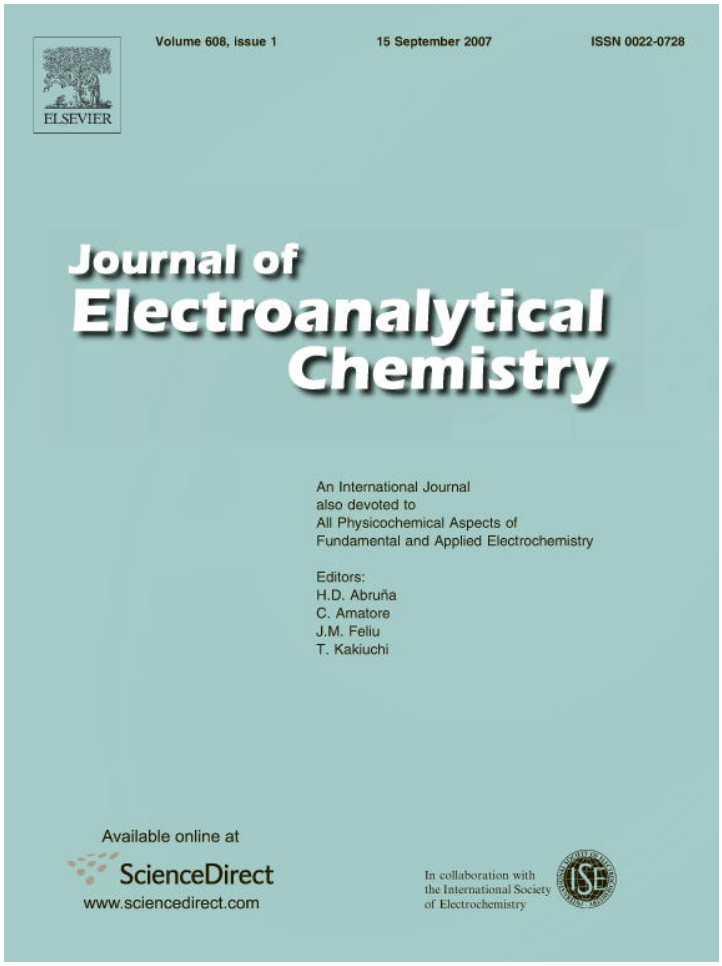

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Journal of Electroanalytical Chemistry 608 (2007) 78–83
Journal of Electroanalytical Chemistry
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Enhanced response induced by polyelectrolyte-functionalized ionic liquid in glucose biosensor based on sol–gel organic–inorganic hybrid material

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Received 3 March 2007; received in revised form 18 April 2007; accepted 10 May 2007
Available online 24 May 2007

Abstract

In this work, a polyelectrolyte-functionalized ionic liquid (PFIL) was firstly incorporated into a sol–gel organic–inorganic hybrid material (PFIL/sol–gel). This new composite material was used to immobilize glucose oxidase on a glassy carbon electrode. An enhanced current response towards glucose was obtained, relative to a control case without PFIL. In addition, chronoamperometry showed that electroactive mediators diffused at a rate 10 times higher in the apparent diffusion coefficient in PFIL-containing matrices. These findings suggest a potential application in bioelectroanalytical chemistry.

Keywords: Polyelectrolyte; Ionic liquid; Glucose oxidase; Biosensor; Sol–gel

1. Introduction

In recent years, sol–gel derived silica materials have been used extensively in electrochemistry [1–3], especially in bioelectrochemistry [4–10] due to simple preparation, ease of immobilization, chemical inertia, mechanical stability and high biocompatibility. From this class of silicate-based materials, hybrid organic–inorganic materials are of particular interest. With these materials, functionalization of the inorganic network affords tunable chemical and physical properties [1,3,5–7] with application to various chemically modified electrodes. For example, Dong and co-workers immobilized biomolecules within functionalized sol–gel materials to construct a series of biosensors which are highly biocompatible, exhibit limited swelling and almost unleachable [5–7]. However, most sol–gel derived materials are electronic insulators with low charge-transfer efficiency and substrate diffusion. In order to solve the problem, many works have been done [1,2,9–16]. Moreau and coworkers have prepared a silica gel containing thiophene units. Thiophene unit was an electron-rich conjugated molecule which upon polymerization led to a highly electroactive and electro-conductive material [12]. Lev and co-workers introduced a ceramic–carbon composite electrode where a dispersion of conductive powder such as nano-crystalline gold and graphite afforded electron conductivity [2,14–17]. Moreover, multi-walled carbon nanotubes have also been incorporated into sol–gel matrix for improved conductivity [18,19], etc.

