# Ionic Liquid-hemoglobin-carbon Paste Composite Bioelectrode and Its Electrocatalytic Activity

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A new carbon ionic liquid paste bioelectrode was fabricated by mixing hemoglobin (Hb) with graphite powder, ionic liquid 1-ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$) and liquid paraffin homogeneously. Nafion film was cast on the electrode surface to improve the stability of bioelectrode. Direct electrochemistry of Hb in the bioelectrode was carefully investigated. Cyclic voltammetric results indicated that a pair of well-defined and quasi-reversible electrochemical responses appeared in pH 7.0 phosphate buffer solution (PBS), indicating that direct electron transfer of Hb was realized in the modified electrode. The formal potential ($E^{0'}$) was calculated as -0.316 V (vs. SCE), which was the typical characteristic of the electrochemical reaction of heme Fe(III)/Fe(II) redox couple. Based on the cyclic voltammetric results the electrochemical parameters of the electrode reaction were calculated. This bioelectrode showed high electrocatalytic activity towards the reduction of trichloroacetic acid (TCA) with good stability and reproducibility.

**Keywords:** Hemoglobin; Nafion; Ionic liquid; Carbon paste electrode; Direct electrochemistry.

## INTRODUCTION

Direct electron transfer between redox proteins and electrodes has established the basis for the development of biosensors and bioreactors, which can provide the mechanistic investigation on the electron transfer in the biological system.[1] But the electron exchange rate of protein with bare electrode is often slow due to the deep burying of the electroactive center and the unfavorable orientation of protein on the electrode surface.[2] This problem can be solved by using the redox mediators or fabricating a specific interface to fasten the electron transfer rate.[3-5] In recent years different kinds of modified electrodes had been devised for the third-generation biosensors with different methods or materials such as sol-gel,[6,7] surfactant,[8,9] hydrogel,[10,11] biopolymer,[12,13] composite film[14,15] or nanoparticles.[16,17]

Carbon paste electrode had been widely studied in the field of electroanalytical chemistry with the advantages such as low cost and easy preparation. By applying different kinds of modifier incorporated in the carbon paste, chemically modified carbon paste electrodes show many specific functions and applications. Various modifiers such as organic, inorganic and biomolecules have been used in the fabrication processes. For example Matuszewski et al.[18] firstly mixed glucose oxidase with graphite powder and silicon oil to get a carbon paste bioelectrode. Gorton[19] reviewed the progress of carbon paste bioelectrodes with various biological substances including enzyme, tissues and cells. The carbon paste enzyme electrode had been demonstrated to show excellent acidic and thermal ability, which was attributed to the conformational rigidity of the biocatalyst within the highly hydrophobic pasting liquid.[20,21]

In recent years ionic liquids (ILs) had been used in the field of electrochemical biosensors due to the higher ionic conductivity and wider electrochemical windows. ILs can be used as not only the modifier but also the binder in the preparation of the chemically modified electrodes. Wei et

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al.[22] reviewed the application of ILs in the electrochemical sensors. Maleki et al.[23] applied N-octylpyridinum hexafluorophosphate as a binder for the construction of carbon ionic liquid electrode (CILE) and investigated its electrochemical behaviors. Qiao[24] investigated a gellan and IL modified CILE for the direct electrochemistry of hemoglobin (Hb). Our groups also fabricated different kinds of ILs modified carbon paste electrode and applied to the detection of some electroactive substances.[25,26]

1-Ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$) is a hydrophilic ionic liquid with higher conductivity and smaller viscosity, also the hydrophilic solvent is benefit for the maintenance of structure and activity of protein. $EMIMBF_4$ had been used as the modifier for the preparation of ionic liquid modified carbon paste electrode and used as the substrate electrode for the furthur modification by multi-walled carbon nanotube (MWCNT). Direct electrochemistry of Hb on MWCNT/CILE was realized and carefully investigated.[27] In this paper Hb and hydrophilic IL of $EMIMBF_4$ were mixed with traditional carbon paste to get a carbon ionic liquid paste bioelectrode. A Nafion film was cast on the bioelectrode surface to improve the stability of electrode. Electrochemical behaviors of Hb in the bioelectrode were carefully investigated with a pair of well-defined redox peaks appeared, indicating the realization of direct electron transfer process. The fabricated bioelectrode showed good electrocatalytic ability to trichloroacetic acid (TCA), which provided a potential approach to the fabrication of the third-generation mediator-free biosensors.

