

# Improvement of carbon paste-based enzyme electrode using a new ionic liquid [Pmim][PF<sub>6</sub>] as the binder

Lu Lu · Xirong Huang · Yinbo Qu

Received: 10 March 2012 / Accepted: 6 May 2012 / Published online: 24 May 2012  
© Springer-Verlag 2012

**Abstract** A new carbon ionic liquid electrode (CILE) has been constructed using a low melting point (39 °C) hydrophobic ionic liquid (IL) 1-propyl-3-methylimidazolium hexafluorophosphate ([Pmim][PF<sub>6</sub>]) as the binder. Both cyclic voltammetry and electrochemical impedance spectroscopy demonstrate that, in addition to the composition optimization of the IL/graphite composite, heating the composite at a temperature a little higher than the melting point of [Pmim][PF<sub>6</sub>] can also lower the background current and enhance the mechanical strength of the CILE. The heated CILE is more sensitive than the traditional carbon paste electrode for the detection of H<sub>2</sub>O<sub>2</sub>. Glucose oxidase (GOx) can be easily entrapped in the bulk IL/graphite composite. Heating the GOx-modified CILE (GOx-CILE) at the melting point of [Pmim][PF<sub>6</sub>] does not lower the catalytic activity of GOx. As compared with *n*-octylpyridinium hexafluorophosphate (melting point 65 °C) as the binder, [Pmim][PF<sub>6</sub>]-based CILE is much better in signal-to-noise ratio. Under the optimum conditions, the [Pmim][PF<sub>6</sub>]-based GOx-CILE has a linear amperometric response to glucose over a concentration range of 2.0–26 mM with the detection limit as low as 0.39 mM. It follows that choosing an IL with a melting point of ca. 40 °C as a binder to fabricate enzyme-

entrapped CILEs is a good strategy for the enhancement of the performance of the electrode.

**Keywords** Low melting point solid ionic liquid · Carbon ionic liquid electrode · Glucose oxidase · Enzymatic activity

## Introduction

Carbon paste electrodes (CPEs), which were first introduced by Adams [1], are a mixture of a graphite powder and a nonconductive, chemically inert liquid binder such as mineral oil, paraffin oil, or Nujol. Due to their low cost, ease of fabrication, low background current, relatively wide electrochemical windows, renewable surface and tunable composition, CPEs have been widely used in electrochemistry and electroanalytical chemistry fields [2–4]. The main disadvantages of CPE are that the liquid binders are not conductive, which to some extent weaken the electrochemical signal [5], and that the components from different sources are not constant and some unaccounted ingredients may engender unpredictable influences on the performance of the electrode.

Ionic liquids (ILs) are molten salts, which have some unique physicochemical properties, such as high ionic conductivity, wide electrochemical windows, nonflammability, good chemical and thermal stability, negligible vapor pressure, etc. [6, 7]. As supporting electrolytes [8–10] or modifying materials of electrodes [11–14], ILs have been widely used in the field of bioelectrochemistry and bioelectrocatalysis. The high viscosity of ILs limits their application in many fields [15], but it favors for the fabrication of IL-based CPEs. Recently, there are many reports on the fabrication of CPEs using ILs as binders [called carbon ionic liquid electrodes (CILEs)], which have been used in electrochemical

**Electronic supplementary material** The online version of this article (doi:10.1007/s10008-012-1772-x) contains supplementary material, which is available to authorized users.

L. Lu · X. Huang (✉)  
Key Laboratory of Colloid and Interface Chemistry of the  
Education Ministry of China, Shandong University,  
Jinan 250100, People's Republic of China  
e-mail: xrhuang@sdu.edu.cn

X. Huang · Y. Qu  
State Key Laboratory of Microbial Technology of China,  
Shandong University,  
Jinan 250100, People's Republic of China

fields [16–20]. With reference to CPEs, CILEs have more unique properties such as low resistance, large potential windows, low overpotential, antifouling, etc. One drawback is their high background current, which limits their further application.

*n*-Octylpyridinium hexafluorophosphate (OPFP) is an IL that is solid at room temperature (melting point 65 °C). Using OPFP as a binder, Maleki et al. [21] fabricated a CILE and found that heating the composite material at a temperature higher than the melting point of OPFP could not only obtain a uniform and robust carbon paste but also lower the background current and improve the electrochemical behaviors of many electroactive compounds on the CILEs [22]. However, heating the CILE fabricated using a liquid IL at room temperature could not decrease the background current [21]. Recently, Musameh et al. [23] prepared an enzyme-containing CILE by entrapping glucose oxidase (GOx) in the CILE mixture of OPFP and graphite powder. They adjusted the ratio of OPFP to graphite powder to lower the background current of the CILE, but no heating was made to avoid the decrease or even loss of the catalytic activity of GOx. It follows that an IL with the melting point slightly higher than room temperature as a binder will be a good candidate for the fabrication of high-performance enzyme-containing CILEs.

