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Covalent modification of ordered mesoporous carbon with glucose oxidase for fabrication of glucose biosensor

Elaheh Ghasemi [a], Esmaeil Shams [a,*], Najmeh Farzin Nejad [b]

[a] Chemistry Department, University of Isfahan, Isfahan 81746-73441, Iran
[b] Petroleum Refining Technology Development Division, Research Institute of Petroleum Industry, Tehran, Iran

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ABSTRACT

In this paper, a new methodology is developed for the modification of glassy carbon electrode (GCE) with covalently modified ordered mesoporous carbon (OMC) with glucose oxidase (GOD) as a model of electroactive biomolecules. In this methodology the following steps were followed: (1) preparing the OMC modified GCE (OMC/GCE) by simple casting, (2) functionalization of OMC/GCE with 4-nitrophenyl (NP) group by electrochemical reduction of 4-nitrobenzenediazonium salt in nonaqueous media, (3) converting the surface NP to aminophenyl (AP) through electrochemical reduction, (4) covalent attachment of 2,4,6-trichloro-1,3,5-triazine (TCT) to the surface of electrode through its reaction with AP and (5) enzyme immobilization through reaction between GOD and surface TCTs. The direct electrochemistry and catalytic activity of the immobilized GOD on OMC are investigated. Although the cyclic voltammetry (CV) revealed direct electron transfer (DET) between GOD and the OMC modified electrode, however, the biosensor shows response to glucose only in the presence of oxygen, indicating the DET capability and enzymatic activity occurred on different immobilized GODs. The surface coverage of GOD with DET property and GOD with enzymatic activity are 2.94×10^{-10} and 6.87×10^{-12} mol cm^{-2}, respectively. The obtained glucose biosensor shows excellent analytical performance for glucose determination.

1. Introduction

Developing new procedure for effective and stable immobilization of enzymes is very important to design enzyme biosensing and bioelectronics devices. The immobilization procedure must maintain the enzymes close to the transducer surface, while maintaining its biological activity. These objectives may be accomplished when suitable procedures are adopted for immobilization of enzymes on suitable substrates.

A great effort is put in finding new materials and immobilization procedures to improve direct electron transfer (DET) between redox enzyme and supporting matrix and to keep the bioactivities of enzymes. These materials include carbon nanotubes [1], metal or metal oxide nanoparticles [2,3], and polymers [4].

Besides the materials mentioned above, the ordered mesoporous carbons (OMCs) are the types of the new advanced carbon materials, initially synthesized in 1999 [5]. Ever since, the OMCs have been the matter of concern due to their unique properties such as high specific surface area, ordered mesostructure, tunable pore size, low density, high conductivity, chemical stability, and biocompatibility [6]. The high density of edge plane defect sites on OMC may provide many favorable sites for electron transfer to biomolecules which make the OMC a potential new materials for investigating the electrochemical behavior of the substances [7]. These materials are used to modify electrodes in order to determine some biological molecules, including L-cysteine [8], morphine [9], and epinephrine [10]. In addition, OMCs are used as protein immobilization supports in studying the direct electrochemistry of hemoglobin (Hb) [11,12], myoglobin [13], and fabricating glucose [13,14]and alcohol [15,16] biosensors. Accordingly, it is deduced that the available reports indicate a strong evidence where OMC could be an effective candidate for fabricating new types of electrochemical sensors and biosensors [17].

According to the investigations made here no study is conducted on the covalent attachment of biomolecules to the surface of OMCs. The objective of this study is to introduce a new and simple approach for the covalent immobilization of glucose oxidase (GOD), as a model of electroactive enzyme, on the OMC. For this purpose, first the OMC is synthesized and fixed on a glassy carbon electrode (GCE) surface. Next, the OMC/GCE is functionalized with amine groups. Then 2,4,6-trichloro-1,3,5-triazine (TCT) is covalently attached on the surface amino groups and finally GOD is covalently immobilized on the surface of the modified GCE. The

* Corresponding author.
E-mail address: e_shams@chem.ui.ac.ir (E. Shams).