## RESULTS AND DISCUSSION

### SEM images of modified electrodes

Scanning electron microscopic images of different modified electrodes were recorded and shown in Fig. 1. On the surface of CILE, an uniform surface could be observed without separated graphite layers (Fig. 1a), which was due to the embedment of high viscosity $EMIMBF_4$ between the layers of graphite particles. On Hb-CILE (Fig. 1b) many spots were found homogeneously distributed on the surface, which indicated that Hb molecules were dispersed in the carbon ionic liquid paste. After Nafion was cast on the carbon ionic liquid bioelectrode, a layer of film was formed (Fig. 1c). Nafion is a commonly used ion-exchange polymer with the characteristics such as good film forming ability, excellent biocompatibility and chemical resistance.[28,29] So the presence of Nafion can form a stable film on the electrode surface, which can prevent the leakage of Hb from dissolving into the buffer solution.

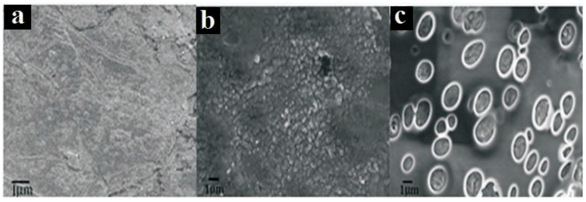

Fig. 1. SEM images of (a) CILE, (b) Hb-CILE and (c) Nafion/Hb-CILE.

### Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) is often used to investigate the impedance changes during the modification process, which can reflect the interface changes of electrode surface. The semicircular part at higher frequencies corresponds to electron transfer limited process and its diameter is equal to electron transfer resistance (Ret), which controls the electron-transfer kinetics of the redox probe at the electrode interface. In the frequencies range from 0.05 to $10^5$ Hz with $[Fe(CN)_6]^{3-/4-}$ redox couples as the electrochemical probe, the Nyquist plots of (a) Nafion/Hb-CPE, (b) Nafion/Hb-CILE and (c) Nafion/CILE were shown in Fig. 2. The Ret value (191.4 Ω) of Nafion/Hb-CILE (curve b) was bigger than that of Nafion/CILE (52.94 Ω) (curve c), indicating the existance of Hb in the CILE. The Ret value (595.9 Ω) of Nafion/Hb-CPE (curve a) was

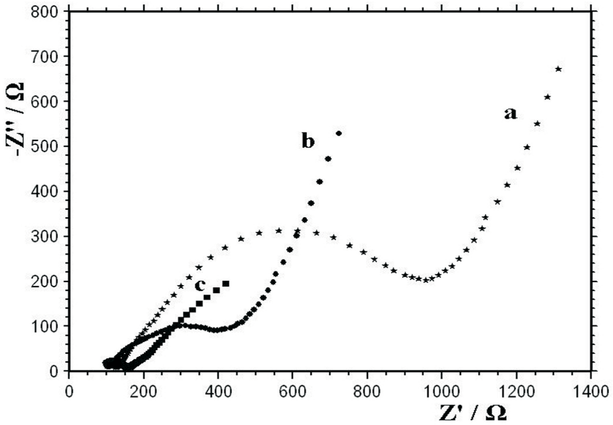

Fig. 2. Electrochemical impedance spectra for (a) Nafion/Hb-CPE, (b) Nafion/Hb-CILE, (c) Nafion/CILE in the presence of 10.0 mmol $L^{-1}$ $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol $L^{-1}$ KCl with the frequencies swept from $10^5$ to 0.05 Hz.

greatly larger than that of Nafion/Hb-CILE and Nafion/CILE. This might be due to the presence of non-conductive liquid paraffin in the carbon paste, which hindered the electron transfer rate and made it more difficult for the electron transfer reaction to take place.