In the present paper, a hydrophobic IL 1-propyl-3-methylimidazolium hexafluorophosphate ([Pmim][PF<sub>6</sub>]) with a melting point of 39 °C is chosen as a binder to fabricate CILE. By entrapping an appropriate amount of GOx in the CILE, an amperometric glucose electrode was prepared. Both the adjustment of the ratio of [Pmim][PF<sub>6</sub>] to graphite powder and heating measures are taken to decrease the background current, to increase the signal-to-noise ratio, and to enhance mechanical strength of the CILE. The results demonstrate that the present strategy does improve the performance of the enzyme-containing electrode.

## Experimental

### Chemicals

ILs [Pmim][PF<sub>6</sub>] (melting point 39 °C, purity 99 %) and OPFP (melting point 65 °C, purity 99 %) were provided by Shanghai Chengjie Chemical and used without further purification; paraffin oil (A. R.), KCl (A. R.), and D-(+)-glucose (A. R.) were all purchased from Sinopharm Chemical Reagent; graphite powder (average particle size 4 μm) was purchased from Shanghai Huayi Group Huayuan Chemical Industry; K<sub>3</sub>[Fe(CN)<sub>6</sub>] was obtained from Reagent No. 1 Factory of Shanghai Chemical Reagent; H<sub>2</sub>O<sub>2</sub> (A. R., 30 wt.%) was purchased from Tianjin Bodi Chemical Industry Stock; GOx (EC 1.1.3.4, from *Aspergillus niger*,

22.5 U mg<sup>-1</sup>) was purchased from Fluka and used as received; 0.1 M phosphate buffers with various pH values were prepared by mixing stock solutions of Na<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> and then adjusting the pH with 0.1 M H<sub>3</sub>PO<sub>4</sub> or NaOH. All other reagents were of analytical grade and used as received. Triply distilled water was used throughout the experiments.

### Preparation of electrodes

CILE was prepared by hand-mixing [Pmim][PF<sub>6</sub>] and graphite powder in a weight ratio using pestle and mortar for 20 min. An appropriate amount of the resulting paste (ca. 15.7 mm<sup>3</sup>) was packed firmly into a clean glass tube. The electrode (its geometric area is 3.14 × 10<sup>-2</sup> cm<sup>2</sup>) was then heated in an oven at 40 °C for 10 min. Prior to cooling, a copper wire was inserted into the paste to establish an electrical contact. For comparison, another CILE was prepared in the same way without heating.

GOx-CILE was prepared by hand-mixing a required amount of GOx and the mixture of [Pmim][PF<sub>6</sub>] and graphite powder (50 mg, in an optimum ratio) for another 10 min. An appropriate amount of the resulting paste was packed firmly into a clean glass tube. The electrode was then heated in an oven at 40 °C for 10 min. Prior to cooling, a copper wire was inserted in the paste to establish an electrical contact. For comparison, another GOx-CILE was prepared in the same way without heating.

The procedure for CPE fabrication was reported elsewhere [21].

GOx-CPE was prepared by hand-mixing an optimum amount of GOx (5 mg) and the mixture of paraffin oil and graphite powder [50 mg, 3/7 (w/w)] for 10 min. An appropriate amount of the resulting paste was then packed into a clean glass tube. Finally, a copper wire was inserted in the paste to establish an electrical contact.

The procedure for fabricating OPFP-based GOx-CILE was reported elsewhere [23].

Prior to use, the surface of the as-prepared electrodes were smoothed on a weighing paper and rinsed carefully with triply distilled water. When not in use, the aforementioned electrodes were stored at 4 °C.

### Apparatus

Cyclic voltammetric and amperometric measurements were performed on a CHI 630 C electrochemical workstation (Shanghai Chenhua, China). Electrochemical impedance spectroscopy (EIS) was recorded on a Zahner IM6 electrochemical workstation. The three-electrode system was composed of a working electrode (CILE, GOx-CILE, CPE, or GOx-CPE), a platinum wire counter-electrode and a saturated calomel electrode (SCE) reference electrode. Cyclic

voltammetry and EIS experiments were carried out under quiescent conditions. The amperometric measurement was made under a hydrodynamic condition. All the potentials given in this paper were versus SCE. All the measurements were carried out at ca. 25 °C.

