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A novel enzymatic glucose biosensor and sensitive non-enzymatic hydrogen peroxide sensor based on graphene and cobalt oxide nanoparticles composite modified glassy carbon electrode

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ABSTRACT

In the present study, we have demonstrated the fabrication of novel enzymatic glucose biosensor using glucose oxidase (GOD) as a model enzyme which has been immobilized onto the graphene (GF) and cobalt oxide nanoparticles (Co_3O_4-NPs) composite modified electrode. The GF/Co_3O_4-NPs composite was prepared by hydrothermal method and characterized by using scanning electron microscopy, X-ray diffraction and elemental analysis. The GOD immobilized GF/Co_3O_4-NPs modified electrode shows a well defined redox behaviour indicating the reversible proton and electron transfer reaction of GOD. A heterogeneous electron transfer rate constant (K_s) of immobilized GOD has been calculated to be 3.52 s^{-1} which is much higher than that of GOD immobilized GF supports. The fast electron transfer of GOD is attributed to the excellent biocompatibility of Co_3O_4-NPs and high conductivity of the GF. The fabricated glucose biosensor exhibits a wider linear response for glucose from 0.5 mM to 16.5 mM with the sensitivity of 13.52 $\mu A\,mM^{-1}\,cm^{-2}$. In addition, a non-enzymatic H_2O_2 sensor has been further developed using GF/Co_3O_4-NPs composite modified electrode. The GF/Co_3O_4-NPs composite electrode shows an excellent electrocatalytic activity towards H_2O_2 with the response time of <10 s. The H_2O_2 response at GF/Co_3O_4-NPs composite modified electrode displays a linear response ranging from 0.2 to 211.5 μM with a limit of detection of 0.06 μM.

1. Introduction

In recent years, graphene (GF) has flourished in the scientific field for the use of different potential applications that include solar cells, drug delivery, chemical sensors, biological sensors and many more [1–4]. GF is also known as a mother of all graphitic forms which shows remarkable electrical, thermal, mechanical and structural properties with a high theoretical surface area (2630 $m^2\,g^2$) which is higher than that of other carbon nanomaterials like carbon nanotubes and fullerenes [5,6]. Over the past few years, GF based composites are extensively studied and used in the various fields due to their fascinating unique properties like large specific surface area, high electrical conductivities, and unique mechanical properties [7]. Most of the GF based composites have been prepared using chemical reduction of reduced graphene oxide (RGO) and the corresponding metal salt solutions [8]. The active surface area of GF is greatly abridged in the RGO based composites due to the aggregation of each sheet owing to the strong π–π interaction of individual GF sheets [9]. Furthermore, the special properties of GF are largely affected in RGO due to chemical molecules that are involved in the RGO composites [9]. To overcome these problems, pristine GF is used as an alternative material to prolong the special properties for the fabrication of composites.

So far, a large number of GF based composites have been synthesized and used for the energy storage devices, biosensor and electrocatalytic applications, which include metal nanoparticles, metal oxides, conducting polymers, carbon nanomaterials and so on [10–13]. Among different nanomaterials, particularly cobalt oxide nanoparticles have attracted considerable attention due to the high ratio of surface atoms and high surface area to volume ratio with high chemical stability [14–16]. The interesting properties of cobalt oxide nanoparticles have been successfully used for a wide range of applications, including energy storage devices, biosensing and electrocatalysis [17–21]. Thus far, only a very few attempts

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have been made in the synthesis of GF-cobalt oxide nano composites for electrocatalytic applications [9]. In most of the cases, the unaided cobalt oxide nanoparticles have been used for the electrochemical sensing or biosensing [21–23]. However, there are no reports available for the immobilization of redox active proteins on the GF-cobalt oxide composite for the biosensor applications. It is well known that the direct electron transfer of GOD at bare graphene matrix is a challenging task and impossible one, due to the hydrophobic nature of GF [24]. Thus different materials or approaches have been tried with GF to wring the GOD on the GF surface. For example, nafion [25] and chitosan [26] have been widely used to enhance the biocampatibility of GF that can easily interact with GOD by physical adsorption. Herein, we have used the cobalt oxide nanoparticles (Co_3O_4-NPs) as a biocompatible material with GF for the immobilization of GOD. Certainly the fabricated GOD immobilized GF/Co_3O_4-NPs composite reveals a faster direct electron transfer towards the electrode surface than GOD immobilized at pristine GF based supports.

In this paper, we have constructed a novel glucose biosensor based on GF/Co_3O_4-NPs composite modified electrode. The GF/Co_3O_4-NPs composite was synthesized by hydrothermal method. Glucose oxidase (GOD) was used as a model enzyme for the construction of glucose biosensor. The immobilized GOD shows a well-defined redox active behaviour at GF/Co_3O_4-NPs composite and the subsequent biosensor shows a good electrocatalytic activity towards the glucose. Additionally, we have also found that the GF/Co_3O_4-NPs composite is a promising electrode material for the detection of H_2O_2. The composite electrode shows a fast amperometric response, low detection limit and wider linear response range for H_2O_2.

2. Experimental

2.1. Chemicals

Graphene flakes, Cobalt chloride hexahydrate ($CoCl_2{\cdot}6H_2O$) and glucose oxidase were purchased from Sigma–Aldrich. D+ glucose and H_2O_2 were obtained from Wako pure chemical industries. The supporting electrolyte was prepared by using 0.05 M Na_2HPO_4 and NaH_2PO_4 solutions in doubly distilled water. All the chemicals used in this work were of analytical grade and all the solutions were prepared using doubly distilled water without any further purification.