### Direct electrochemistry of Nafion/Hb-CILE

Fig. 3 showed the typical cyclic voltammograms of different modified electrodes at the scan rate of 100 mV $s^{-1}$ in pH 7.0 PBS. No electrochemical responses appeared on the Nafion/CILE (curve b), which indicated no electroactive substances existed on the electrode. On the Nafion/Hb-CPE a pair of redox peaks appeared with small electrochemical response (curve c), indicating the direct electron transfer of Hb in the CPE could be realized but with slow rate. While on the Nafion/Hb-CILE (curve a) a pair of well-defined and stable redox peaks appeared, and the electrochemcial responses were bigger than that of Nafion/Hb-CPE. The results indicated that the presence of IL in the carbon paste greatly improved the electron transfer rate of Hb. Since ILs exhibit the characteristics including high ionic conductivity, wide electrochemical windows and good biocompatibility. Proteins and enzymes are usually active in IL that containing tetrafluoroborate ($BF_4^-$) or hexafluorophosphate ($BF_6^-$) anions.[30] Since the Hb molecules and ILs were co-existed in the carbon paste, then the direct electron transfer of Hb was greatly improved within the IL matrix. From curve a it can be seen that the cathodic (Epc) and anodic (Epa) peak potentials appeared at -0.253 V and -0.378 V (vs. SCE), respectively, and the formal potential ($E^{0'}$) was calculated as -0.316 V, which was in agreement with the typical characteristics of heme Fe(III)/Fe(II) redox couples. The peak-to-peak separation (ΔEp) was calculated as 125 mV, indicating that Hb immobilized in the carbon ionic liquid paste bioelectrode displayed a quasi-reversible electron transfer reaction. According to the references,[20, 21] the mixture of enzyme with carbon paste was an attractive method for the preparation of versatile and renewable reagentless biosensors, which showed the improvement to the stability of enzyme in the carbon paste microenvironment. The enzyme can also retain the biocatalytic activity in the hostile paste environment, which is related to the enzymology in the nonaqueous media such as the organic-phase environments. ILs are also green media for enzymes to retain their bioactivity, so the presence of ILs in the carbon paste can also provide a biocompatible microenvironment for enzymes. On the other hand, the presence of Nafion on the electrode surface exhibited good ability to retain Hb on the electrode surface and prevent interfering substances in the solution from reaching the electrode interface. So the direct electron transfer of Hb was successfully realized in the fabricated carbon ionic liquid paste bioelectrode.

### Influence of scan rate

The influence of scan rate on the electrochemical response of Nafion/Hb-CILE was investigated with the results shown in Fig. 4. A pair of well-defined redox peaks appeared with almost equal height of redox peak current, which indicated that all the electroactive ferric hemoglobin [Hb Fe(III)] was reduced to ferrous hemoglobin [Hb Fe(II)] on the forward scan and then the Hb Fe(II) produced

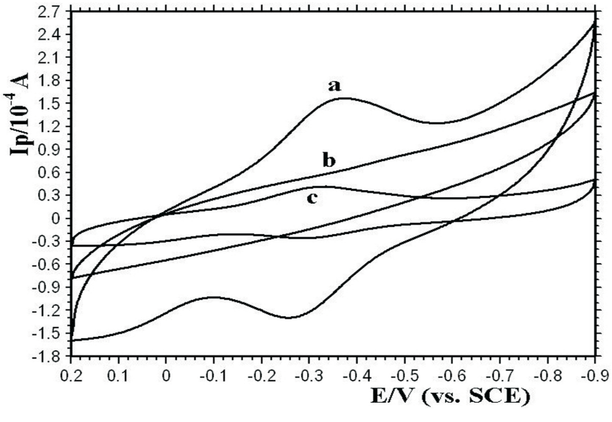

Fig. 3. Cyclic voltammograms of (a) Nafion/Hb-CILE, (b) Nafion/CILE and (c) Nafion/Hb-CPE in pH 7.0 PBS at the scan rate of 100 mV $s^{-1}$.

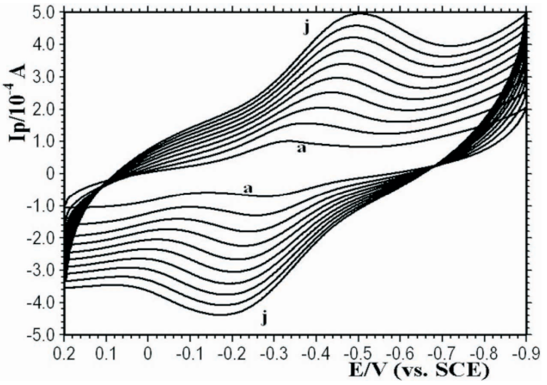

Fig. 4. Cyclic voltammograms of Nafion/Hb-CILE with different scan rates (from a to j: 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 mV $s^{-1}$) in pH 7.0 PBS.

was reoxidized to Hb Fe(III) on the reverse scan. Both the redox peak currents were proportional to scan rate in the range from 50.0 to 500.0 mV $s^{-1}$, which indicated that the electrode reaction was a surface-controlled process. The linear regression equations between the redox peak currents and scan rate were calculated as Ipa(μA) = -663.31 υ (V $s^{-1}$) -53.97 (γ = 0.997) and Ipc(μA) = 629.33 υ (V $s^{-1}$) +60.17 (γ = 0.999), respectively.