Due to their unique chemical and physical properties [20–22], room-temperature ionic liquids (RTILs) have attracted much attention as novel environmentally benign solvents in chemical synthesis, catalysis and separation [21,23–27]. Particularly, they have relatively large potential windows, good solubility and high conductivity, and allow

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doi:10.1016/j.jelechem.2007.05.004

electrochemistry studies without addition of supporting electrolytes [28–30]. Recently ionic liquids (ILs) have been used widely as a reaction medium in biocatalysts [31–36]. In these works, the activity of biomolecules in ILs is typically observed to be comparable to or higher than activity observed in conventional organic solvents. Also, enhanced enzyme stability has been observed in ILs.

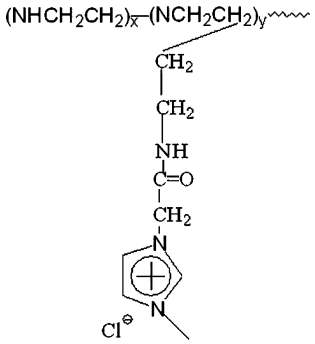
Our research group has previously reported development of a polyelectrolyte-functionalized IL (PFIL) by covalent attachment of an IL unit (a carboxyl terminated methylimidazole) onto a polyelectrolyte, polyethylenimine (PEI), and described how this PFIL could be immobilized onto various substrates via casting [37]. The IL unit in the PFIL improved the charge transportation in the film matrix and exhibited direct electrocatalysis towards the oxidation of NADH [37]. Furthermore, PFIL could also be used as a film to enhance the electrochemical response in the solutions without addition of supporting electrolytes [38,39]. This method provided an economical way to immobilize ILs on solid supports and indicated a potential application in electroanalytical chemistry.

In this work, PFIL was incorporated into a sol–gel organic–inorganic hybrid material for the purpose of improving the ionic conductivity of the resulting composite. The resulting novel material was used to embed glucose oxidase (GOx) and construct biosensors via casting. The role of the PFIL in the enhancement of electrochemical response due to its high ion conductivity, biocompatibility and fast substrate diffusion was also explored.

2. Experimental

2.1. Reagents

Polyelectrolyte-functionalized IL (Scheme 1) and grafting copolymer of poly (vinyl alcohol) and 4-vinylpyridine (PVA-g-P (4-VP)) were prepared as described previously [37,40]. When the copolymer was doped into silica sol, the sol–gel organic–inorganic hybrid material was obtained. Glucose oxidase (GOx from Aspergillus niger, EC 1.1.3.4, 100–250 units/mg) was obtained from Sigma. 1,1′-Ferrocene dicarboxylic acid (FDCA) and α-D-(+)-glucose were obtained from Aldrich. All other chemicals were



Scheme 1. The molecular structure of PFIL.

of analytical grade, and aqueous solutions were prepared in doubly distilled water. A phosphate buffer solution (PBS 0.1 M) of pH 7.4 was used during the experiments. Stock solution of glucose was allowed to maturate at room temperature for 24 h before use.

2.2. Apparatus

UV-vis spectra were recorded on a Cary 500 UV–Vis spectrometer. Electrochemical experiments were performed with a CHI660 (CHI Instruments, USA). All experiments were carried out with a conventional three-electrode system with the enzyme electrode as the working electrode and a platinum wire as the auxiliary electrode. All the potential given here were relative to an Ag/AgCl (saturated KCl) reference electrode. All experiments were conducted at ambient conditions. Particularly, glassy carbon electrode (GC, 3 mm diameter) was employed in cyclic voltammetric (CV) and amperometric experiments. Pt microelectrode ($d = 20$ μm) was employed in chronoamperometry. GC electrode and Pt microelectrode were polished before each experiment with 1.0, 0.3, 0.5 μm α-alumina powder, respectively. The electrode was rinsed thoroughly with double distilled water between each polishing step, sonicated in doubly distilled water for 5 min, and then dried under nitrogen flow.

2.3. Preparation of GOx/PFIL/sol–gel/GC electrode

The enzyme electrodes were prepared by a simple casting method. The sol–gel organic–inorganic hybrid material was obtained by mixing PVA-g-P (4-VP) and silica sol as previous [5,6]. The PFIL was incorporated into above hybrid material by mixing with the copolymer and the silica as follows.

