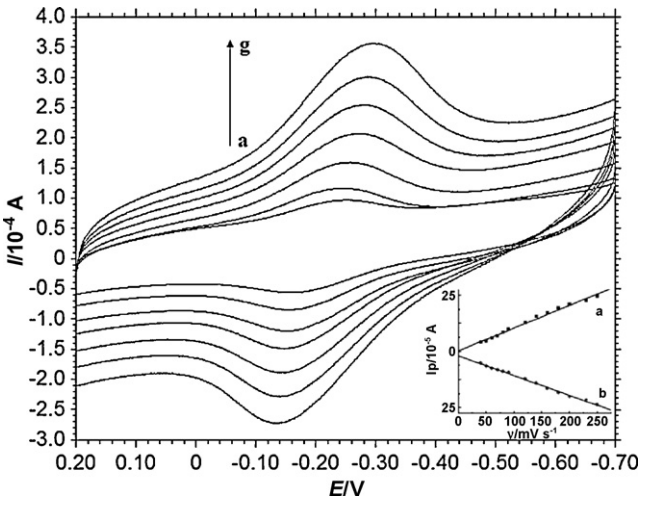

Fig. 5. Cyclic voltammograms of Nafion/Hb/Au/CILE in pH 7.0 PBS with the scan rates from a to g as 40, 60, 100, 120, 160, 200 and 250 mV/s, respectively. Inset: linear relationship of the cathodic (a) and anodic (b) peak current versus scan rate (v).

quasi-reversible redox peaks appeared. The results indicated that the presence of gold nanoparticle on the CILE surface greatly improved the direct electron transfer rate of Hb, which may be attributed to the high metal conductivity and three-dimensional structure of gold nanoparticle. The redox peak potentials were observed with the reduction potential (E_{pc}) as −0.257 V and the oxidation potential (E_{pa}) as −0.162 V. The formal peak potential [$E^{0\prime} = (E_{pc} + E_{pa})/2$] was estimated to be −0.210 V, which was the characteristic of the Hb heme Fe(III)/Fe(II) redox couple. The results showed that the Au/CILE interface was suitable for the electron transfer process between Hb and the electrode. For comparison the similar modified electrodes were prepared with carbon paste electrode (CPE) as the substrate electrode and the cyclic voltammograms were recorded at the same conditions. As shown in Fig. 4B no redox peaks appeared on the bare CPE, Au/CPE and Nafion/Hb/CPE (curve a–c) indicating no electrochemical reaction took place on the electrodes. On the Nafion/Hb/Au/CPE a pair of redox peak appeared with redox peak current much smaller than that of Nafion/Hb/Au/CILE. The results also indicated the superiority of CILE than CPE. CILE has been demonstrated to have the advantages of high conductivity, good stability and anti-fouling ability. On the surface of CILE a layer of IL was also present, which provided a specific interface for the electrodeposition of gold nanoparticle. So the electrochemical response of immobilized Hb was greatly improved with Au/CILE.

Cyclic voltammograms of Nafion/Hb/Au/CILE at different scan rates were recorded in the range from 40 to 250 mV/s with the results shown in Fig. 5. A pair of well-defined redox peaks appeared and the peak currents increased linearly with the scan rate, which indicated a typical surface-confined thin-layer electrochemical behavior [23]. Based on the following equation: $Q = nAF\Gamma^*$, the surface concentration of electroactive Hb (Γ^*) was calculated as 2.62×10^{-9} mol/cm^2, which accounted for 26.5% of the total amount of Hb cast on the electrode surface. The value was much higher than that of Hb monolayer (1.89×10^{-11} mol/cm^2) on the electrode surface [24], which indicated that several layers of Hb in the film took part in the electrode reaction. The reason can be attributed to the presence of Au nanoparticle on the surface of CILE provided a three-dimensional interface for Hb immobilization with the increased effective surface area. The redox peak potentials also exhibited linear relationship with the logarithm of the scan rate and two equations were calculated as $E_{pa}(V) = -0.034 \ln v - 0.346$ ($r = 0.980$) and $E_{pc}(V) = 0.015 \ln v - 0.114$ ($r = 0.968$). According to the Laviron's equation [23], the values of electron transfer coefficient (α) and electron transfer rate constant (k_s) were calculated as 0.573 and 0.412 s^{-1}, respectively.