The surface concentration ($\Gamma^*$) of electroactive Hb can be further calculated by the integration of the reduction peak on the cyclic voltammograms based on the equation of $\Gamma^* = Q/nAF$. The integration of the reduction peak at different scan rates from 50.0 to 500.0 mV $s^{-1}$ gave a nearly constant value of charge (Q), so the average value of $\Gamma^*$ was got as $1.332 \times 10^{-8}$ mol $cm^{-2}$, which was much higher than the reported value.[31] The result indicated that Hb molecules in the carbon ionic liquid paste matrix had closer and effective contact to graphite powder with ILs as the medium.

With the increase of scan rate the redox peak potentials were also shifted with the increment of the peak-to-peak separation (ΔEp). Based on the Laviron's equations:[32,33]

$$Epa = E^{0'} + \frac{RT}{(1-\alpha)nF} \ln \upsilon$$

$$Epc = E^{0'} - \frac{RT}{\alpha nF} \ln \upsilon$$

$$Ep = E^{0'} + \frac{RT}{\alpha nF} \ln \frac{RTK_S}{\alpha nF} - \frac{RT}{\alpha nF} \ln \upsilon$$

$$\log k_S = \alpha \log(1-\alpha) + (1-\alpha)\log \alpha - \log \frac{RT}{nF\nu} - \frac{nF\Delta Epa(1-\alpha)}{2.3RT}$$

The electrochemical parameters can be calculated based on the relationship of the redox peak potentials with lnυ. The linear regression equations were got as Epa(V) = 0.046lnυ – 0.147 (γ = 0.990) and Epc(V) = -0.072lnυ – 0.551 (γ = 0.997), respectively. From the slope and the intercept, the values of electron transfer coefficient (α) and electrode reaction standard rate constant (*ks*) were calculated as 0.390 and 0.568 $s^{-1}$, respectively. The *ks* value was bigger than some other reported values,[34,35] which indicated a fast electron transfer rate of Hb in the carbon ionic liquid bioelectrode.

**Electrocatalysis towards trichloroacetic acid**

Due to the presence of Hb molecules in the modified electrode, the Nafion/Hb-CILE showed good electrocatalytic ability to the reduction of trichloroacetic acid (TCA). The catalytic activity of Nafion/Hb-CILE toward different amounts of TCA was investigated with the typical cyclic voltammograms shown in Fig. 5. With the increase of the TCA concentrations, the reduction peak currents increased gradually at -0.30 V (vs. SCE) with the simultaneously disappearance of the oxidation peak (curve a-g), which was a typical electrocatalytic reduction on the Hb modified electrode. The results suggested that the Hb Fe(II) had reacted with TCA to produce Hb Fe(III), which was further reduced on the electrode to form a catalytic cycle. The catalytic reduction mechanism can be expressed as follows:

$$\text{Hb heme Fe(III)} + e \leftrightarrow \text{Hb heme Fe(II) at electrode} \quad (1)$$

$$Cl_3CCOOH \leftrightarrow Cl_3CCOO^- + H^+ \quad pKa = 0.89 \quad (2)$$

$$\text{2Hb heme Fe(II)} + Cl_3CCOOH + H^+ \leftrightarrow \text{2Hb heme Fe(III)} + Cl_2HCCOOH + Cl^- \quad (3)$$

The linear relationship between the reduction peak current and the TCA concentration was constructed in the range of 33.0~154.0 mmol $L^{-1}$ with the linear regression equation as Ip(μA) = 4.17C (mmol $L^{-1}$) + 218.84 (γ = 0.998) and the detection limit was calculated as 8.5 mmol $L^{-1}$ (3σ). At a higher concentration of TCA in the buffer solution, the calibration curve tended to level off and reached a constant value, which indicated a typical Michaelis-Menten kinetic process. Based on the electrochemical version of the Lineweaver-Burk equation,[36]