First, Silica sol was prepared by mixing TEOS, water, 0.1 M hydrochloric acid and ethanol in a 1:4:0.6:0.2 ratio. The mixture was sonicated for 30 min and subsequently stored at room temperature for 3–4 h.

For preparation of a GOx/PFIL/sol–gel/GC electrode, 1 mg of GOx was dissolved in 15 μL of PBS, then 60 μL of grafting copolymer, 15 μL of silica sol and 60 μL of PFIL saturated solution were successively added into the enzyme solution, and the resulting solution was mixed. Next, 5 μL of the mixture was dropped on the surface of GC electrode and allowed to dry at 4 °C for 48 h. Then, a GOx/PFIL/sol–gel/GC electrode was obtained. The control experiment was done by the same procedures except that PBS was used instead of PFIL saturated solution. Thus, a GOx/sol–gel/GC electrode was obtained. Blank experiment was performed on a PFIL/sol–gel/GC electrode (without GOx).

2.4. Chronoamperometry experiments

The diffusion coefficient was determined by chronoamperometry measurements. The experiments were

performed by applying a potential step from an initial potential of 0.2 V to a final potential of 0.7 V. Plotting of current vs. the reciprocal of the square root of time, for a time domain from 0.8 to 1.6 s can give a linear relationship corresponding to the modified Cottrell equation [41–43].

$$I(t) = \frac{nFAD^{1/2}C}{\pi^{1/2}t^{1/2}} + \pi FnDCr$$

where r is the electrode radius, A is the geometric area of the microelectrode, n is the number of electrons transferred per mole of species, F is the Faraday constant, and the D and C are the diffusion coefficient and the concentration of the redox probe in the film, respectively. The quantity $D^{1/2}C$ and DC are obtained from the linear regression of the slope and the intercept. Finally, both D and C can be obtained.

2.5. Enzymatic assay experiments

Enzymatic assay of the immobilized GOx was done by UV–Vis experiments based on the Trinder reaction [44]. The glucose (0.4 mM) was added with a certain concentration of 4-aminoantipyrine, phenol and horseradish peroxidase to a constant volume of solution (3 ml) in PBS. The absorbance from 600 to 375 nm was recorded so as to observe the color reactions.

3. Results and discussion

The PFIL was a kind of hydrogels, it could form a relatively stable film on the electrode surface alone [37]. However, it was easily to be stripped of under furious stirring or a long time dipping in solution. Here, the PFIL was introduced into the PVA-g-P (4-VP) doped silica, which was reported to be a highly stable material [5–7]. A rather stable matrix was obtained because the PFIL component could interact with hydroxyl groups of silicate and PVA through electrostatic and hydrogen-bonding forces. Moreover, PFIL also played an important role in the immobilization of enzyme because it was positively charged and favor of immobilization of negatively-charged GOx (isoelectric point = 5.5) in pH 7.4 PBS via electrostatic interaction.

The new biosensor was successfully characterized by amperometry. Upon dipping it into the glucose solution, glucose was oxidized to gluconic acid by reduction of a flavin group. The reduced flavin group could then be reoxidized by oxygen, which was converted to hydrogen peroxide. The whole reaction is depicted as follow [45]:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconic acid} + H_2O_2 \quad (1)$$

$$H_2O_2 - 2e^- \rightarrow O_2 + 2H^+ \quad (2)$$

Hydrogen peroxide generated in the vicinity of the surface of the electrodes was immediately oxidized, resulting in an anodic current, which was used for electrochemical quantification of glucose. Here, 1050 mV was selected as the working potential [5], and pH 7.4 was used in the experiments. The current–time plots of the modified electrodes on successive step changes of glucose concentration during stirring in PBS solution are demonstrated in Fig. 1A. The response currents at the GOx/sol–gel/GC electrode (curve a) and GOx/PFIL/sol–gel/GC electrode (curve b) were compared. It is obvious that the current response at the GOx/PFIL/sol–gel/GC electrode with increase of glucose concentration is much higher (ca. twice) than that at the GOx/sol–gel/GC electrode, as shown clearly in Fig. 1B. Blank experiment was done on a PFIL/sol–gel/GC electrode without GOx and no current response occurred when adding glucose (data is not shown here). So the change in current originated only from the enzyme catalysis. The higher response in current at the GOx/PFIL/sol–gel/GC electrode might result from the high ion conductivity of the IL unit just as the reports earlier [28–30,38,39]. The inset of Fig. 1A shows that the GOx/PFIL/sol–gel/GC electrode could reach 95% steady-state current within 8 s while the GOx/sol–gel/GC electrode needed ca. 11 s. Such