3.4. Electrocatalytic reduction of trichloroacetic acid

TCA is an analogue of acetic acid in which the three hydrogen atoms of the methyl group have all been replaced by chlorine atoms. It is widely used in biochemistry for the precipitation of macromolecules, such as proteins, DNA and RNA, and cosmetic treatments, such as chemical peels and tattoo removal. It also is an environmental pollutant which can kill normal cells. For these reasons, the determination of TCA has received great attentions in recent years [25,26]. The electrocatalytic reduction of TCA on Nafion/Hb/Au/CILE was examined by cyclic voltammetry with the results as shown in Fig. 6. After the addition of TCA into the deoxygenated pH 7.0 PBS, an obvious reduction peak at −0.20 V appeared with the decrease of the corresponding oxidation peak current, which was a typical electrocatalytic reaction [13]. With the increase of TCA concentration in PBS a new reduction peak appeared at −0.42 V, which could be attributed to the formation of highly reduced form of Hb [27,28]. The catalytic peak current at −0.20 V had a good linear relationship with TCA concentration in the range from 0.2 to 18.0 mmol/L with the regression equation as $I_p(\mu A) = 0.533\,C\,(\text{mmol/L}) + 2.442$ ($r = 0.990$). The detection limit was calculated as 0.16 mmol/L based on the S/N = 3, which was lower than some previous reports [24,29]. When the TCA concentration was more than 20.0 mmol/L, the reduction peak current turned to level off, indicating a typical Michalies–Menten kinetic process. So the apparent Michalies–Menten constant (K_M^{app}) was estimated by using the electrochemical version of the Lineweaver–Burk equation [30]. By exploring the relationship of $1/I_{SS}$ versus $1/C_{TCA}$, the K_M^{app} value was calculated as 0.499 mmol/L, which was lower than some reported values [14,29]. A small K_M^{app} value suggests that the Hb on the modified electrode has higher affinity to the substrates, which can be attributed to the favorable microenvironment of Hb on the modified electrode.

3.5. Stability and repeatability of the modified electrode

The stability of the modified electrode was evaluated. After two-week storage at 4 °C, 97.3% of the initial current signal was obtained and 95.0% remained after four-week storage, indicating that the

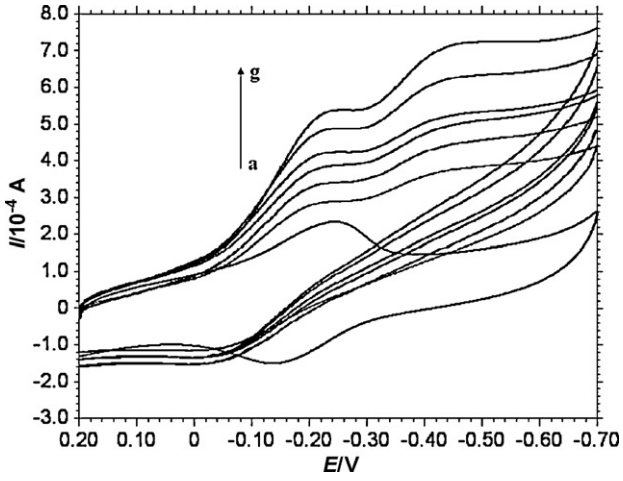

Fig. 6. Cyclic voltammograms obtained at a Nafion/Hb/Au/CILE in pH 7.0 PBS after the addition of 0, 2.0×10^{-3}, 5.0×10^{-3}, 8.0×10^{-3}, 1.0×10^{-2}, 1.4×10^{-2} and 1.8×10^{-2} mol/L TCA (a–g), respectively, at the scan rate of 100 mV/s.

prepared electrode had excellent long-term stability. To investigate the repeatability of the modified electrode, seven electrodes were made by the same procedure independently for the determination 1.0 mmol/L TCA and the relative standard deviation (RSD) value was got as 3.50%. While the RSD of 2.89% was estimated with one electrode for seven successive determinations of 1.0 mmol/L TCA, which indicated that the modified electrode had good reproducibility.

4. Conclusions

Gold nanoparticle was electrodeposited on the surface of CILE to get an effective supporting platform and Hb was immobilized on the electrode surface with Nafion film to get a Hb modified electrode. Due to the presence of gold nanoparticle direct electrochemistry of Hb was accelerated on the modified electrode and a pair of well-defined redox couples appeared with the formal peak potential ($E^{0\prime}$) as −0.210 V (*vs.* SCE). The results indicated the gold nanoparticle decorated carbon ionic liquid electrode provided an excellent interface to promote the direct electron transfer between Hb and the electrode. The Hb modified electrode showed good electrocatalytic activity to TCA with lower detection limit and wider dynamic range, which had the potential application as the third-generation electrochemical biosensor in the field of pathological research, environmental protection and quality control.