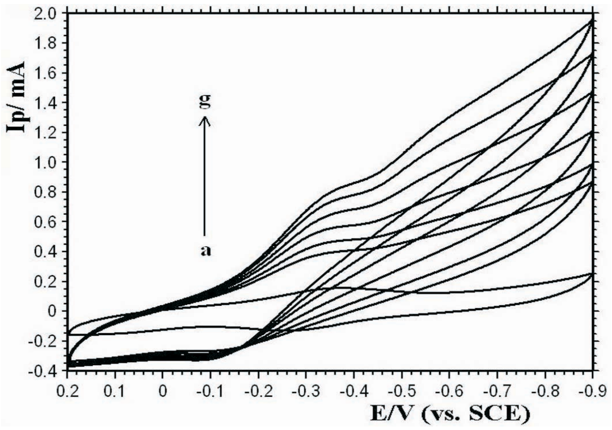

Fig. 5. Cyclic voltammograms of Nafion/Hb-CILE in pH 7.0 PBS before (a) and after the addition of 38.0, 48.0, 72.0, 100.0, 124.0, 128.0 mmol $L^{-1}$ of TCA (curve b-g), respectively, at the scan rate of 100 mV $s^{-1}$.

$$\frac{1}{I_{SS}} = \frac{1}{I_{\max}} + \frac{K_M^{app}}{I_{\max} C}$$

the apparent Michaelis-Menten constant ($K_M^{app}$) can be calculated, where C is the concentration of TCA in the solution, $I_{ss}$ is the steady current after the addition of TCA, and $I_{max}$ is the maximum current measured under saturated substrate condition. According to this equation, the $K_M^{app}$ value for Nafion/Hb-CILE based TCA biosensor was calculated as 0.0969 mol $L^{-1}$, which was similar to a previous reported value.[37] It is known that the smaller $K_M^{app}$ value indicates the higher catalytic activity, so the Hb molecules in the Nafion/Hb-CILE show good bioactivity.

### Stability of the modified electrode

The stability of the Nafion/Hb-CILE was firstly tested by comparing the cyclic voltammetric peak currents of Hb with time intervals of 2 h. Only slight changes happened in cyclic voltammograms after 2 days, which indicated the presence of Nafion on the electrode surface could form a stable film. Nafion can be served as an effective membrane to prevent the leakage of Hb and protect the electrode surface from interference. After two weeks storage of the modified electrode at 4 ºC, 97.3% of its initial current response was retained, demonstrating the Nafion/Hb-CILE had good long-term storage stability.

## CONCLUSION

In this paper, a new Hb modified carbon ionic liquid paste bioelectrode was fabricated by mixing hemoglobin, ionic liquid $EMIMBF_4$, graphite powder and liquid paraffin together in an agate mortar. Nafion was used as a stable film material to retain the Hb molecules on the surface of electrode. Direct electron transfer of Hb was realized and enhanced on the electrode with a pair of well-defined redox peaks appeared. The results can be attributed to the presence of biocompatibility and higher ionic conductivity of $EMIMBF_4$, which provided a suitable microenviroment for Hb to mix homogeneously with graphite powder. Compared with another ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIMPF_6$) used for the fabrication of the paste bioelectrode,[38] the smaller value of $\Delta Ep$ with the bigger values of $\Gamma^*$ and *ks* appeared in this paper showed the faster electron transfer rate. The results were attributed to the different physicochemcial properities of $EMIMBF_4$, which was a hydrophilic solvent with high conductivity and small viscosity than the hydrophobic $BMIMPF_6$. The fabricated bioelectrode showed good electrocatalytic ability to the reduction of TCA, which provided a new way for fabricating third generation biosensor.

## EXPERIMENTAL

### Apparatus and reagents

Bovine hemoglobin (Hb, MW. 64 500, Tianjin Chuanye Biochemical Company, China), 1-ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$, Hangzhou Kemer Chemical Company, China), graphite powder (average particle size 30 μm, Shanghai Colloid Chemical Company, China), liquid paraffin (Laiyang Shuangshuang Chemical Limited Company, China) and Nafion (0.5% ethanol solution, Sigma) were used as received without further purification. All the electrochemical measurements were carried out with 0.1 mol $L^{-1}$ phosphate buffer solution (PBS) as supporting electrolyte. Other chemicals used were of analytical grade and doubly distilled water was used throughout.

