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Original Article

Investigation of the effect of substrate conditions on electron transfer of glucose oxidase

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Abstract

Direct electron transferring of glucose oxidase was investigated on reduced graphene and graphene oxide templates. The direct electrochemistry glucose oxidase on graphene showed a cyclic voltammograms corresponding to the FAD/$FADH_2$ redox couple with an anodic, cathodic and formal potential of -430, -460 and -445 mV, respectively in 0.1 M phosphate buffer solution and air saturated condition for similarity of in vivo usage. The cyclic voltammograms of glucose oxidase on graphene is reversible. Also, the voltammograms results show, the current intensity of glucose oxidase on graphene is high, due to fast electron transferring. Moreover, the linear rang concentration of glucose on Gr are 0.4–9 µM. These studies make useful insight into the enzyme immobilization on nanoparticles for biosensors and bio-fuel cell preparation.

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Introduction

In the recent years, graphene (Gr) has great interest among physicists, chemists and biologist. Graphene is a two-dimensional (2-D) sheet of carbon atoms in a hexagonal configuration with atoms bonded by sp^2 bonds [1]. Graphene can now be produced relatively easily by mechanical exfoliation of graphite, [2] or by heating SiC, [3] or by the reduction of graphene oxide (GrO) [4]. Also, in comparison with carbon nonotubes (CNTs), graphene have many advantages, as follows: a) no metallic impurity, b) cheap and easy production [4, 5]. These properties have been caused that, the graphene to be ideal candidate for electrochemistry investigation and chemical sensors and biosensors fabrication. Graphene has been widely applied in synthesizing nanocomposites, fabricating chemical sensors and biosensors [6].

Graphene is an ideal material for electrochemistry, because of its very large 2-D electrical conductivity, large surface area, wide potential windows, fairly inert electrochemistry, good electrocatalytic activity for many redox reactions and low cost [7, 8].

Graphene does not contain metallic impurities as CNTs do. In many cases, such impurities dominate the electrochemistry of CNTs (so far, such negative influence is known for hydrazine, hydrogen peroxide, halothane, glucose, amino acids and short regulatory peptides even at <100 ppm levels of impurities in CNTs) and lead to misleading conclusions [9, 10].

Furthermore, in compared with CNTs, graphene can be obtained easily by chemical conversion of the inexpensive graphite. Recently, many attentions were reported for protein immobilization on graphene and hybrid of graphene was reported. In this study direct electron transferring of glucose oxidase was investigated on reduced graphene and graphene oxide templates for enzyme immobilization on nanoparticles for biosensors preparation.

Materials and methods

Apparatus and procedure

FT-IR spectra were obtained with a Nicolet FT-IR spectrometer. Electrochemical experiments were performed with an Autolab potentiostat (PGSTAT 101). A working glassy carbon electrode with a diameter of 3 mm, a silver/silver chloride (Ag/AgCl) reference electrode, containing 3 M, KCl and a platinum rod auxiliary electrode were used from metrohm. All potentials were measured and reported versus the Ag/AgCl reference electrode. Cyclic voltammetry experiments were performed at 0.1 V/s. The amperometric experiments were carried out by applying the desired potential and allowing the transient current to reach the steady-state value prior to the addition of the analyte and the subsequent current monitoring.

Reagents

Glucose oxidase (GOx) (EC 1.1.3.4, Type X-S from *Aspergillus Niger*) and β-d-(+)-glucose were purchased from Sigma (USA) and used as received. Graphite powder was purchased from Fisher (Chemical Scientific Grade#38, UK). Dihydrogen phosphate (KH_2PO_4),

dipotassium hydrogen phosphate (K_2HPO_4), $K_2S_2O_8$, hydrochloric acid and hydrogen peroxide were purchased from Merck. A 0.1 M phosphate buffer solution (pH: 7.4) was employed as supporting electrolyte. Ultrapure water from a Millipore-MilliQ system was used for preparing all solutions. All the reagents were used as received, without further purification and all experiments were carried out at room temperature (25°C).

