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Direct electrochemistry of glucose oxidase based on its direct immobilization on carbon ionic liquid electrode and glucose sensing

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ABSTRACT

Direct electrochemistry of glucose oxidase (GOx) has been achieved by its direct immobilization on carbon ionic liquid electrode (CILE) with a conductive hydrophobic ionic liquid, 1-butyl pyridinium hexafluophosphate ([BuPy][PF_6]) as binder for the first time. A pair of reversible peaks is exhibited on GOx/CILE by cyclic voltammetry. The peak-to-peak potential separation (ΔE_P) of immobilized GOx is 0.056 V in 0.067 M phosphate buffer solution (pH 6.98) with scan rate of 0.1 V/s. The average surface coverage and the apparent Michaelis–Menten constant are 6.69×10^{-11} mol·cm^{-2} and 2.47 μM. GOx/CILE shows excellent electrocatalytic activity towards glucose determination in the range of 0.1–800 μM with detection limit of 0.03 μM ($S/N = 3$). The biosensor has been successfully applied to the determination of glucose in human plasma with the average recoveries between 95.0% and 102.5% for three times determination. The direct electrochemistry of GOx on CILE is achieved without the help of any supporting film or any electron mediator. GOx/CILE is inexpensive, stable, repeatable and easy to be fabricated.

1. Introduction

Glucose oxidase (GOx), containing a flavin adenine dinucleotide (FAD) redox center that catalyzes the electron transfer from glucose to gluconolactone, has been extensively used to monitor the glucose in biochemistry, clinical chemistry, food processing and fermentation [1]. Many methods have been developed for the determination of glucose, especially amperometric biosensors [2]. It is well known that the active redox centre of GOD, FAD is deeply embedded in a protective protein shell, which makes the direct electron transfer with the conventional solid electrodes extremely difficult [3]. Hence, the different biocompatible immobilizing materials, including polymer [4], silica sol–gel film [5], poly acrylamide microgel matrix [6] and nano gold particles-ionic liquid-*N*, *N*-dimethylformamide etc. [7], are used to promote the electron transfer.

Ionic liquids (ILs) are molten salts with the melting point close to or below room temperature, low flammability, high ionic conductivity, minimal vapour pressure, high thermal stability and a wide electrochemical window [8–11]. They are composed of two asymmetrical ions of opposite charges that only loosely fit together (usually bulky organic cations and smaller anions).

In recent years, direct electrochemistry of biologically important enzymes has been studied with ILs in both theoretical and practical application because ILs are considered to be suitable media for supporting biocatalytic processes with high polarity, non-coordination power, high selectivity, fast rate and great enzyme stability [12]. Enzymes are usually active and protein refolding is improved in ILs [13]. Zeng and co-workers constructed a modified glassy carbon electrode by entrapping GOx into nano gold particles-ionic liquid-*N*, *N*-dimethylformamide composite film [7]. Sun and co-workers constructed a modified imidazolium-based carbon ionic liquid electrode by entrapping Hb into sodium alginate hydrogel film [14]. Safavi and co-workers constructed a modified pyridinium-based carbon ionic liquid electrode by the use of [OcPy][Cl] to immobilize directly Hb on CILE [15].

In this paper, a new GOx/CILE has been constructed by directly immobilizing GOx on CILE, which comprises of graphite powder and ionic liquid ([BuPy][PF_6]). The direct electrochemistry and electrocatalytic behaviour of GOx on CILE is investigated. Compared to our previous work [16], background current can be decreased highly on the proposed CILE. To our best knowledge, this is the first report on direct electrochemistry of GOx on CILE by direct immobilization of GOx without the need for modification of the electrode surface with a special film, nanoparticles or polymer as supporting materials.

2. Experimental

2.1. Reagents

Glucose oxidase (E.C. 1.1.3.4, 182 U/mg, *Aspergillus niger*, Sigma, USA). β-D-glucose, butane chloride, ethylacetate, methanol,

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hexafluorophosphate acid and pyridinium were of analytical grades. [BuPy][PF$_6$] was synthesized and purified following the similar procedures described in the literature [17]. High purity graphite powder (SP, China). All aqueous solutions were made with deionized water, which was further purified with a Milli-Q system (Millipore Co., USA).

2.2. Apparatus

A CHI660A electrochemical workstation (Shanghai Chenhua Co. Ltd., China) controlled by a microcomputer with CHI660A software was employed for all electrochemical measurements in the experiment. DL–180 ultrasonic cleaning machine (35 KHz, Zhejiang Haitian Electron Instrument Factory, China) was used to dissolve and form homogeneous solution. A three-electrode system, where a standard saturated calomel electrode (SCE) served as reference electrode, a platinum wire electrode as the auxiliary electrode and the prepared electrodes as the working electrode. All the electrochemical experiments were conducted at room temperature. All potentials reported were versus the SCE.

2.3. Preparation of the CILE

CILE was prepared by hand-mixing of synthesized [BuPy][PF$_6$] and graphite powder with a ratio of 50/50 (w/w) in the manner described in the literature [18]. A portion of the resulting paste was packed firmly into the cavity (2.0 mm i.d.) of a Teflon holder. In order to better homogeneity in the composite and lower background current, the electrode should be heated to a temperature above the melting point of IL (m.p. 65 °C) prior to its use [18]. A new surface was attained by polishing the electrode on fine grit sandpaper firstly and then smoothing on weighing paper. For preparing GOx/CILE, 4 μL 15 mg/mL GOx was immobilized on the surface of CILE by dropping method and dried in 4 °C for 6 h. After this process, the GOx/CILE was rinsed with water and then with 0.067 M phosphate buffer solution (PBS, pH 6.98). The GOx/CILE was stored at 4 °C in a refrigerator when not in use.

