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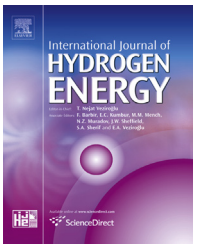

Immobilization of glucose oxidase on 3D graphene thin film: Novel glucose bioanalytical sensing platform

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ABSTRACT

Electrochemical biosensors are responsible for quantification of analytes for medical diagnostics applications. They are considered as a promising means to investigate the content of a biological sample owing to the direct exchange of a biological process to an electronic output signal. Novel characteristics of nanocarbon materials attracted much attention for fabrication of numerous electrochemical biosensors with developed analytical capacities. This paper aims to provide perceptions of 3D graphene-based electrochemical biosensors and to demonstrate its application in glucose detection. The developed glucose biosensing platform exhibits excellent catalytic activity towards glucose detection over a wide linear range of up to 6 mM with sensitivity of 1.63 μA mM^{-1} cm^{-2} and the stability of electrode is around 76.9% after one month. The facile and easy electrochemical approach used for the preparation of 3DG–GOD modified GCE may open up new horizons in the production of cost-effective biosensors.

Introduction

The exceptional and interesting chemical and physical properties of nanoparticles make them as a truly appropriate candidate to design novel and developed sensors, especially electrochemical biosensors. Great deals of attempts have been made technologically in order to estimate, monitor and control chemical species [1]. The great need for analytical information using biosensors open up promising perspectives in various applications, including healthcare and veterinary medicine, the food, pharmaceutical, biomedical and many others. In addition, the enhanced collections of data as well as their automated analysis are able to assist in the management and diagnosis of chronic and episodic diseases like diabetes, congestive heart failure, and cardiac dysrhythmias. Therefore, biosensors deliver encouraging influence on identifying, monitoring and controlling health [2].

Recent enhancements have been introduced toward utilization of nanotechnology in order to decrease the dimensions of electrochemical sensor elements, which lead to an increment of signal-to-noise ratio and signal per event. A multidisciplinary comprehension of bio- and

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electrochemistry, solid-state and surface physics, bioengineering, integrated circuit technology and signal processing pave the way for introducing new generation of greatly specific, sensitive, selective and reliable biochemical sensors and biosensors [3–6].

The very first produced biosensor, which has been used to measure glucose in diabetic patients' blood has been commercially introduced in 1975 by Springs Instruments (Yellow Springs, OH, USA). One of the fundamental parts of an electrochemical biosensor is a proper enzyme in the biorecognition layer offering electroactive substances intended for detection by the physico-chemical transducer providing the measurable signal [7–9].

One of the leading causes of death and disability all around the world is diabetes, which is caused by abnormal blood glucose concentration. Real time intensive care of physiological glucose transport is essential for acquisition of new understanding of this epidemic disease. The growing trend of diabetic patients can be controlled by employing CNTs to design highly sensitive glucose biosensors [10–13]. In this kind of biosensors, glucose concentration can be measured by electrochemical monitoring of H_2O_2 through catalyzing glucose to gluconic acid and hydrogen in the existence of oxygen. Correspondingly, a research conducted by Yu et al. [14] exhibited direct electrochemistry of glucose oxidase (GOD) on 3D porous CNT-based electrodes.

Researches have been proved that utilization of an immobilization matrix with excellent electrical conductivity, stability, and antifouling characteristics is significantly required for biosensing devices. In other words, enzyme immobilization not only enhances the practical stability, but also brings about the controlled diffusion induced by augmented amount of enzyme. Besides, consumption of free enzymes by cells in enzyme immobilization contributes to stability of immobilized enzymes in order to encourage the growth and restore the defect. The simplest reversible route of immobilization in biomedical applications is the enzyme encapsulation via electroporation [15,16]. Immobilization methods are performed mainly to prevent enzyme loss. It was assumed that deficient immobilization in biosensors would reveal a greater level of degradation in sensor performance [17].

3D graphene (3DG) foam can be a promising potential as biosensor's support with notable sensitivity and low detection limit. This remarkable performance can be credited to some unique properties of 3DG including (i) large surface area; (ii) excellent charge transfer rate caused by the extraordinarily great conductivity of graphene as well as 3D multiplexed conductive architecture of graphene foam; and (iii) the macroporous structure of 3DG, which establish effective mass transport of the diffusional redox species. Moreover, 3DG has gained researchers' attention due to its capability to be functionalized or hybridized with other organic (e.g., enzymes) or inorganic materials with large capacity and ability of molecules detection with high oxidation or reduction potential [18–21]. In this contribution, the glassy carbon electrode has been modified with 3DG–GOD biocomposite and its electroanalytical applications towards the glucose determination have been investigated in details.

Experimental

Apparatus

All electrochemical measurements were performed using a computer controlled Potentiostat/Galvanostat (302N Autolab) in a 50 ml cell with conventional three electrode system with modified glassy carbon electrode (GCE) with a diameter of 3 mm as working electrode, a thin Pt wire as counter electrode and Ag/AgCl (3 M KCl) as a reference electrode. Field-emission scanning electron microscope images were obtained by means of Carl Zeiss SUPRA 35VP. Bruker-D8 Advance Powder X-ray diffraction (XRD) was employed using CuKa radiation of 1.5416 Å at a scan rate of 0.02 2θ s^{-1}. The morphology was studied by field emission electron microscopy (FESEM) and high resolution transmission electron microscopy (HRTEM) Hitach, Japan.

Reagents and materials

Graphite powder, H_2SO_4, H_3PO_4, $KMnO_4$, Na_2HPO_4, NaH_2PO_4, KCl and GOD were purchased from Sigma–Aldrich. The supporting electrolyte used for electrochemical studies was 0.1 M phosphate buffer solution (PBS), prepared using Na_2HPO4, NaH_2PO_4 and KCl dissolved in deionized water (DI Water) with pH of 7.

Preparation of 3D graphene as a biocatalyst support

The fundamental material for preparation of 3DG is graphite in order to synthesize graphite oxide by modified Hummer's method [22,23]. Graphene oxide was produced by oxidation of graphite flakes with specific amounts of chemicals including H_2SO_4 (120 ml), H_3PO_4 (13 ml) and $KMnO_4$ (6 g). The obtained mixture was stirred for 72 h to make sure of completed oxidation of the graphite. Throughout this step, the color of the mixture turned to dark brown. Ice cubes and H_2O_2 (7 ml) were added to the mixture in order to halt the oxidation process. Thus, yellowish color of the mixture revealed the high oxidation level of graphite. The obtained graphite oxide was washed consecutively with HCl and DI Water until the pH of 4–5 was achieved. As a result of this stage, the graphite oxide was subjected to exfoliation which causes the production of graphene oxide gel [24].

In order to get a homogeneous solution a mixture of GO aqueous dispersion (1 ml) and DI Water (40 ml) was stirred for 20 min. During the stirring process, hydrazine (10 μl) was added to the mixture and pH adjusted to 9. The obtained mixture sealed in a 50 ml stainless steel autoclave and maintained at 80 °C for one day. The produced 3DG kept in a freeze drier for two days to suck out the adsorbed water. Dried 3DG stored into the sealed Schott bottle for further actions [25].

Fabrication of GCE/3DG–GOD modified electrode

Initially, a test tube filled with 3DG and 5 ml ethanol ultrasonicated for half an hour to produce a homogeneous solution. After cooling down, 5 mg of glucose oxidase enzyme

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