Analytica Chimica Acta 671 (2010) 36–40

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Electrochemical characterization of a single-walled carbon nanotube electrode for detection of glucose

Xuan-Hung Pham[a], Minh-Phuong Ngoc Bui[a], Cheng Ai Li[a], Kwi Nam Han[b], Jun Hee Kim[b], Hoshik Won[b], Gi Hun Seong[a,*]

[a] Department of Bionano Engineering, Hanyang University, Ansan 426-791, South Korea
[b] Department of Applied Chemistry, Hanyang University, Ansan 426-791, South Korea

ARTICLE INFO

Article history:
Received 2 March 2010
Received in revised form 2 May 2010
Accepted 5 May 2010
Available online 13 May 2010

Keywords:
Single-walled carbon nanotube
Glucose detection
Amperometric sensor
Nafion

ABSTRACT

We developed glucose biosensing electrodes using single-walled carbon nanotube (SWCNT) films on flexible, transparent poly(ethylene terephthalate). The homogeneous SWCNT films were fabricated by a vacuum filtration method, and the averaged resistivity and transparency of the fabricated flexible SWCNT films were 400 Ω sq^{-1} and 80%, respectively. The glucose sensing electrodes were constructed by encapsulating glucose oxidase (GOx) by Nafion binder into the SWCNT film, and the variation in current response as a function of enzyme loading amount, Nafion thickness were investigated. 30 mg mL^{-1} GOx and 2% Nafion was optimal for the detection of glucose. When ferrocene monocarboxylic acid (FMCA) was introduced as diffusional electron mediator, the current responses toward glucose of the Nafion/GOx/SWCNT electrodes in glucose solution containing FMCA were dramatically improved, and the developed sensor was independent of oxygen. In the application of GOx immobilized SWCNT films for glucose detection, a linear electrical response was observed for concentrations ranging from 0.25 to 3.0 mM, and the detection limit and the sensitivity were assessed to be 97 μM and 9.32 μA mM^{-1} cm^{-2}, respectively. Moreover, according to the Lineweaver–Burk plot, the apparent Michaelis–Menten constant was calculated to be 23.8 mM, and the current responses did not interfere with coexisting electroactive species, indicating that Nafion is an effective permselective polymer barrier.

1. Introduction

Carbon nanotubes (CNTs) have received considerable attention due to their electrical, mechanical, and optical properties [1,2]. In particular, CNTs have extensively been used in electrochemical biosensing studies as immuno/enzyme sensors [3–6] and DNA sensors [7–9]. One promising approach for fabricating CNT based biosensors is the fabrication of a field effect transistor in which a single semiconducting CNT connects the source electrode to the drain electrode while the gate electrode is used to manipulate the nanotube's conductivity [10]. These devices have provided excellent sensitivity for biosensing of biomolecules such as NO_2 [11], NH_3 [12], cytochrome c [13], streptavidin [14], antibodies [15], DNA [16], and glucose [17]. However, there is substantial variation between the different devices, which is due to variations in the electronic characteristics of individual nanotubes [10]. In addition, the interface between the nanotube and the metallic contact can vary from device to device. Therefore, specialized techniques are needed either to mount or grow an individual CNT at a predetermined location, and, as such, placement is difficult rendering the technique impractical for mass fabrication [10].

In another application of CNTs for biosensing, CNTs have been used as anionic dopants in the preparation of enzyme-based electrodes [18–20]. Here, CNTs were randomly dispersed into a proper solvent with enzyme or/and various binders under sonication followed by casting the resultant suspensions onto the electrode to confine CNTs onto the electrode matrix. As a result, various types of CNT based biosensors have been developed to detect some important species, such as insulin [21,22], cytochrome c [23], NADH [24], glucose [25,26], amino acids [27], trace elements [28,29], and ethanol [30]. However, the biggest obstacle to using CNTs as dopants is the distribution of CNTs on the electrode matrix. CNTs generally exist as highly tangled ropes and are insoluble in most solvents, thereby greatly hindering their capacity of forming uniform and stable films [31], resulting in low reproducibility [32].

Traditional electrodes such as carbon paste, gold, and indium tin oxide (ITO) that are most commonly used suffer from the difficulty of fabrication, limit in sensor design, and high fabrication cost. For example, gold is highly conductive but is not transparent and is expensive, while ITO, though transparent and highly conductive, is fragile. In contrast, SWCNTs are a promising elec-

* Corresponding author. Tel.: +82 31 400 5202; fax: +82 31 436 8148.
E-mail address: ghseong@hanyang.ac.kr (G.H. Seong).

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doi:10.1016/j.aca.2010.05.010

trode material for biosensing due to their excellent electrical and mechanical properties.

In this study, we have used a flexible, transparent CNT film as an electrode platform for biosensing. Homogeneous single-walled carbon nanotube (SWCNT) films produced by the vacuum filtration method were fabricated on flexible, transparent poly(ethylene terephthalate) (PET) substrates. The fabricated SWCNT electrodes were used for the electrochemical detection of glucose by encapsulating glucose oxidase (GOx) by Nafion binder into the SWCNT film. The electrochemical properties of the SWCNT electrodes were characterized and the variation in current response as a function of enzyme loading amount and Nafion thickness were investigated to optimize the electrodes.

2. Experimental

2.1. Materials

SWCNT solutions (0.3 mg mL^{-1}) and PET films were purchased from TOP NANOSYS Co. (Sung Nam, South Korea). Glucose oxidase from *Aspergillus niger* (GOx, EC 1.1.3.4, 211 units mg^{-1}), glucose, a Nafion solution (5 wt% in 15–20% water/lower aliphatic alcohols), $K_3Fe(CN)_6$, KNO_3, ferrocene monocarboxylic acid (FMCA), and sodium dodecyl sulfate (SDS) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Phosphate-buffered saline (PBS, pH 7.0) consisted of 150 mM NaCl, 4 mM KCl, 8.1 mM Na_2HPO_4, and 1.47 mM KH_2PO_4 in deionized water. All other chemicals were of analytical grade and aqueous solutions were prepared with deionized (DI) water.