Graphene oxide and graphene synthesis

Graphene oxide (GrO) was synthesized from graphite using the Hummers method [11] and reduced graphene (RGr) was obtained by reduction of GrO with $K_2S_2O_8$. Briefly, graphite, sodium nitrate and potassium permanganate were added to concentrated sulfuric acid. After heating at 35°C for 30 min, the reaction mixture turned greenish and pasty. Then, the reaction was carefully quenched by the slow addition of water. The paste was kept at 100°C for 15 min and turned brownish. After further dilution with water it was allowed to cool to 30°C for 30 min, during which it turned yellow. Hydrogen peroxide was carefully added to form colorless soluble manganese sulfate. The resulting GrO was isolated while still warm by filtration and the yellow-brown filter cake was washed with warm 5% diluted hydrochloric acid and finally with water. The resulting stable and brownish GrO aqueous solution was reduced by 1:1 $Gr/K_2S_2O_8$ mass ratio, at room temperature for overnight. The graphene black precipitate was filtrated and washed with ultra pure water. Photograph of RGr (black) and GrO (brown) was shown in Figure 1.

Figure 1. RGr (right) and GrO (left) photograph**.**

FT-IR Spectroscopic characterization of Graphene

Figure 2 shows the spectroscopic FT-IR characterization of GrO and RGr. The IR spectrum of graphene oxide show bands attributed to oxygen containing groups, which confirmed the successful oxidation of graphite. These bands are assigned to (O-H) stretching vibration mode of intercalated water (3400 cm−1); (C-O) stretching (1730 cm−1); (CO epoxy) stretching (1170 cm−1); and (CO alkoxy) stretching vibration (1014 cm−1) [11]. It is obvious that, the intensity of the absorption peaks for reduce graphene were decreased. This figure proves that, the GrO was successfully reduced to RGr.

Electrode preparation

The graphene was washed in phosphate buffer (0.1 M, pH: 7.4). Then 5 µl (1 µg/ml) of graphene was dropped on the glassy carbon electrode. Then 2 µl (0.1 mg/ml) of GOx solution was dropped on the graphene. After 10 min the cyclic voltammogram (CV) of GOx immobilized on graphene was recorded in 0.1 M phosphate buffer solution (pH 7.4) at air saturated condition.

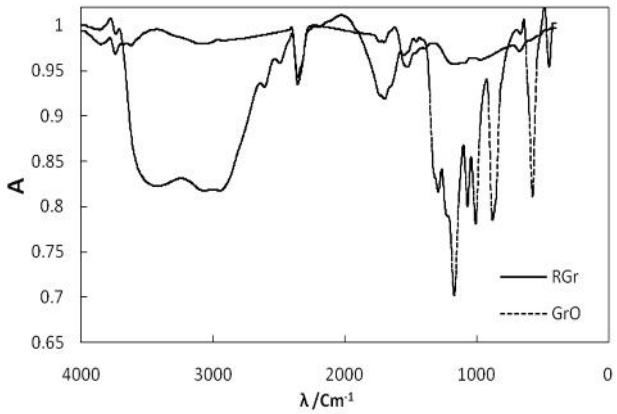

Figure 2. FT-IR spectra of GrO (---) and RGr (—).

Results and discussion

Figure 3 shows the cyclic voltammograms (CVs) of GOx immobilized on RGr and GrO glassy carbon electrodes in air saturated PBS (0.1 M, pH: 7.4). It is obvious that the GOx/GrO on glassy carbone electrode do not show any response in this potential window. By immobilization of GOx on RGr, a couple of well defined reversible peak was observed with the anodic and cathodic peak potentials of -430 and -460 mV (vs. Ag/AgCl), respectively (d). The peak potential separation was determined as 30 mV. The formal potential (E^0) of GOx has been calculated as the average of cathodic and anodic peak potentials (−445 mV vs. Ag/AgCl).

