Sensors and Actuators B 197 (2014) 20–27

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Enzyme biosensor based on an N-doped activated carbon fiber electrode prepared by a thermal solid-state reaction

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ARTICLE INFO

Article history:
Received 5 November 2013
Received in revised form 11 February 2014
Accepted 17 February 2014
Available online 22 February 2014

Keywords:
Enzyme biosensor
Sensor performance
Activated carbon fiber
Thermal solid-state reaction
Functional group

ABSTRACT

The sensitivity of a biosensor electrode was increased by introducing hydrophilic N-groups onto the surface of a polyacrylonitrile (PAN) based activated carbon fiber. The electrospun carbon fiber was activated using KOH to improve the adsorption of glucose oxidase (GOx) enzymes through pore production and the introduction of oxygen functional groups. The activated carbon fibers (ACFs) were then reacted with urea to increase their hydrophilicity by doping their nitrogen groups. The sensor sensitivity and the Michaelis-Menten constant, K_m, were altered by varying the percentages of functional groups on the electrode surfaces. Whereas the value of K_m was affected by the kind of functional groups, the sensitivity of the biosensor electrode was chiefly affected by the amount of functional groups introduced urea modification because the enzyme was better immobilized onto urea-modified activated carbon fibers. Quantitatively, the sensitivity was two- to three-fold higher for the biosensors based on urea-treated ACFs than those based on untreated ones. This increased sensitivity is attributed to the nitrogen and oxygen functional groups on the urea-modified ACFs.

1. Introduction

Glucose biosensor design is an active area of research that aims to enhance sensor performance, which is critical in clinical applications such as diabetes testing and in the food industry for quality control and the detection of hazardous contaminants [1]. Due to the large demand for glucose biosensors in various fields, many glucose biosensors are fabricated, and improvements to glucose detection methods using biosensors are actively pursued. The detection method is used to analyze glucose concentrations during one-time blood sugar testing or during continuous real-time glucose monitoring [2]. Generally, the human body regulates blood glucose levels within the range of 4–8 mM (70–120 mg dL^{-1}). Therefore, glucose biosensors are designed to detect blood glucose levels of 2–30 mM (30–500 mg dL^{-1}) [3,4].

Biosensors rely on electrochemical transduction, employing glucose oxidase (GOx) or glucose dehydrogenase (GDH) enzymes and the measuring materials appropriate for the analyzed matrices. Electrochemical biosensors are based on the entrapment of these enzymes in polymer matrices or membranes deposited on a metal or carbon working electrode, which is utilized as the transducer, and a mediator, which is utilized as the medium that transfers the electrons from the reduced enzyme to the electrode. The mediator has the several functions: One is the direct electrical communication between the enzyme and the electrode surface. The other function is to reduce any re-oxidation at the electrode. The mediator also regenerates its oxidized form [5–8].

Carbon nano-materials are used as substrates in the adsorption of biomaterials because they allow for the loading of high quantities of enzymes and for the sensing of electrical signals in a microenvironment. In addition, these materials can be incorporated for rapid electron transfer and enhanced sensitivities. Carbon nanomaterials have several forms including graphite, activated carbon fiber, and carbon nanotubes, all of which have been studied for use as biosensor electrodes [7–12].

However, carbon nano-materials have a weakness that prevents their wide use as biosensors; the surface of the carbon nanomaterials is composed of hydrophobic elements, but the biosensor must operate in a hydrophilic environment. The poor interfacial affinity between the surface of hydrophobic carbon nanomaterials and the hydrophilic biomaterial results in low sensitivity due to decreased electron transfer. Thus, the hydrophilic properties of the carbon surface must be enhanced by doping it with hydrophilic groups [13–15]. The doping elements include various heteroatoms (N, B, and P) that network with the carbon materials. Doping with these heteroatoms has been found to enhance the electrical conductivity

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http://dx.doi.org/10.1016/j.snb.2014.02.054
0925-4005/

and electrode biomaterial interaction of carbon nano-materials by increasing their charge-transfer ability and surface hydrophilicity, respectively [16,17].

In this study, biosensor electrodes were prepared by using N-doped activated carbon fibers (ACFs) based on polyacrylonitrile (PAN). The prepared carbon fiber was activated using KOH to increase the quantity of immobilized glucose oxidase enzyme. To increase the sensitivity of the biosensor, nitrogen groups were doped onto the surface of the activated carbon fiber by urea treatment. The amperometric and the current-voltage curve methods were utilized to examine the effects of the conductivity, and interfacial affinity at the contact area between the N-doped activated carbon electrode and glucose oxidase (GOx) on the glucose-sensing ability.

2. Experimental

2.1. Materials

The polymer and the solvent for the electrospinning solution were PAN (polyacrylonitrile, Aldrich, San Louis, MO, USA) and DMF (N,N-dimethyl formamide, 99.0%, Samchun, Korea), respectively. Potassium hydroxide (KOH, 95.0%, Samchun, Korea) was used to produce pores and introduce oxygen groups onto the carbon surface. Urea (H_2NCONH_2, 99.0–100.5%, ACS reagent) was used as a reacting agent to produce amine groups onto the carbon surface. Glucose (D-(+)-glucose, ACS reagent, San Louis, MO, USA) was prepared in a stock solution that was further used for glucose sensing analysis. Glucose oxidase (GOx, Type X-S, *Aspergillus niger*, 211 U mg^{-1}, San Louis, MO, USA) was used as an enzyme. Potassium hexacyanoferrate ($K_4[Fe(CN)_6]$, 98.5–102.0%, ACS reagent, San Louis, MO, USA) was used as the electron-transfer mediator between the GOx and the electrode. A-screen-printed electrode (SPE, Pine Research Ins., Canada,) was used as the sensing electrode to load the prepared glucose-sensing material for the electrochemical measurements.