2.4. Experimental procedure

A 10 mL PBS (0.067 M, pH 6.98) with proper amount of glucose was transferred into a 10 mL cell and the three-electrode system was immersed into the solution. CV and DPV experiments were performed between −0.8 and −0.2 V at scan rate of 0.1 V/s. The peak currents and potentials of GOx were recorded by CHI660A workstation. The glucose content in the samples was obtained by using DPV. All experiments were performed at room temperature.

3. Results and discussion

3.1. Cyclic voltammetry of GOx/CILE

Cyclic voltammograms of CILE and GOx/CILE are shown in Fig. 1. Under the same conditions and in the absence of GOx, bare CILE (Fig. 1a) does not show any peak in the working potential range mentioned above. While a pair of well-defined redox peaks is observed for GOx/CILE (Fig. 1b) with oxidation potential (E_{pa}) of −0.445 V, reduction potential (E_{pc}) of −0.501 V, ΔE_P of 56 mV and I_{pa}/I_{pc} of 1. This indicates that the electrochemical reaction is almost reversible, which is better than that reported in literatures [7,19–21] and that [BuPy][PF$_6$] is favorable to the immobilization of GOx and the direct electron transfer between GOx and CILE. It is thought that [BuPy][PF$_6$] can improve the activity and stability of GOx.

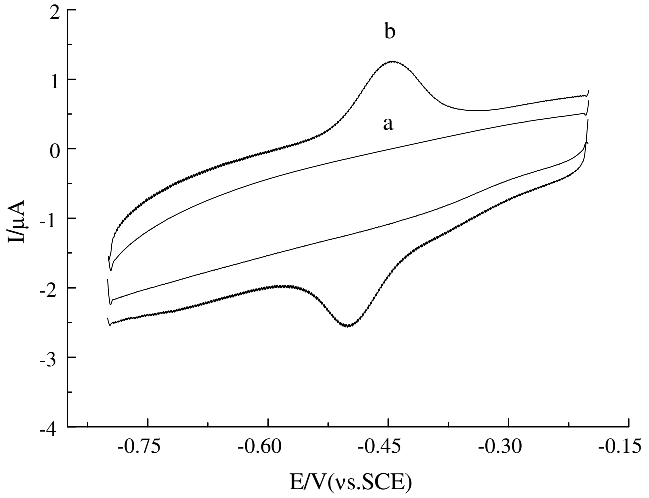

Fig. 1. The CV curves of CILE (a) and GOx/CILE (b) in 0.067 M PBS (pH 6.98) at 100 mV/s.

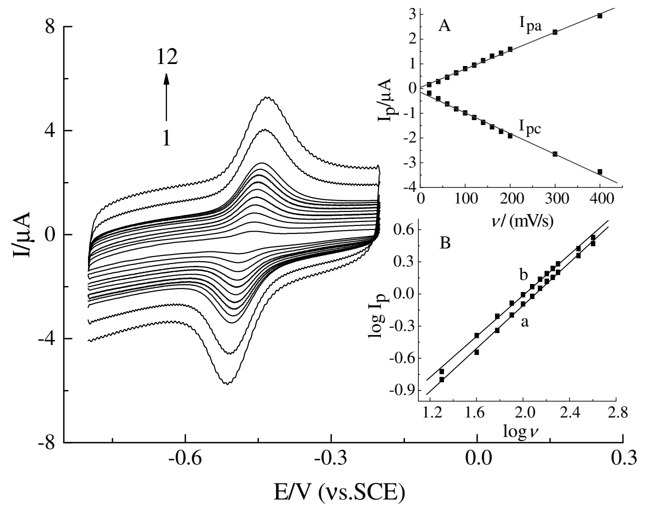

Fig. 2. The CV curves of GOx in 0.067 M PBS (pH 6.98) at different scan rate on CILE. Scan rate 1–12: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 300, 400 mV/s. Inset A is linear relationship of I_p and v at GOx/CILE. Inset B is linear relationship of log I_p and log v at GOx/CILE (a log I_{pa}, b log I_{pc}).

3.2. Effect of scan rate

The cyclic voltammograms for GOx/CILE are studied at different scan rates (v) in the range of 20–400 mV/s in Fig. 2. The results exhibit about symmetrical anodic and cathodic peaks of approximately equal heights for GOx at different scan rates. The peak currents (I_p) are linearly with v in range mentioned above in Fig. 2 inset A. The linear regression equation is I_{pa} (μA) = $4.904 \times 10^{-2} + 7.440 \times 10^{-3}$ v (mV/s), $R = 0.9984$; I_{pc} (μA) = $-0.1420 - 8.370 \times 10^{-3}$ v (mV/s), $R = 0.9969$, respectively. This indicates that the electron transfer process for GOx/CILE is a surface-confined mechanism in potential scope mentioned above.

The logarithm of peak currents (log I_p) versus logarithm of scan rate (log v) shows linear relationships in (Fig. 2) inset B. The slope value of 1.001 for anodic currents and 0.9855 for cathodic currents is very close to the theoretical value of 1 expected for thin layer electrochemical behavior [22].

According to the equation of $I_p = n^2F^2vA\Gamma^*/4RT = nFQv/4RT$ [23], n is calculated as 1.72, meaning that 2e transfer is involved [24].