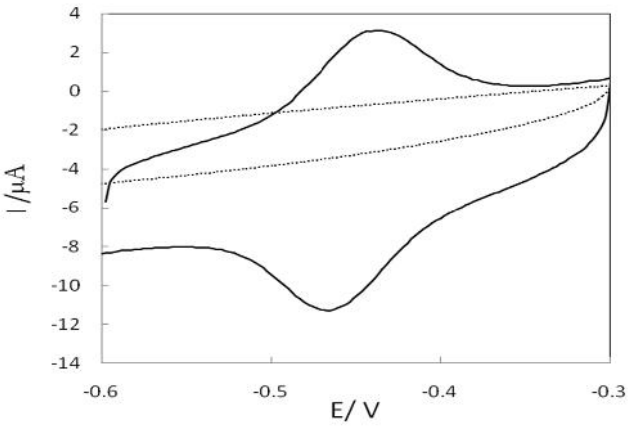

Figure 3. CVs of GOx immobilized on GrO (---) and RGr (—) at glassy carbon electrode in 0.1 M PBS (pH: 7.4)

Figure 4 shows the plot of cathodic and anodic peaks current (I_p) against the scan rate (υ). Both the anodic and cathodic peak currents increased linearly with square scan rate in the region of 30–1300 mVs−1 indicating surface controlled redox reaction [12]. Because, the peak potential separation Δp <200 mV, therefore, the calculation of kinetic parameters such as α and K_s is not possible.

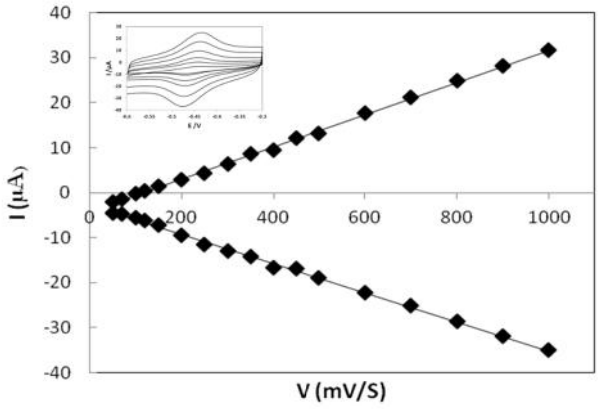

Figure 4. CVs of GOx/RGr on glassy carbon electrode in 0.1 M PBS (pH: 7.4) at various scan rates (ν) for plot of I_p vs ν.

The effect of PBS concentration on electron transferring of GOx

The plot of I_{pc} (A) and E° vs. different concentration of PBS for GOx/RGr on glassy carbon electrode in pH: 7.4 were shown in Figure 5. The results show, the I_p and E° was increased from 10 to 50 mM and after that I_p and E° to be fixed. With increase of electrolyte concentration, the conductivity of supporting electrolyte was increased.