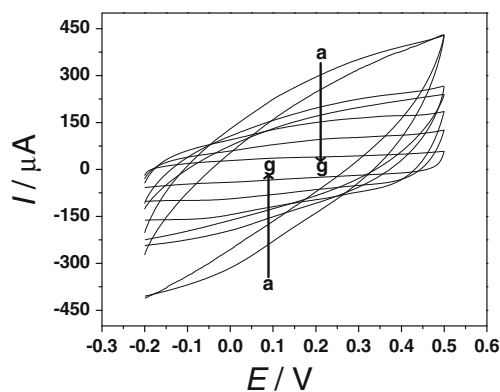
The surface morphologies of different electrodes were characterized with a JEOL JSM-6700F field emission scanning electron microscope (SEM). The Fourier transform infrared (FT-IR) spectra of GOx in unheated and heated materials were collected on FT-IR spectrometer (VERTEX-70, Bruker).

## Results and discussion

### Optimization of the IL/graphite ratio in CILE

Figure 1 shows the effect of the ratio of IL to graphite powder on the background current of the heated CILE in phosphate buffer (0.1 M, pH 7.5). It is seen that the CILE with 20 % loading of the IL shows very large background current. Moreover, it was found that the carbon paste fell off gradually from the surface of the CILE, indicating low stability of this composition. With the increase of IL loading, a dramatic decrease in background current as well as an improved stability of the CILE was observed. This is because IL is needed to bind graphite particles together. In addition to the  $\pi$ - $\pi$  interaction between the IL and graphite, which favors for the mechanical stability, the filling of the interstice between particles with the IL also enhances the electrical contact and prevents water penetration into the bulk composite material, leading to lower background currents [23].

To further evaluate the performance of the CILE, we investigated the electrochemical behaviors of  $K_3[Fe(CN)_6]$  at the CILE. Online Resource 1 (Table 1) shows the



**Fig. 1** Cyclic voltammograms of heated CILE prepared in different ratios of IL to graphite powder: 2/8 (a), 3/7 (b), 4/6 (c), 5/5 (d), 6/4 (e), 7/3 (f), and 8/2 (g), respectively. Supporting electrolyte: phosphate buffer (0.1 M, pH 7.5). Scan rate: 100 mV s<sup>-1</sup>

electrochemical parameters of 5.0 mM  $K_3[Fe(CN)_6]$  in 0.5 M KCl solution at heated CILEs. It can be seen that at the CILE with the IL/graphite ratio of 6/4, the peak current, both the anodic and the cathodic one, is the largest, the ratio of the anodic to cathodic peak current is the closest to 1, and the peak potential separation is the closest to 56.5 mV (25 °C). These results indicate that, in addition to the largest electrochemical response, the reversibility of the electron transfer of  $K_3[Fe(CN)_6]$  is the best at this CILE. Taking into account the good conductivity and relatively low background current, the 60 % loading of IL was used to prepare CILE for the subsequent experiments.

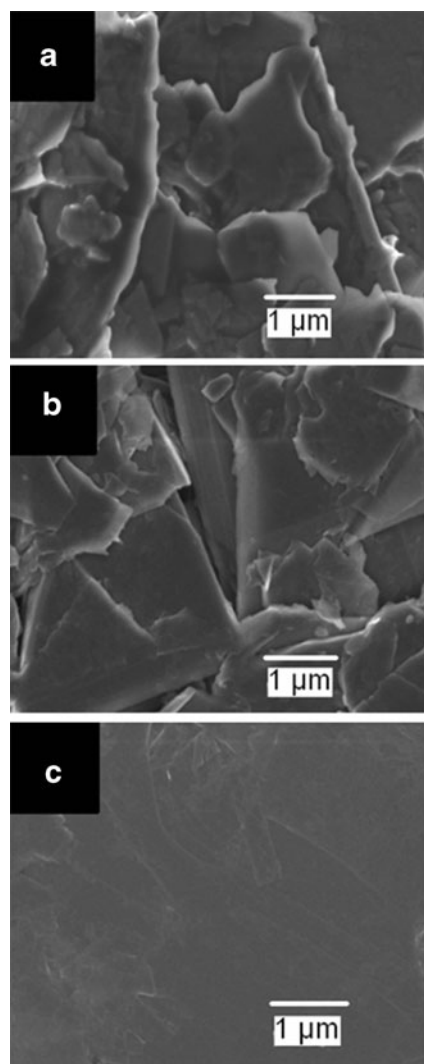
### Characterization of CILE

#### Morphological characterization

Scanning electron microscopy (SEM) was used to characterize the morphologies of different electrodes. As shown in Fig. 2, on the surfaces of CPE and unheated CILE, there exist some irregularly shaped graphite flakes, which are stacked together with the help of binders. The surface of the heated CILE is, however, uniform, and no separated graphite layer could be observed. This is because the melted IL is easy to fill into the interstice between graphite layers and therefore bridge the carbon flakes [5]. It follows that, in addition to mechanical mixing of ILs with graphite powder to enhance the  $\pi$ - $\pi$  interaction [24], heating the ILs with their melting point higher than room temperature is a pivotal step to obtain a smooth electrode surface.