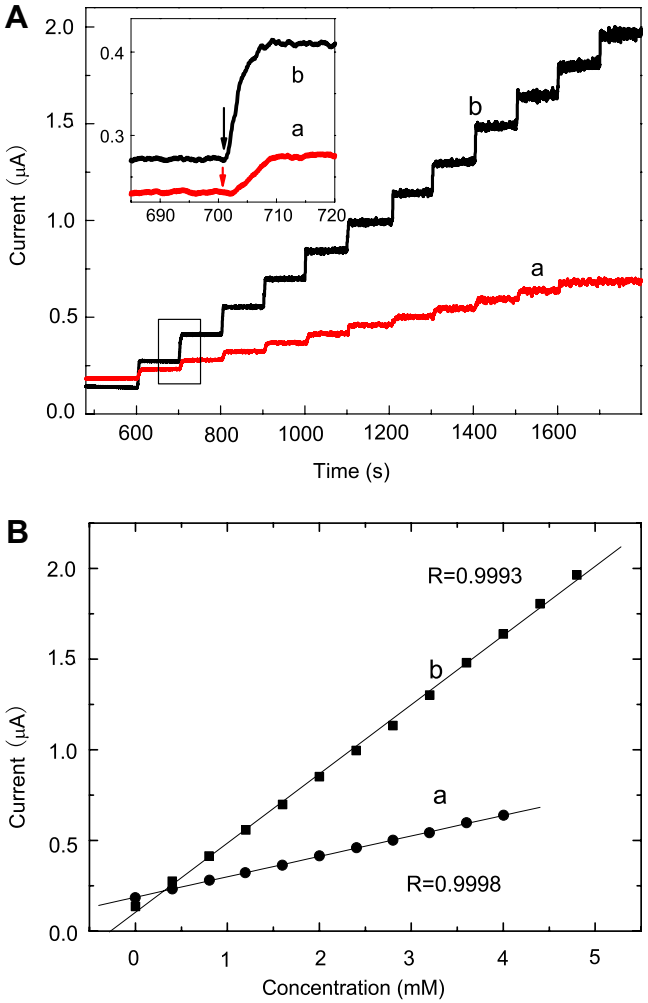


Fig. 1. (A) Steady-state response of (a) GOx/sol–gel/GC and (b) GOx/PFIL/sol–gel/GC electrodes with successive addition of glucose concentration (0.4 mmol/L each step). Applied potential: 1.05 V vs. Ag|AgCl (in saturated KCl). Inset shows a magnification of the second addition of glucose. (B) Calibration curves of the above (a), (b) electrodes.

a faster response should originate from the faster diffusion of substrate molecule as well as the improved ionic conductivity in the GOx/PFIL/sol–gel film matrix caused by the ionic liquid unit. In this case, there are the problems of sensitivity to O_2 concentration and significant interference due to the high overpotential [46].

In order to decrease the overpotential required for the oxidation of hydrogen peroxide and exclude the effect of O_2 concentration, the enzyme electrodes in the presence of electroactive mediators were characterized further. Fig. 2 compares the voltammetric responses for 8 mM glucose at the GOx/sol–gel/GC electrode and the GOx/PFIL/sol–gel/GC electrode in N_2-saturated PBS containing 2 mM FDCA electroactive mediator. Curve a and b give the CVs of GOx/sol–gel/GC electrode and GOx/PFIL/sol–gel/GC electrode in FDCA/PBS solution without the addition of glucose, respectively. The pair of peaks ascribed to the redox behavior of FDCA can be observed. The differences on the reversibility and reduction currents between the curve a and curve b were due to the individual differences of the electrodes. The curve c and d give the CV responses of GOx/sol–gel/GC electrode and GOx/PFIL/sol–gel/GC electrode in FDCA/PBS solution with 8 mM glucose, respectively. It can be found that the anodic peak currents at the two modified electrodes both increased upon addition of glucose (8 mM) to the solution, suggesting that the oxidation of glucose can be well catalyzed by GOx at both of the enzyme modified electrodes. The mechanism is illustrated as follows [46]