Acknowledgements

The authors appreciate the financial support of the National Natural Science Foundation of China (nos. 20635020, 20405008) and the Foundation of University Teacher International Training Project of Shandong Province.

References

[1] F.A. Armstrong, H.A.O. Hill, N.J. Walton, Acc. Chem. Res. 21 (1988) 407.
[2] P.M. Guto, J.F. Rusling, Electrochem. Commun. 8 (2006) 455.
[3] W. Sun, D.D. Wang, R.F. Gao, K. Jiao, Electrochem. Commun. 9 (2007) 1159.
[4] H. Zhang, N.F. Hu, Biosens. Bioelectron. 23 (2007) 393.
[5] N. Maleki, A. Safavi, F. Tajabadi, Anal. Chem. 78 (2006) 3820.
[6] D. Wei, A. Ivaska, Anal. Chim. Acta 607 (2008) 126.
[7] H.Y. Xiong, T. Chen, X.H. Zhang, S.F. Wang, Electrochem. Commun. 9 (2007) 2671.
[8] H. Liu, P.G. He, Z. Li, C. Sun, L. Shi, Y. Liu, G. Zhu, J. Li, Electrochem. Commun. 7 (2005) 1357.
[9] H. Xiong, T. Chen, X. Zhang, S. Wang, Electrochem. Commun. 9 (2007) 1648.
[10] A. Safavi, N. Maleki, F. Tajabadi, E. Farjami, Electrochem. Commun. 9 (2007) 1963.
[11] N. Maleki, A. Safavi, E. Farjami, E. Farjami, Anal. Chim. Acta 611 (2008) 151.
[12] W. Sun, X.Q. Li, Y. Wang, X. Li, C.Z. Zhao, K. Jiao, Bioelectrochemistry 75 (2009) 170.
[13] W. Sun, R.F. Gao, K. Jiao, J. Phys. Chem. B 111 (2007) 4560.
[14] W. Sun, D.D. Wang, G.C. Li, Z.Q. Zhai, R.J. Zhao, K. Jiao, Electrochim. Acta 53 (2008) 8217.
[15] C.X. Lei, S.Q. Hu, G.L. Shen, R.Q. Yu, Talanta 59 (2003) 981.
[16] M. Yang, F. Qu, Y. Li, Y. He, G. Shen, R. Yu, Biosens. Bioelectron. 23 (2007) 414.
[17] Y. Zhang, R. Yan, F. Zhao, B. Zeng, Colloid Surf. B 71 (2009) 288.
[18] J. Hong, Z. Dai, Sens. Actuators B 140 (2009) 222.
[19] J.F. Hicks, F.P. Zamborini, A.J. Osisek, R.W. Murray, J. Am. Chem. Soc. 123 (2001) 7048.
[20] K.A. Mauritz, R.B. Moore, Chem. Rev. 104 (2004) 4535.
[21] C.F. Ding, F. Zhao, R. Ren, J.M. Lin, Talanta 78 (2009) 1148.
[22] A.J. Bard, L.R. Faulkner, Electrochemical Methods, 2nd ed., Wiley, New York, 2001, p. 213.
[23] E. Laviron, J. Electroanal. Chem. 101 (1979) 19.
[24] S.F. Wang, T. Chen, Z.L. Zhang, X.C. Shen, Z.X. Lu, D.W. Pang, K. Wong, Langmuir 21 (2005) 9260.
[25] T.A. Piggot, R.W. Norris, Br. J. Plast. Surg. 41 (1988) 112.
[26] H.K. Bhat, M.F. Kanz, G.A. Campbell, G.A.S. Ansari, Fund. Appl. Toxicol. 17 (1991) 240.
[27] X. Ma, X.J. Liu, H. Xiao, G.X. Li, Biosens. Bioelectron. 20 (2005) 1836.
[28] A.E.F. Nassar, J.M. Bottitt, J.D. Stuart, J.F. Rusling, J. Am. Chem. Soc. 117 (1995) 10986.
[29] H.Y. Ma, N.F. Hu, J.F. Rusling, Langmuir 16 (2000) 4969.
[30] R.A. Kamin, G.S. Willson, Anal. Chem. 52 (1980) 1198.