#### Electrochemical characterization

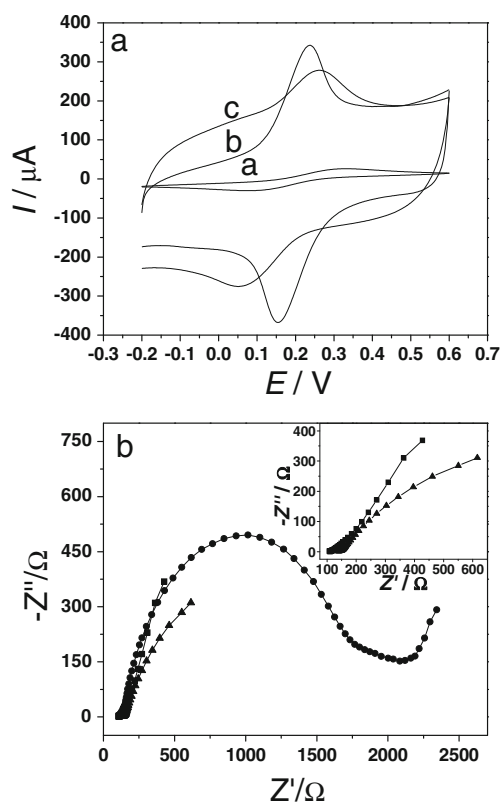
Figure 3a shows the cyclic voltammograms of 5.0 mM  $K_3[Fe(CN)_6]$  in 0.5 M KCl solution at traditional CPE (a), heated CILE (b), and unheated CILE (c), respectively. As compared with the CPE, the heated and unheated CILEs produce much larger peak currents. These changes can be explained as follows: (1) due to the good adherence of the conductive IL to the graphite particles and the favorable  $\pi$ - $\pi$  interaction between the imidazole ring on IL and graphite [24], the carbon paste with conductive IL as the binder has a lower resistance and larger electrochemical response than that with nonconductive paraffin oil as the binder; (2) as reported elsewhere [24], the IL has electrocatalytic activity toward the oxidoreduction of the probe; (3) some  $K_3[Fe(CN)_6]$  can pass through the hydrophobic but polar IL/electrolyte interface and discharge at the graphite/IL interface and that results in an increase in the electroactive area of CILE. For CILE, the reversibility of the heated one is much better than that of the unheated one. This is because heating the IL can decrease the background current and increase the voltammetric response [21, 22]. Online Resource 2 (Table 2) shows that the signal-to-



**Fig. 2** SEM images of different electrodes: **a** CPE, **b** unheated CILE and **c** heated CILE

noise ratio at the heated CILE is much bigger than that at the unheated one.

EIS is a powerful technique for the characterization of the electrode performance. The semicircular part at higher frequencies corresponds to the electron transfer limited process and the linear part at lower frequencies corresponds to the diffusion-limited process. The electron transfer resistance, which reflects the interfacial electron transfer ability, can be obtained from the diameter of the semicircle in Nyquist plot. Figure 3b shows the Nyquist plots of the three electrodes using  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  as the probe with the frequencies swept from  $10^5$  to 0.1 Hz. Similar to the voltammetric signals, the impedance responses also show a significant difference between the CPE and the CILE. As shown in Fig. 3b, a semicircle with a large diameter in higher frequency range is observed at the CPE, indicating a low electron transfer rate at the electrode surface due to a high electron transfer resistance. However, no semicircle is observed in the

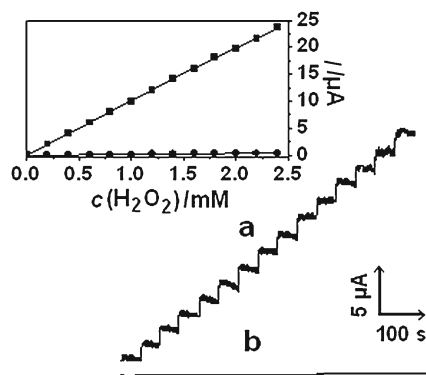


**Fig. 3** **a** Cyclic voltammograms of 5.0 mM  $\text{K}_3[\text{Fe}(\text{CN})_6]$  in 0.5 M KCl at CPE (**a**), heated CILE (**b**), and unheated CILE (**c**), respectively. Scan rate:  $100 \text{ mV s}^{-1}$ . **b** EIS of CPE ( $\bullet$ ), heated CILE ( $\blacksquare$ ), and unheated CILE ( $\blacktriangle$ ) in the aqueous solution of 10 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  and 0.1 M KCl with the frequencies swept from  $10^5$  to 0.01 Hz. Inset Amplified EIS of heated CILE ( $\blacksquare$ ) and unheated CILE ( $\blacktriangle$ )

Nyquist plot of the CILE, indicating a high electron transfer rate at the CILE. This is because the IL has a mass of caves in its molecule structure which favors for holding more charges [25, 26]. The good conductivity of the IL also facilitates fast electron transfer. It follows that ILs play a great role in improving the electrochemical performance of these electrodes.