2.2. Electrochemical measurements

Electrochemical measurements were carried out with a CHI600C electrochemical analyzer (CH Instruments, Inc., USA) controlled by a personal computer. A three-electrode configuration was employed, in which a Nafion/GOx/SWCNT electrode (a working electrode) was placed into a cell with clean platinum wire (a counter electrode) and Ag/AgCl (saturated in 3 M NaCl) (a reference electrode). All cyclic voltammetric measurements were performed in 0.1 M PBS buffer (pH 7.0) as the electrolyte with a scanning rate of 50 mV s^{-1}. Amperometric measurements were obtained in a 10 mL electrochemical cell with an applied potential of +0.8 V and continuous stirring at 300 rpm.

2.3. Preparation of glucose sensing electrodes using SWCNT films

Homogeneous SWCNT films were produced using a vacuum filtration method [33]. The SWCNT mixture was sonicated for 1 h and then centrifuged at 14,000 rpm for 10 min. The pre-suspended solution was further diluted by a factor of 50 with DI water and filtered through an anodic aluminum oxide membrane of 200 nm pore size and 47 mm diameter. SWCNT were trapped on the membrane surface of the filter, creating a homogeneous gray layer. Then, the filtered film was rinsed with DI water to remove SDS surfactant until no bubbles could be observed. The alumina membrane under the SWCNT thin-layer was easily removed in a 3 M NaOH solution and the thin SWCNT layer was then transferred to a flexible PET film directly after adjusting the solution to neutral pH using DI water. Next, the SWCNT film was dried in an oven at 80 °C for 30 min. To fabricate the glucose sensor electrode, 5 μL of GOx (30 mg mL^{-1}) prepared in 0.1 M PBS buffer (pH 7.0) was dropped onto the SWCNT films surface (0.5 cm × 0.5 cm) and dried for 12 h. The enzyme-adsorbed surface was coated with a secondary layer of 5 μL of 1% Nafion in PBS buffer (pH 7.0) and was then dried at 4 °C to generate a uniform surface. The resulting electrodes were thoroughly washed with PBS buffer (pH 7.0) and kept in a refrigerator when not in use.

3. Results and discussion

3.1. Preparation and characterization of SWCNT electrodes

To fabricate the homogeneous SWCNT films in a large area, the vacuum filtration method was adopted. The thickness of the SWCNT films was controlled to be ~100 nm by adjusting the concentration and volume of the SWCNT suspension. The averaged resistivity and transparency of the fabricated flexible SWCNT films were 400 Ω sq^{-1} and 80%, respectively. SWCNT films showed high flexibility with negligible change in resistivity when undergoing hard bending. Other methods of SWCNT film production including drop-drying from solvent, airbrushing, and Langmuir–Blodgett deposition have presented limitations in terms of the film quality or production. However, the vacuum filtration method guarantees the homogeneity of the SWCNT films by the process itself. In addition, the film thickness can be controlled with nanoscale precision by adjusting the SWCNT concentration and volume of the suspension. GOx was immobilized onto SWCNT films by a Nafion binder, which is a well-known perfluorosulfonate polymer bearing a polar side chain. Nafion has unique ion-exchange, discriminative, and biocompatible properties, thus it has been widely used for modifying electrode surfaces and constructing amperometric biosensors [34,35]. This insulating polymer has been recently demonstrated to be useful for the preparation of CNT based biosensors due to its ability to confine the CNTs and enzymes onto substrate electrodes [25,36]. GOx solution was dropped onto the SWCNT film and then the enzyme-adsorbed surface was coated with Nafion as a secondary layer. The morphological changes of the SWCNT films were investigated by SEM (Fig. 1). SWCNTs having dense and homogeneous cylindrical morphology were observed on SWCNT film electrode. However, this typical morphology was not obtained in SWCNT film electrodes modified with GOx/Nafion, where a rough surface covered by particles resulting from evaporation of Nafion polymer solution was clearly revealed. The significant difference in surface morphologies of the two electrodes demonstrates the presence of GOx and Nafion on the SWCNT film.

The electrical performance of the SWCNT film electrode was evaluated by cyclic voltammetry (CV) measurements using the redox couple $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ as a probe. Fig. 2A showed the CVs of the pristine SWCNT film electrode and the SWCNT film electrode modified with Nafion and GOx in 0.4 M KNO_3 solution containing 1 mM $K_3(FeCN)_6$ at a scanning rate of 50 mV s^{-1}. Because of the significant electrocatalytic activities of SWCNTs, a pair of well-defined oxidation and reduction peaks with a symmetric shape was observed for the SWCNT film electrode at 0.55 and −0.20 V, respectively. The electrical potential separation was 750 mV, corresponding to a formal potential ($E^{0\prime}$) of 0.175 V. In contrast, the charge current obtained for the Nafion/GOx/SWCNT film electrode was two-fold less than at the pristine SWCNT electrode. The electrical potential separation between the anodic and cathodic peaks shifted positively by 25 mV compared to that of the pristine SWCNT electrode. These results come from a slower electron transfer rate due to the lower inherent conductivity of Nafion and GOx than that of the SWCNT materials. Even though the oxidation–reduction process became slower at the surface of the Nafion/GOx/SWCNT electrode, the selectivity was remarkably improved as mentioned in next section. In addition, the anodic and cathodic current responses were linearly proportional to the square root of the scan rate ranging from 10 to 200 mV s^{-1} (Fig. 2B). The