$$\text{Glucose} + \text{GOx}_{(\text{ox})} \rightarrow \text{Gluconic acid} + \text{GOx}_{(\text{red})} \quad (3)$$

$$\text{GOx}_{(\text{red})} + 2\text{FDCA}^+ \rightarrow \text{GOx}_{(\text{ox})} + 2\text{FDCA} + 2\text{H}^+ \quad (4)$$

$$2\text{FDCA} - 2\text{e}^- \leftrightharpoons 2\text{FDCA}^+ \quad (5)$$

The deep discussion on the electron transfer mechanism between $GOx_{(red)}$ and the ferricinium derivatives was also reported [46]. From the distinctive difference of oxidative current between curve a and c, curve b and d, it can be seen that the response current at the electrode with PFIL is much larger than that without PFIL, which is in well agreement with the electrochemical behavior shown in Fig. 1. Fig. 3A illustrates steady-state response of the GOx/PFIL/sol–gel/GC electrode on successive addition of glucose under the existence of 2 mM FDCA. As the glucose was added into the stirring solution, GOx/PFIL/sol–gel/GC electrode responded rapidly to the substrate. The linear range of glucose was from 1 to 25 mM with a correlation coefficient of 0.9994 (Fig. 3, inset). Meanwhile, a steady-state response at the GOx/sol–gel/GC electrode with the same successive addition of glucose can not be observed due to the big noise and slow response (as shown in Fig. 3B). These results confirmed that PFIL enhanced the current response of the enzyme electrode towards oxidation of glucose and improved the diffusion rate of the substrate in the film. It was also deduced that enzyme might keep a higher activity in the presence of PFIL due to its high biocompatibility [31–35]. The hydrogen bond and the electrostatic interaction between IL and enzyme might result in a high stability of the enzyme as previously reported [9].

In order to further discuss the influence of substrate diffusion, chronoamperometry was used to determine the apparent diffusion coefficient of the redox probe (FDCA) in the film. First, it is necessary to establish that diffusion controlled conditions are valid [47]. Fig. 4 depicts the cyclic voltammograms recorded at GOx/PFIL/sol–gel/GC electrode in pH 7.4 PBS containing 2 mM FDCA at different scan rates. It can be clearly seen that, with increase of scan rates, the anodic peak currents increase and the peak currents vary linearly with the square roots of scan rates from 0.01 to 0.3 V/s, indicating an ideal diffusion-controlled process. Typical current–time transient measurements associated with FDCA oxidation at the GOx/PFIL/sol–gel/Pt

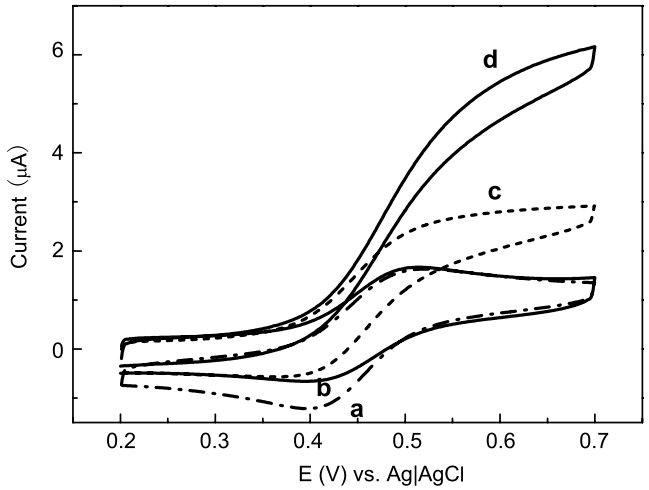


Fig. 2. Cyclic voltammograms of GOx/sol–gel/GC electrode (a and c) and GOx/PFIL/sol–gel/GC electrode (b and d) in 2 mM FDCA/0.1 M PBS solution in the absence (a and b) and presence of 8 mM glucose (c and d), respectively.