#### Electrochemical response of $\text{H}_2\text{O}_2$ at the heated CILE

Figure 4 shows a typical amperometric response of the heated CILE (**a**) and CPE (**b**) at a potential of +0.9 V on the successive addition of 0.2 mM  $\text{H}_2\text{O}_2$ . With the addition of  $\text{H}_2\text{O}_2$  into the stirred phosphate buffer, the heated CILE responded rapidly to the analyte and a maximum steady-state current was achieved within 5 s. By contrast, the electrochemical response of  $\text{H}_2\text{O}_2$  was hardly observed at the CPE. The corresponding calibration curves are also shown in Fig. 4 (see inset). It is seen that over a range of 0.2–2.4 mM  $\text{H}_2\text{O}_2$ , the calibration curve at the heated CILE is linear, indicating that  $\text{H}_2\text{O}_2$  can be detected effectively at the heated CILE.



**Fig. 4** Current response of the heated CILE (a) and CPE (b) at +0.9 V on the successive addition of 0.2 mM H<sub>2</sub>O<sub>2</sub> into the stirred phosphate buffer (0.1 M, pH 7.5). *Inset* Calibration curves of the CILE (■) and CPE (●) in the phosphate buffer containing various H<sub>2</sub>O<sub>2</sub> concentrations

*Characterization of enzyme-entrapped CILE*

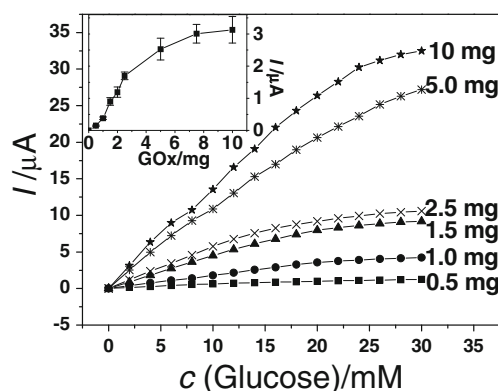
GOx, which is a frequently used model, is used to characterize the enzyme-entrapped CILE. GOx is a dimer with a molecular weight of ca. 150–180 kDa and contains two tightly bound flavine adenine dinucleotide (FAD) cofactors [27]. The quantitative detection of glucose here is based on the GOx-catalyzed conversion of glucose to H<sub>2</sub>O<sub>2</sub> and the amperometric response of the heated GOx-CILE toward H<sub>2</sub>O<sub>2</sub>.

*Optimization of the experimental conditions*

To obtain large electrochemical signal, the amperometric conditions were optimized. Online Resource 3 (Fig. S1) shows the effect of the pH on the steady-state response current at the heated GOx-CILE. Over the pH range of 5.0–8.0, a positive amperometric response of the electrode toward 2.0 mM glucose in 0.1 M phosphate buffer was observed with the maximum response at pH 7.5, which was chosen for the subsequent experiments.

The effect of the applied potential on the response current was also investigated. As shown in Online Resource 4 (Fig. S2), with the increase of the applied potential (from +0.5 to +1.0 V), the response current of the heated GOx-CILE toward 2.0 mM glucose first increases then levels off at the applied potential higher than +0.9 V. For sensitive detection, +0.9 V was chosen as the optimum potential.

The GOx-CILE containing different GOx loadings was prepared by mixing a required amount of GOx in 50 mg IL/graphite [6/4 (w/w)] composite. Figure 5 shows the calibration plots of glucose at the heated GOx-CILE containing different GOx loadings. It is seen that the response current increases with the increase of the concentration of glucose, indicating that glucose can be detected at the enzyme electrode. At the GOx loading of 5.0 mg, the linear range of the calibration curve is as wide as 26 mM. To obtain an

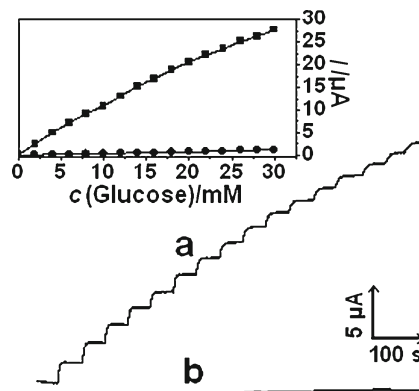


**Fig. 5** Calibration curves of glucose using the heated GOx-CILE which contains 0.5 mg (■), 1.0 mg (●), 1.5 mg (▲), 2.5 mg (×), 5.0 mg (\*), or 10 mg (★) GOx, respectively. *Inset* Current responses of the heated GOx-CILE toward 2.0 mM glucose at different GOx loadings. Applied potential: +0.9 V. Supporting electrolyte: phosphate buffer (0.1 M, pH 7.5)

optimum GOx loading, the response current of the heated CILE toward 2.0 mM glucose is plotted against the GOx loading (see inset in Fig. 5). It is seen that the signal increases with the increase of the GOx loading, but it levels off at the GOx loading higher than 7.5 mg. Taking account into the linear range, a loading of 5.0 mg GOx was chosen to fabricate the enzyme electrode.