All the electrochemical experiments including cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed on a CHI 750 B electrochemical workstation (Shanghai CH Instrument, China). A conventional three-electrode system was employed with a Hb modified electrode as working electrode, a platinum wire as counter electrode and a saturated calomel electrode (SCE) as reference electrode. Scanning electron microscopy (SEM) was performed with a JSM-6700F scanning electron microscope (Japan Electron Company, Japan).

### Preparation of ionic liquid-graphite powder-Hb composite paste electrode

Carbon paste (CP) was prepared by simply mixing graphite powder and liquid paraffin (3 g: 1 mL) thoroughly in an agate mortar. Carbon ionic liquid paste (CIL) was obtained by mixing graphite powder with liquid paraffin and $EMIMBF_4$ (3 g: 0.25 mL: 0.75 mL). The Hb modified CIL (Hb-CIL) was prepared by directly mixing Hb with the carbon ionic liquid paste at the weight ratio of 3:5 and ground homogeneously in an agate mortar. As for comparison, the Hb modified CP (Hb-CP) was prepared by the similar procedure with the same weight ratio. Portions of the modified carbon paste was packed into one end of glass tube ($\Phi = 4.2$ mm) and copper wire was inserted through the opposite end to establish an electrical contact. Then different kinds of chemically modified carbon paste electrodes (CILE, Hb-CPE, Hb-CILE) were fabricated and these electrodes were stored at 4 ºC when not in use. Prior to use the surfaces of the prepared electrodes were gently polished on a piece of weighing paper and a 8 μL volume of 0.5% Nafion solution was cast on the electrode surface to form a stable film, which could prevent the leakage of Hb from electrode surface and increase the stability of the modified electrode. Then these modified electrodes were demonstrated as Nafion/CILE, Nafion/Hb-CPE and Nafion/Hb-CILE, respectively.

### Electrochemical procedure

The three-electrode system was placed in a 10 mL electrochemical cell containing 0.1 mol $L^{-1}$ pH 7.0 PBS and cyclic voltammetric experiments were performed in the potential range from +0.2 to –0.9 V (vs. SCE) at the scan rate of 100 mV $s^{-1}$. Electrochemical impedance spectroscopy (EIS) was preformed in a 0.1 mol $L^{-1}$ KCl solution containing 10.0 mmol $L^{-1}$ $[Fe(CN)_6]^{3-/4-}$ with the applied AC voltage amplitude as 5 mV and the voltage frequencies swept from $10^5$ to 0.05 Hz. All the buffers were purged with high-purity nitrogen for 15 min before the experiments and the electrochemical experiments were carried out at 25 ± 2 ºC.