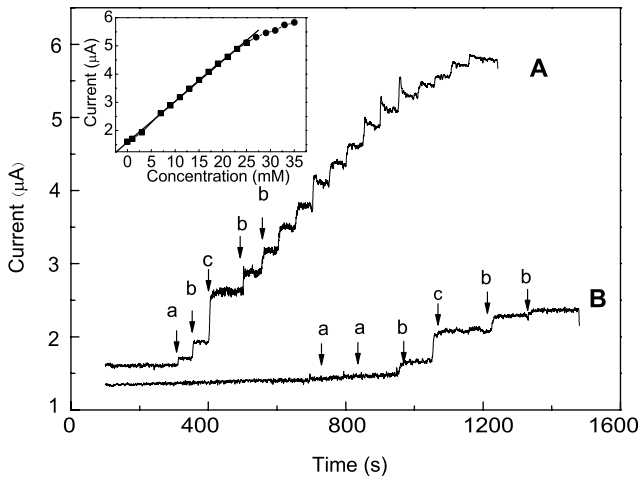


Fig. 3. Current–time response of (A) GOx/PFIL/sol–gel/GC electrode and (B) GOx/sol–gel/GC electrode on injecting (a) 1 mM (b) 2 mM (c) 4 mM of glucose solution under the existence of 2 mM FDCA. Applied potential: 0.55 V. Inset shows a calibration curve at GOx/PFIL/sol–gel/GC electrode.

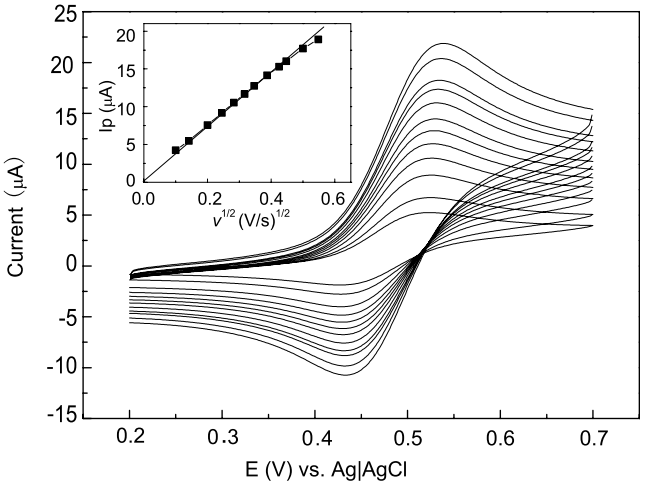


Fig. 4. Cyclic voltammograms of 2 mM FDCA in PBS solution at the GOx/PFIL/sol–gel/GC electrode at scan rates of 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.15, 0.18, 0.2, 0.25 and 0.3 V s^{-1} (from inner to outer). Inset: the plot of peak current vs. the square root of scan rates.

and GOx/sol–gel/Pt microelectrode interface have been done and the plots of I vs. $t^{-1/2}$ are both linear, as shown in Fig. 5. As indicated early, the slope of the plot yields the product $D^{1/2}C$, and the intercept yields the product DC, so that D can be evaluated. The obtained D values for FDCA in PFIL/sol–gel and sol–gel are 6.56×10^{-7} cm^2/s and 5.35×10^{-8} cm^2/s, respectively. It can be found that the resulting D value for FDCA in PFIL/sol–gel is 10 times larger than that in sol–gel. This result confirms that such a faster ion exchange in the PFIL/sol–gel is due to introduction of IL units. The possible processes can be considered as: the positively-charged imidazolium moieties attract the carboxyl groups of FDCA in solution and prompt their aggregation in the film/solution interface, and then counter-anions exchange between the imidazolium salts and the FDCA begins. So a faster diffusion of FDCA in PFIL/sol–gel film matrix is observed. Moreover, this stimulative diffusion based on the electrostatic interaction also promotes the charge transfer between the electrode and the substrate molecules during the electrochemical redoxidation, which is helpful in increasing the current response. In addition, IL usually acts as a good solvent [48] due its broad solubility, ILs with aromatic ions are more capable of π–π and n–π type interactions with solutes. So the introduction of IL component into the film matrix also improves the affinity of the film to the FDCA and substrate, facilitating their diffusion in the film. Finally, an enhanced response was resulted based on the electrostatic interaction, high conductivity and good affinity of IL moieties.