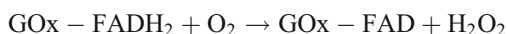
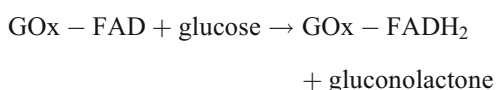
*Amperometric response of the heated GOx-CILE toward glucose*

Figure 6 shows a typical amperometric response of the GOx-CILE (a) and GOx-CPE (b) toward glucose under the optimum conditions. For clear observation on the response of the heated GOx-CILE toward glucose and easy comparison with that of GOx-CPE, 2.0 mM glucose was added each time. As shown in Fig. 6, with the addition of glucose



**Fig. 6** Current response of the heated GOx-CILE (a) and the GOx-CPE (b) at +0.9 V on the successive addition of 2.0 mM glucose into the stirred phosphate buffer (0.1 M, pH 7.5). *Inset*: Calibration curves of the heated GOx-CILE (■) and the GOx-CPE (●) at +0.9 V in the phosphate buffer containing various glucose concentrations

into the stirred phosphate buffer, the heated GOx-CILE responded rapidly and a maximum steady-state current was achieved within 10 s. Under the same conditions, however, the response was hardly observed at the GOx-CPE. The calibration curve of glucose at the heated GOx-CILE (see inset in Fig. 6) shows a good linear line over a glucose concentration range of 2.0–26 mM with the detection limit as low as 0.39 mM (calculated based on three times the noise), indicating that the heated GOx-CILE has good biosensing ability toward glucose. It is worth to mention that the linear range for glucose at the heated GOx-CILE is not in accordance with that for H<sub>2</sub>O<sub>2</sub> at the heated CILE. This is because the response current at the heated GOx-CILE comes from the enzymatically liberated H<sub>2</sub>O<sub>2</sub> [28]:

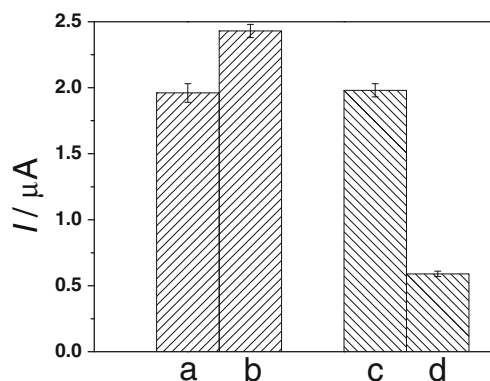


In fact, not all the added glucose is converted to H<sub>2</sub>O<sub>2</sub> via the enzymatic reaction.

For the determination of glucose in a complex sample, the applied potential of +0.9 V was too high and the selectivity deteriorated. This paper focused on the improvement of the enzyme-containing CILE by decreasing the background current and maintaining the catalytic activity of the enzyme. For the sensitive detection described above and for the reasonable comparison of the present improved electrode with others, we herein chose +0.9 V as the detecting potential. Improvement of the selectivity of the enzyme electrode for real applications is in progress.