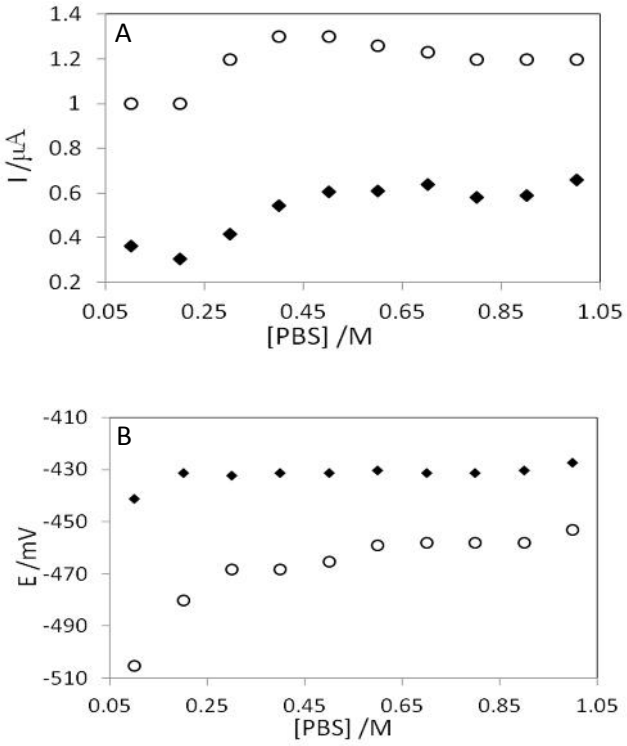

Figure 5. The plot of I_{pc} (A) and E° (B) vs. different concentration of PBS for GOx/RGr on glassy carbon electrode in pH: 7.4. The scan rate is 100 mV/s at air saturated condition.

So, high intensity of electron in GOx was transferred. Also, the wide range stability of Ip and E° in different PBS concentration shows that, the immobilization of GOx on graphene was occurred with chemical binding. In fact, various positive or negative charged can be decorated on the GOx graphene based on the electrostatic adherence.

Electrocatalytic activity of GOx on graphene/glassy carbon electrode

When glucose was added into air-saturated PBS, with the increase of the concentration of glucose, the reduction peak currents of GOx on graphene/glassy carbon electrode gradually decreased (Fig. 6). According to the following enzyme-catalyzed reaction:

Glucose + GOx (FAD) → gluconolactone + GOx ($FADH_2$)

It can be explained that glucose is the substrate of GOx, whose presence will result in an enzyme-catalyzed reaction and decrease the concentration of the oxidized form of GOx.

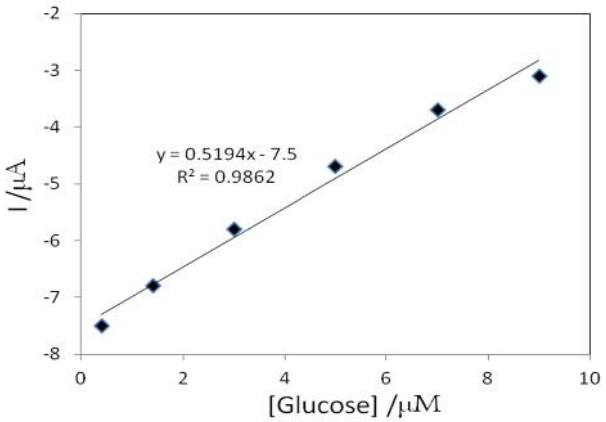

Figure 6. Calibration curve for the glucose by GOx/RGr on glassy carbon electrode by choronograms in 0.1 M PBS (pH: 7.4) containing various concentrations of glucose.

Thus, the addition of glucose restrains the electrocatalytic reaction and leads to the decrease of the reduction current. Based on the decrease of the reduction current, the concentration of glucose can be detected without the interference of coexistied electroactive-substance, which is different from the common glucose chronoamperometric sensors based on the detection of the consumption of oxygen or the production of hydrogen peroxide [13]. Figure 6 shows the relationship between the decrease of the reduction peak current and the glucose concentration of GOx on graphene/glassy carbon electrode. The current values linearly change with the concentration of 0.4–9 μM with a correlation coefficient (r) of 0.9862 and with detection limit of 0.1 μM.

Conclusion

The study and preparation of enzyme template is very important and requirement. We investigated graphene and graphene oxide as a biocompatible and conductive substrate for direct electron transferring of GOx. The impedance results show graphene is more conductive than from graphene oxide. Although graphene oxide with the

posses of hydrated groups, seems to be the biocompatible environment for immobilized enzyme, but it's very week conductive substrate for enzyme electron transferring. Graphene with having of high current intensity is completely reversible for enzyme electron transfer.
Therefore, graphene is high conductive and biocompatible substrate for enzyme immobilization. In this work, graphene was used for GOx biosensor, with having low detection limit for glucose determination. These studies make useful insight for biosensors and bio-fuel cell preparation. This linear concentration rang of biosensor is expended, and the life time of biosensor is more than 4 month.

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