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## REFERENCES

1. Armstrong, F.-A.; Wilson, G.-S. *Electrochim. Acta* **2000**, *45*, 2623.
2. Rusling, J.-F. In *Protein Architecture: Interfacing Molecular Assemblies and Immobilization Biotechnology*; Mohwald, H.; Lvov, Y., Eds.; Marcel Dekker: New York, 2000.
3. Willner, I.; Katz, E. *Angew. Chem. Int. Ed.* **2000**, *39*, 1180.
4. Dai, Z.-H.; Liu, S.-Q.; Ju, H.-X.; Chen, H.-Y. *Biosens. Bioelectron.* **2004**, *19*, 861.
5. Zhai, Z.-Q.; Wu, J.; Sun, W.; Jiao, K. *J. Chin. Chem. Soc.* **2009**, *56*, 561.
6. Liu, Y.; Wang, M.-J.; Li, J.; Li, Z.-Y.; He, P.; Liu, H.-T.; Li, J.-H. *Chem. Commun.* **2005**, *13*, 1778.
7. Chen, X.; Jia, J.-B.; Dong, S.-J. *Electroanalysis* **2003**, *15*, 608.
8. Lin, R.; Bayachou, M.; Greaves, J.; Farmer, P.-J. *J. Am. Chem. Soc.* **1997**, *119*, 12689.
9. Nassar, A.-E.; Willis, W.-S.; Rusling, J.-F. *Anal. Chem.* **1995**, *67*, 2386.
10. Ray, A.; Feng, M.; Tachikawa, H. *Langmuir* **2005**, *21*, 7456.
11. Lu, H.-Y.; Yang, J.; Rusling, J.-F.; Hu, N.-F. *Electroanalysis* **2006**, *18*, 379.
12. Sun, W.; Wang, D.-D.; Gao, R.-F.; Jiao, K. *Electrochem. Commun.* **2007**, *9*, 1159.
13. Li, X.-Q.; Wang, Y.; Sun, X.-Y.; Zhan, T.-R.; Sun, W. *J. Chem. Sci.* **2010**, *122*, 271.
14. Lu, X.-B.; Hu, J.-Q.; Yao, X.; Wang, Z.-P.; Li, J.-H. *Biomacromolecules* **2006**, *7*, 975.
15. Wang, D.-D.; Liu, H.-J.; Zhao, C.-Z.; Hui, N.; Sun, W. *J. Chin. Chem. Soc.* **2010**, *57*, 99.
16. Luo, X.-L.; Killard, A.-J.; Smyth, M.-R. *Electroanalysis* **2006**, *18*, 1131.
17. Sun, W.; Gao, R.-F.; Jiao, K. *J. Phys. Chem. B* **2007**, *111*, 4560.
18. Matuszewski, W.; Trojanowica, M. *Analyst* **1988**, *113*, 735.
19. Gorton, L. *Electroanalysis* **1995**, *7*, 23.
20. Wang, J.; Liu, J.; Cepra, G. *Anal. Chem.* **1997**, *69*, 3124.
21. Wang, J.; Musameh, M.; Mo, J.-W. *Anal. Chem.* **2006**, *78*, 7044.
22. Wei, D.; Ivaska, A. *Anal. Chim. Acta* **2008**, *607*, 126.
23. Maleki, N.; Safavi, A.; Tajabadi, F. *Anal. Chem.* **2006**, *78*, 3820.
24. Qiao, L.-F.; Zheng, J.-B.; Gao, R.-F.; Sheng, Q.-L. *J. Chin. Chem. Soc.* **2010**, *57*, 718.
25. Sun, W.; Zhai, Z.-Q.; Wang, D.-D.; Liu, S.-F.; Jiao, K. *Bioelectrochemistry* **2009**, *74*, 295.
26. Zhao, R.-J.; Jiang, Q.; Sun, W.; Jiao, K. *J. Chin. Chem. Soc.* **2009**, *56,* 158.
27. Sun, W.; Li, X.-Q.; Wang, Y.; Zhao, R.-J.; Jiao, K. *Electrochim. Acta* **2009**, *54*, 4141.
28. Huang, Q.-D.; Lu, Z.-Q.; Rusling, J.-F. *Langmuir* **1996**, *12*, 5472.
29. Nadzhafova, O.-Y.; Zaitsev, V.-N.; Drozdova, M.-V.; Vaze, A.; Rusling, J.-F. *Electrochem. Commun.* **2004**, *6*, 205.
30. Park, S.; Kazlauskas, R.-J. *Curr. Opin. Biotechnol.* **2003**, *14*, 432.
31. Lu, X.-B.; Zhou, J.-H.; Lu, W.; Liu, Q.; Li, J.-H. *Biosens. Bioelectron.* **2008**, *23*, 1236.
32. Laviron, E. *J. Electroanal. Chem.* **1974**, *52*, 355.
33. Laviron, E. *J. Electroanal. Chem.* **1979**, *101*, 19.
34. Zhao, Y.-D.; Bi, Y.-H.; Zhang, W.-D.; Luo, Q.-M. *Talanta* **2005**, *65*, 489.
35. Li, Q.-W.; Luo, G.-A.; Feng, J. *Electroanalysis* **2001**, *13*, 359.
36. Kamin, R.-A.; Wilson, G.-S. *Anal. Chem.* **1980**, *52*, 1198.
37. Sun, W.; Wang, D.-D.; Li, G.-C.; Zhai, Z.-Q.; Zhao, R.-J.; Jiao, K. *Electrochim. Acta* **2008**, *53*, 8217.
38. Sun, W.; Gao, R.-F.; Li, X.-Q.; Wang, D.-D.; Yang, M.-X.; Jiao, K. *Electroanalysis* **2008**, *20*, 1048.