#### Stability and reproducibility of the heated GOx-CILE

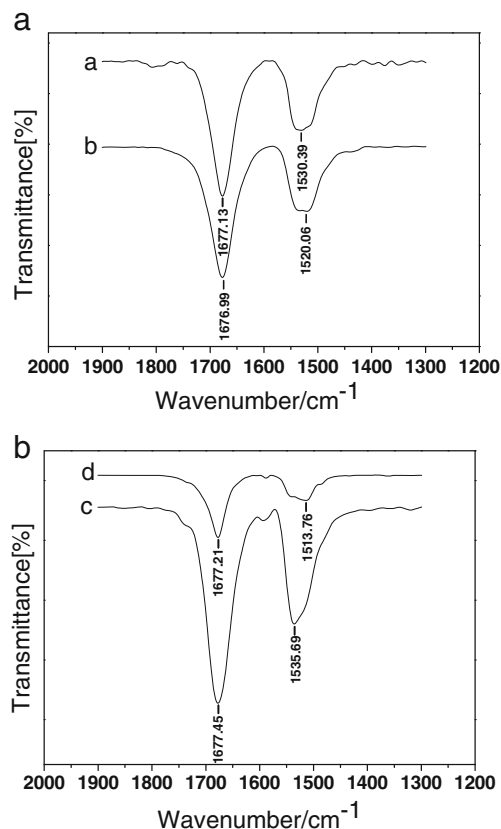
The electrochemical response toward glucose at the heated GOx-CILE was relatively stable. For 2.0 mM glucose at the heated GOx-CILE, ten successive measurements give an average response current of 2.48  $\mu\text{A}$  with the relative standard deviation (RSD) being 3.1 %. During 1-month storage, the response of the heated GOx-CILE was checked against 2.0 mM glucose every three other days. In the first week, no noticeable change was observed in the response current; a month later, the electrode retained about 86.9 % of the initial response current. The reproducibility of the heated GOx-CILE was also good. For five heated GOx-CILE prepared in the same way, an RSD value of 6.8 % in the response current was obtained.



**Fig. 7** Response currents of the unheated GOx-CILE (a, c) and heated GOx-CILE (b, d) toward 2.0 mM glucose at +0.9 V in 0.1 M phosphate buffer (pH 7.5). The binder was [Pmim][PF<sub>6</sub>] (a, b) or OPFP (c, d), and the resulting GOx-CILE was heated at a temperature a little higher than respective melting point for 10 min and then cooled at ca. 25 °C

#### Advantage of [Pmim][PF<sub>6</sub>] over OPFP

Figure 7 shows the response current of 2.0 mM glucose at GOx-CILE prepared using [Pmim][PF<sub>6</sub>] (melting point 39 °C) or OPFP (melting point 65 °C) as the binder with or without heating. For [Pmim][PF<sub>6</sub>] as the binder, the



**Fig. 8** FT-IR spectra of the unheated (a and c) and heated (b and d) mixtures of **a** [Pmim][PF<sub>6</sub>], graphite, and GOx, or **b** OPFP, graphite, and GOx. For clarity, the spectra were offset

currents of the unheated and heated GOx-CILE were  $1.96 \pm 0.07 \mu\text{A}$  and  $2.43 \pm 0.05 \mu\text{A}$ , respectively, indicating that heating favors for the glucose detection. As GOx could maintain its catalytic activity at  $40^\circ\text{C}$ , the increase in the response current caused by heating should be attributed to the decrease of the background current. For OPFP as the binder, the response current of the unheated GOx-CILE was  $1.98 \pm 0.05 \mu\text{A}$ , which was almost the same as that using [Pmim][PF<sub>6</sub>] as the binder, but it decreased to  $0.59 \pm 0.02 \mu\text{A}$  when the heating measure was taken. This is because the activity of GOx decreased greatly at a relatively high temperature of  $65^\circ\text{C}$ . For confirmation, the conformation of GOx in the two CILEs was monitored at different temperatures using FT-IR spectroscopy. It is known that the shape and the position of amide I ( $1700\text{--}1600 \text{ cm}^{-1}$ ) and amide II ( $1600\text{--}1500 \text{ cm}^{-1}$ ) IR bands can provide detailed information on the secondary structure of the polypeptide chain of proteins. The two bands are attributed to the C=O stretching and the combination of N–H bending with C–N stretching, respectively [29]. It is seen from Fig. 8a that the amide I and amide II bands of GOx in the unheated and heated mixture of [Pmim][PF<sub>6</sub>] and graphite were at  $1677.13$ ,  $1530.39 \text{ cm}^{-1}$  and  $1676.99$ ,  $1520.06 \text{ cm}^{-1}$ , respectively. With the exception of the position of amide II band, which shifted toward lower wavenumber after heating, the spectra were almost the same at the two conditions, indicating that heating did not affect the conformation of GOx. In the mixture of OPFP and graphite, however, the IR bands of the amide I and amide II of GOx in the unheated and heated mixture were quite different (Fig. 8b). After heating, the two absorption peaks decreased greatly in addition to large bathochromic shift of amide II band, indicating that heating at a higher temperature of  $65^\circ\text{C}$  greatly affected the secondary structure of GOx. It follows that the melting point of an IL is the dominant factor for the fabrication of high-performance GOx-CILE for the detection of glucose.