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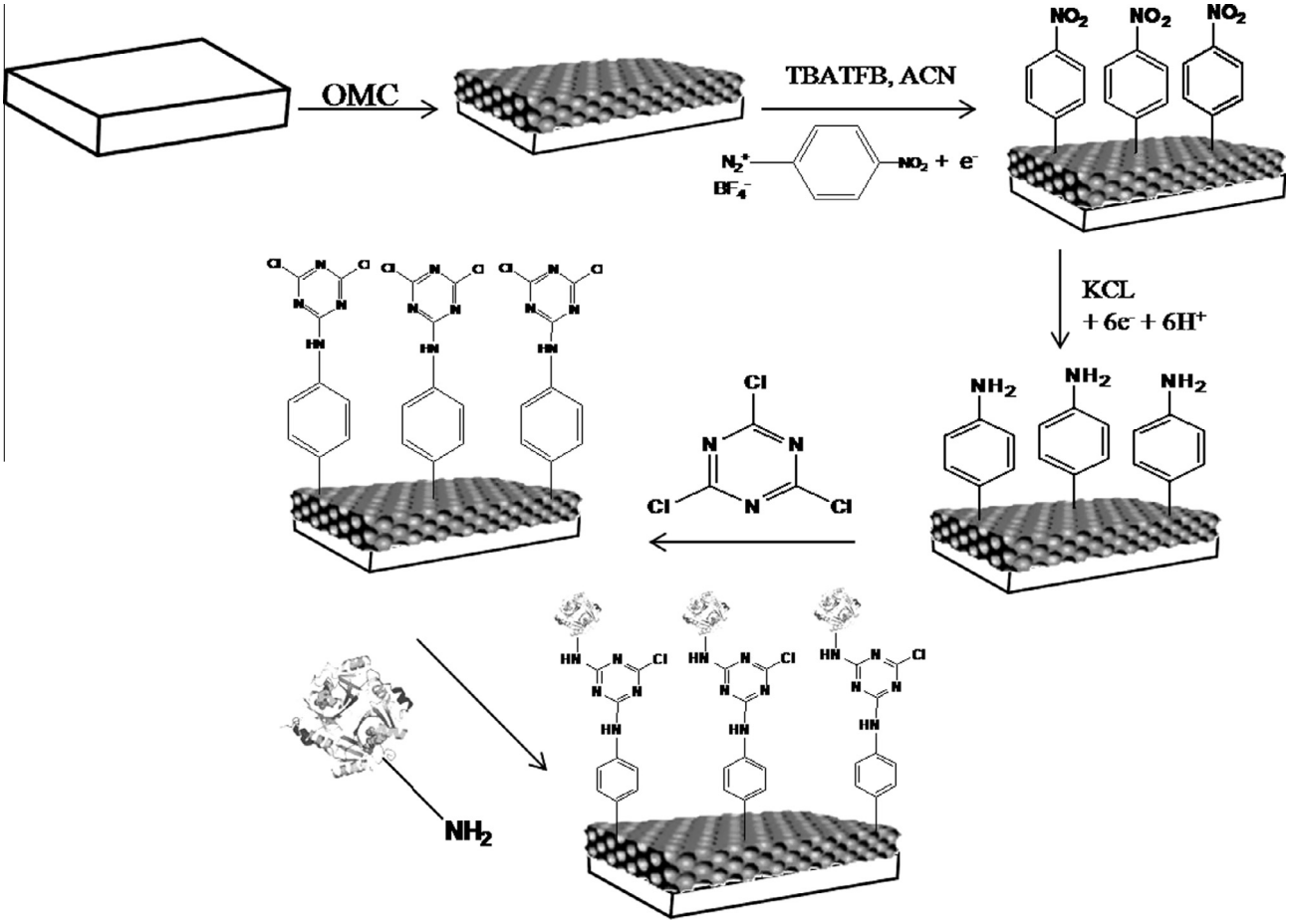

Scheme 1. Schematic representation of different steps of GOD modified GCE.

catalytic activity of the immobilized GOD toward the oxidation of glucose indicates that the proposed strategy could be a good candidate in immobilizing biomolecules on the surface of OMC.

2. Experimental

2.1. Materials and reagents

The OMC is prepared in our laboratory and its synthesis, purification and characterization procedure is reported in our previous articles [18,19]. The transmission electron microscopy (TEM) image and X-ray diffraction (XRD) pattern of the synthesized OMC are shown in Figs. S1 and S2, respectively. GOD (EC 1.1.3.4. Type II: from *Aspergillus niger*) is purchased from Sigma. Other chemicals are of analytical grade from Merck, Fluka, and Aldrich and used without further purification. Distilled water is consumed in all aqueous solutions. The phosphate buffer solutions (0.1 M) are prepared from NaH_2PO_4, Na_2HPO_4 and 0.1 M KCl and their pHs were adjusted using 0.1 M HCl or 0.1 M NaOH solutions using a calibrated pH meter.

2.2. Apparatus

The electrochemical measurements including cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) are performed with a computer-controlled potentiostat, the Autolab electrochemical analyzer model PGSTAT30 (EcoChemie, Utrecht, The Netherlands). A conventional three electrode cell is used, containing a modified GCE as the working electrode, Ag/AgCl (3 M KCl) and Ag/Ag^+ (0.01 M acetonitrile (ACN)) as reference electrodes for electrochemical experiments in aqueous and organic solvents, respectively, and a platinum wire as the counter electrode. pH measurements are made with a Metrohm 827 pH meter.

2.3. Electrode preparation and modification

Prior to electrode modification, the GCE of 3 mm diameter is successively polished to a mirror shine surface by applying 1, 0.3 and 0.05 μm Al_2O_3 powder. After polishing the electrode is thoroughly rinsed with distilled water and sonicated in ethanol and distilled water for 5 min. The obtained GCE is then dried at room temperature.

Five milligram of OMC is dispersed in 1 ml N,N′-dimethyl formamide (DMF) through ultrasonic agitation. Next 2 μl of the mixture is transferred on the GCE surface, where the solvent is evaporated under an infrared lamp. The surface modification of (OMC/GCE) with 4-nitrophenyl (NP) and conversion of NP to aminophenyl (AP) is carried out according to the methodology already described in our research group for modification of bare GCE [20]. The obtained AP/OMC/GCE is transferred into 50 mM TCT in tetrahydrofuran (THF) solution for 90 min. After washing with THF to remove any adsorbed TCT, the TCT-activated electrode is dried at room temperature. This TCT-activated electrode is immersed in phosphate buffer solution (pH 7.0) containing 5 mg ml^{-1} of GOD, for about 1 h for covalent immobilization of GOD. Finally, the obtained glucose biosensor is washed with distilled water and then stored in pH 7.0 buffer solutions in a refrigerator at 4 °C for further use upcoming in experiments. For