The further studies showed that this biosensor exhibited high stability. It retained above 90% of its initial response to the oxidation of glucose after intermittent use over 10 days. At the same time, the electrode kept the initial response after six continuous measurements with a relatively standard deviation of 4.14%. In order to study the activity of the GOx more clearly, enzymatic assay of the

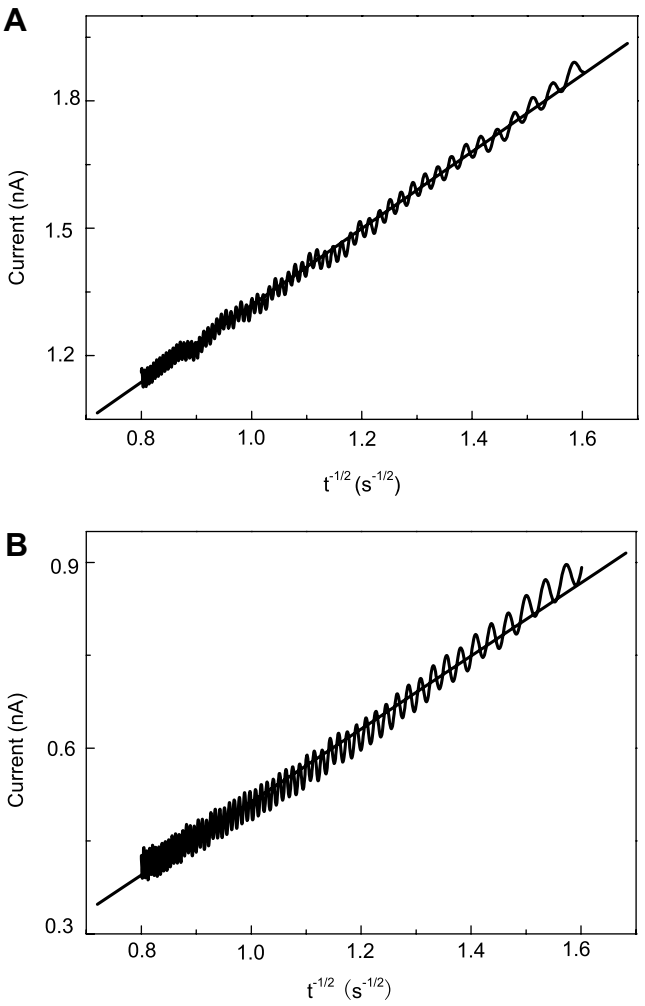


Fig. 5. The plots of current vs. $t^{-1/2}$ in 2 mM FDCA/PBS solution at the (A) GOx/PFIL/sol–gel/Pt and (B) GOx/sol–gel/Pt microelectrodes, respectively.

immobilized GOx was done by UV–Vis experiments based on the Trinder reaction. Fig. 6 gives enzymatic assay of the GOx immobilized on PFIL/sol–gel/GC electrode (a) and of

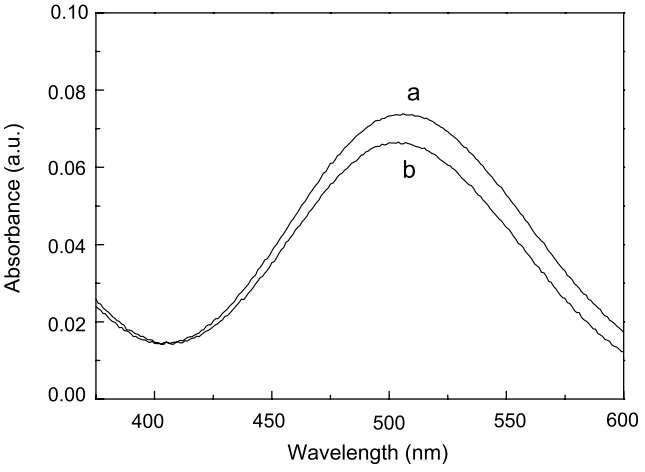


Fig. 6. The enzymatic assay of the immobilized GOx on the PFIL/sol–gel/GC electrode (a) and of the same enzyme electrode after 1 week (b).

the same enzyme electrode after one week (b). The absorbance of quinonimine dye at 507 nm decreases a little from curve a to curve b after one week. It depicted the not seriously decrease of the enzyme activity.

4. Conclusions

A composite material comprising PFIL and a sol–gel organic–inorganic hybrid material for enwrapping the enzyme (GOx) has been reported. An enhanced charge transfer and an improved diffusion rate in the film matrix are obtained due to the introduction of PFIL. This resulting immobilization of GOx in PFIL/sol–gel film exhibits a higher response than the film without PFIL towards glucose due to its high conductivity, good affinity and fast substrate diffusion, which might extend to the fabrication of other sol–gel-based biosensors.

Acknowledgements

The authors are most grateful to the National Science Foundation of China (No. 20475053 and No. 20673109), Department of Science and Technology of Jilin Province (No. 20050102) and Ministry of Science and Technology of China (No. 2006BAKB05).

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