2.2. Apparatus

A computerized electrochemical workstation CHI 750a was used for cyclic voltammetry (CV) and amperometric measurements. Scanning electron microscopy (SEM) was performed using Hitachi S-3000 H electron microscope. An energy-dispersive X-ray (EDX) spectrum was recorded using HORIBA EMAX X-ACT that was attached with Hitachi S-3000 H scanning electron microscope. Amperometric (i–t curve) measurements were performed using a CHI-750a potentiostat with analytical rotator AFMSRX (PINE Instruments, USA). Rotating disc electrode (RDE) with a surface area of 0.24 cm^2 was used for the amperometric i–t measurements. Electrochemical impedance spectroscopy (EIS) studies were performed using IM6ex ZAHNER (Kroanch, Germany). Conventional three-electrode system was used for the electrochemical experiments, glassy carbon electrode (GCE) (active surface area = 0.0798 cm^2) or GF/Co_3O_4-NPs composite modified GCE was used as a working electrode, a saturated Ag/AgCl electrode as a reference electrode and a platinum electrode as the auxiliary electrode. All measurements were carried out at room temperature.

2.3. Synthesis of GF/Co_3O_4-NPs composite and immobilization of GOD

In a typical synthesis of GF/Co_3O_4-NPs composite, 0.5 g of GF flakes were added in 10 mL of distilled water containing 1 mM of $CoCl_2{\cdot}6H_2O$ and 1 mM of urea. Subsequently the mixture was stirred for 10 min and tightly covered by Teflon. The mixture was heated at 130 °C for 18 h and followed by cooling at room temperature. Finally, the mixture was again reheated at 450 °C in a oven for 3 h under a nitrogen atmosphere. After cooling, the product of GF/Co_3O_4-NPs composite was carefully collected and washed several times with distilled water and dried at 50 °C over night. The pure Co_3O_4-NPs were also prepared as described above in the presence of 1 mM of urea without GF flakes.

Fresh GOD stock solutions were prepared by dissolving GOD (5 mg mL^{-1}) in pH 7 solution and stored at 4 °C when not in use. Prior to the electrode modification, the GCE was well polished by using ultra-fine alumina powder. The pre-cleaned GCE was further sonicated in ethanol containing doubly distilled water to remove the adsorbed alumina powder. About 8 μL of GF/Co_3O_4-NPs composite (optimized concentration) was dropped onto the cleaned GCE and dried in an air oven. 6 μL of GOD was drop cast onto the GF/Co_3O_4-NPs composite modified GCE and dried at room temperature. The obtained GOD immobilized composite modified GCE was finally rinsed in distilled water to remove the loosely bound GOD on GF/Co_3O_4-NPs composite and dried at room temperature. The GF/Co_3O_4-NPs-GOD composite modified electrode was used for further electrochemical experiments and it was stored in PBS at 4 °C when not in use.

3. Results and discussion

3.1. Characterization of the GF/Co_3O_4-NPs composite

Fig. 1A shows the typical SEM image of GF, revealing its typical flake structure morphology on the electrode surface. The corresponding EDX profile of GF is shown in Fig. 1B. It can be seen clearly that a sharp elemental peak of carbon appears at 1.0 eV authenticating the pure carbon form of GF. Fig. 1C shows the SEM image of the GF/Co_3O_4-NPs composite. The Co_3O_4-NPs are uniformly distributed on the GF nano flakes with an average diameter of 82 nm, indicating that Co_3O_4-NPs are strongly anchored onto the GF by the strong interaction between Co_3O_4-NPs and GF. The reason might be due to the formation of interfacial Co—O—C bonds by the high reactivity of SP^2 carbon atom from GF with electron-rich oxygen species of Co_3O_4-NPs [17,27]. The synthesized GF/Co_3O_4-NPs composite exhibits a new elemental peak at 2.2 eV in the presence of Co on the GF surface. The formation of GF/Co_3O_4-NPs composite was further confirmed by XRD. Fig. 2A shows the XRD patterns of the prepared GF/Co_3O_4-NPs and GF (inset). The XRD shows the five obvious diffraction peaks ((2 2 0), (3 1 1), (4 0 0), (4 4 0) and (5 11)) for Co_3O_4-NPs, which is similar to the previously reported for Co_3O_4-NPs [16]. The two significant peaks appearing at $2\theta = 26.5$ and 54.6° can be attributed to the reflections of the graphitic carbon planes ((0 0 2) and (0 0 4)) at GF [9]. These results indicate the successful formation of GF/Co_3O_4-NPs composite.

The effect of loading of Co_3O_4-NPs in GF on the electrochemical properties of modified electrode was investigated by using electrochemical impedance spectroscopy. It is well known that EIS is an effective method for probing the interfacial properties of modified electrodes with respect to the electrochemical activity Fig. 2B shows the electrochemical impedance changes of the different loaded Co_3O_4-NPs in GF ($a = 0\%$, $b = 3\%$, $c = 6\%$, $d = 10\%$ and $e = 12\%$) modified GCEs in PBS containing 5 mM $Fe(CN)_6{}^{3-/4-}$ and 0.1 M KCl. The inset shows the Randles equivalent circuit