## Conclusions

Hydrophobic IL [Pmim][PF<sub>6</sub>], which melts at  $39^\circ\text{C}$ , was selected in the present study to fabricate enzyme-containing CILE. Through the optimization of the ratio of the IL to graphite powder and the heating of the IL/graphite composite, the background current of the CILE decreased and the mechanical strength increased. The heated CILE is more sensitive than the traditional CPE for the detection of H<sub>2</sub>O<sub>2</sub>. For the effective enhancement of GOx in the bulk IL/graphite composite, low melting point [Pmim][PF<sub>6</sub>] as a binder is much better than OPFP (melting point  $65^\circ\text{C}$ ). Heating the [Pmim][PF<sub>6</sub>]-based GOx-CILE at a temperature a little higher than the melting point of [Pmim][PF<sub>6</sub>] does not lower the catalytic activity of GOx. Under the optimum conditions, the [Pmim][PF<sub>6</sub>]-based GOx-CILE has a linear current response toward glucose over a concentration range

of  $2.0\text{--}26 \text{ mM}$ . It follows that choosing a hydrophobic IL with a melting point of ca.  $40^\circ\text{C}$  as the binder to fabricate enzyme-entrapped CILEs is a good strategy for the enhancement of the performance of the electrode.

**Acknowledgments** The authors gratefully acknowledge the financial support from State Key Laboratory of Microbial Technology of China, the Provincial Natural Science Foundation of Shandong (Y2008B13), the National Natural Science Foundation of China (20973103, 21173133), and the National Basic Research Program of China (2011CB707400).

## References

- Adams RN (1958) *Anal Chem* 30:1576
- Lawrence NS, Deo RP, Wang J (2004) *Anal Chem* 76:3735–3739
- Švancara I, Vytřas K, Kalcher K, Walcarus A, Wang J (2009) *Electroanalysis* 21:7–28
- Wang J, Lu F (1998) *J Am Chem Soc* 120:1048–1050
- Liu HT, He P, Li ZY, Sun CY, Shi LH, Liu Y, Zhu GY, Li JH (2005) *Electrochem Commun* 7:1357–1363
- Di W, Ivaska A (2008) *Anal Chim Acta* 607:126–135
- Shiddiky MJA, Torriero AAJ (2011) *Biosens Bioelectron* 26:1775–1787
- Lu L, Huang XR, Qu YB (2011) *Colloid Surf B* 87:61–66
- Fu CP, Kuang YF, Huang ZY, Wang X, Yin YF, Chen JH, Zhou HH (2011) *J Solid State Electrochem* 15:2581–2585
- Zein El Abedin S (2012) *J Solid State Electrochem* 16:775–783
- Kachoosangi RT, Musameh MM, Abu-Yousef I, Yousef JM, Kanan SM, Xiao L, Davies SG, Russell A, Compton RG (2009) *Anal Chem* 81:435–442
- Shan CS, Yang HF, Han DX, Zhang QX, Ivaska A, Niu L (2010) *Biosens Bioelectron* 25:1504–1508
- Liu L, Cheng Y, Sun FR, Yang JP, Wu Y (2012) *J Solid State Electrochem* 16:1003–1009
- Siddiquee S, Yusof NA, Salleh AB, Tan SG, Bakar FA (2012) *J Solid State Electrochem* 16:273–282
- Suarez PAZ, Einloft S, Dullius JEL, de Souza RF, Dupont J (1998) *J Chim Phys* 95:1626–1639
- Huang KJ, Niu DJ, Sun JY, Zhu XL, Zhu JJ (2010) *Anal Bioanal Chem* 397:3553–3561
- Huang KJ, Sun JY, Niu DJ, Xie WZ, Wang W (2010) *Colloid Surf B* 78:69–74
- Sun W, Wang DD, Zhong JH, Jiao K (2008) *J Solid State Electrochem* 12:655–661
- Sun W, Jiang Q, Jiao K (2009) *J Solid State Electrochem* 13:1193–1199
- Liu XY, Zeng XD, Mai NN, Liu Y, Kong B, Li YH, Wei WZ, Luo SL (2010) *Biosens Bioelectron* 25:2675–2679
- Maleki N, Safavi A, Tajabadi F (2006) *Anal Chem* 78:3820–3826
- Opallo M, Lesniewski A (2011) *J Electroanal Chem* 656:2–16
- Musameh MM, Kachoosangi RT, Xiao L, Russell A, Compton RG (2008) *Biosens Bioelectron* 24:87–92
- Maleki N, Safavi A, Tajabadi F (2007) *Electroanalysis* 19:2247–2250
- Giraud G, Gordon CM, Dunkin IR, Wynne K (2003) *J Chem Phys* 119:464–477
- Yoshio M, Mukai T, Ohno H, Kato T (2004) *J Am Chem Soc* 126:994–995
- Hecht HJ, Kalisz HM, Hendle J, Schmid RD, Schomburg D (1993) *J Mol Biol* 229:153–172
- Berchmans S, Sathyaj R, Yegnaraman V (2002) *Mater Chem Phys* 77:390–396
- Kauppinen JK, Moffat DJ, Mantsch HH, Cameron DG (1981) *Appl Spectrosc* 35:271–276