2.2. Methods

2.2.1. Preparation of the PAN based fibers by electrospinning

The 10-wt% PAN solution was prepared by DMF and then placed into a 30-cm^3 syringe with a capillary tip (18 G; inner diameter: 1.27 mm). The PAN solution was electrospun using an apparatus equipped with a power supply (NT-PS-25 K, NTSEE Co, Korea). The experimental conditions, such as the concentration of the solution, the voltage, the TCD (tip-to-collector distance), and the pump rate of the syringe, were those previously established by our research group and others for manufacturing PAN-based carbon fibers by electro-spinning [18,19]; thus, the following conditions were applied: an applied voltage of 15 kV, a TCD of 10 cm, a syringe pump rate of 1.5 mL h^{-1}, and a collector speed of 200 rpm [20].

2.2.2. Preparation of activated carbon fiber

The electrospun fiber prepared was stabilized at 260 °C for 4 h in an air atmosphere. To carbonize these stabilized electrospun fibers, they were thermally treated at 1050 °C for 1 h in a nitrogen atmosphere at a heating rate of 5 °C min^{-1}. After stabilization and carbonization, the PAN-based carbon fiber was then activated with 6 M KOH solution as a chemical activation agent to produce mesopores [18,21]. The carbon fiber was immersed in a KOH solution (20 mL/g carbon fiber) for 4 h, with shaking. The immersed sample was placed in an alumina boat and activated in a 750 °C furnace for 3 h in a nitrogen atmosphere. The heating rate was 5 °C min^{-1} and the feed rate of the nitrogen gas was 50 mL min^{-1}. After chemical activation, the resulting sample was washed several times with distilled water until the filtered wash water had a neutral pH. The washed samples were then dried at 110 °C for 1 h [22].

2.2.3. N-doping of the surface of activated carbon fiber

N-doped ACF was prepared by a thermal solid-state reaction method. In a typical experiment, 1 g of as-prepared samples were first finely milled with a different g of urea (1, 3, 5, and 8 g) and then transferred onto an alumina boat. The boat with mixture was placed in the center of a furnace under flowing N_2. After flushing the tube with N_2 for about 30 min, the furnace was heated up to 200 °C at a rate of 5 °C min^{-1} and kept at the temperature for 2 h. After that, the furnace was cooled down to room temperature. The N-doped ACFs was washed with deionized water to remove any residual species adsorbed on the sample surface, and then dried at 110 °C for 1 h [23]. The samples for the sensing electrode were prepared with these ACF: urea weight ratios of 1:1, 1:3, 1:5, and 1:8, which are hereafter denoted as 1U-ACF, 3U-ACF, 5U-ACF, and 8U-ACF, respectively. The weight ratios of prepared electrode were decided by basic experimental results obtained in our laboratory and referencing reported paper [9].

2.2.4. Preparation of the glucose sensor electrode

The final product, which consisted of N-doped ACF (10 mg), was mixed with a GOx solution (4 mg mL^{-1} in 1 mL double-distilled water) by vortexing for 10 s and was stored for 24 h to immobilize the enzyme on the carbon surface. This ACF/GOx mixture (6 μL) was dropped by micropipette onto a working electrode with an area of 4 mm × 5 mm in the prepared SPE. The mixture-loaded SPE was then dried at room temperature for 1 h. This SPE (enzyme electrode) was rinsed with double distilled water to remove unbound enzyme molecules and it was stored at 4 °C for overnight. Hence, this modified electrode was successfully achieved.

2.2.5. Surface analysis of the samples and glucose-sensing measurement

X-ray photoelectron spectra (XPS) were obtained using a MultiLab 2000 spectrometer (Thermo Electron Corporation, UK) and were used to identify the elements present on all sample surfaces.

Cyclic voltammetry (CV) and amperometric studies were performed in a reaction cell containing 30 mL PBS buffer solution (0.1 M, pH 7.0). The glucose-sensing measurements using the N-doped ACFs were processed by cyclic voltammetry (CV) and amperometric methods using Ivium CompactStat (The Netherlands) potentiostat in a reaction cell equipped with three electrodes consisting of N-doped ACFs as the working electrode, platinum electrode as the counter electrode and Ag/AgCl electrode as the reference electrode [24]. The sensitivities of the electrodes prepared with various quantities of N-doped ACFs were measured at glucose concentrations ranging from 0 to 30 mM at pH 7.0. Measurement of amperometric analyses were calculated as an average of five measurements and standard derivations were given as ±SD. All experiments were carried out at ambient conditions.

3. Results and discussion

3.1. The surface element of the N-doped carbon fiber

An XPS elemental analysis was performed to investigate the number of O- and N-containing functional groups on the urea-treated ACF surface. The survey data and deconvoluted C1s and N1s peaks are presented in Figs. 1, 2 and 3, respectively. These results, including the atomic ratio of each element on the surface, are listed in Tables 1 and 2. Fig. 1 provides the XPS survey graphs of both the raw (RACF) and N-doped ACFs (U-ACF), which exhibit distinct carbon and oxygen peaks at approximately 284.5 and 531.0 eV.