Electrochimica Acta 151 (2015) 186–194

Contents lists available at ScienceDirect

Electrochimica Acta

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

A new enzymatic immobilization carrier based on graphene capsule for hydrogen peroxide biosensors

Zengjie Fan [a,c], Qianqian Lin [c], Peiwei Gong [a,b], Bin Liu [c], Jinqing Wang [a,*], Shengrong Yang [a]

[a] State Key Laboratory of Solid Lubrication, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, PR China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100080, PR China
[c] School of Stomatology, Lanzhou University, Lanzhou 730000, PR China

ARTICLE INFO

Article history:
Received 16 August 2014
Received in revised form 31 October 2014
Accepted 5 November 2014
Available online 6 November 2014

Keywords:
Graphene
Biosensor
H_2O_2
Graphene capsules

ABSTRACT

Enzymatic loss and inactivation are two main problems which can affect the performance of the biosensor. In order to resolve these two problems, a new kind of enzymatic biosensor for the amperometric detection of hydrogen peroxide (H_2O_2) was developed using biomimetic graphene capsules (GRCAPS). Horseradish peroxidase was initially encapsulated in GRCAPS using porous $CaCO_3$ as sacrificial templates to mimic the existence form of bio-enzymes in the organisms, and then GRCAPS and graphene-poly(sodium 4-styrenesulfonate) were alternatively assembled onto the substrate of indium tin oxide for constructing multilayer films of the biosensor. Transmittance electron microscopy and field-emission scanning electron microscopy analyses proved that the GRCAPS and multilayer films were prepared. Electrochemical experiment results indicated that easy, direct electrochemistry and good catalytic activity toward H_2O_2 oxidation can be achieved with this biosensor. The resulting biosensor presented a wide linear range of 0.01–12 mmol l^{-1}, a low detection limit of 3.3 μmol l^{-1} (S/N = 3), excellent anti-interference ability, and long-term stability as well.

1. Introduction

In recent years, various methods have been developed for the determination of hydrogen peroxide (H_2O_2) because of its wide and varied applications in many fields, such as in clinical, food, pharmaceutical, and environmental analyses [1–4]. Among these methods, biosensors based on immobilized horseradish peroxidase (HRP) with different nanomaterials are effective and easy instruments for fast and accurately determination of H_2O_2 under complicated test conditions [5,6]. In the past decades, immobilization of various enzymes has been accomplished by multiple approaches: adsorption on the support material; attachment through covalent bonding; microencapsulation and entrapment in the membrane; and film or gel [7–9]. However, the above-mentioned methods have several drawbacks, such as enzymatic loss, enzymatic inactivation, serious diffusion constraints, and low stability [9,10]. In addition, HRP immobilization method cannot provide good protection for the enzyme, and enzyme inactivation would happen in complicated test conditions, such as high ionic-concentration toxicity and microorganism degradation [11]. Thus, finding an effective immobilization method to enhance the service longevity of these biosensors is necessary.

In organisms, various enzymes are encapsulated in a single compartment which represents a microenvironment separated from outer environmental factors; within this compartment, the enzymes are shielded and protected, preventing their denaturation [12]. Inspired by nature, we attempt to construct new graphene capsules (GRCAPS), aiming at mimicking the existing form of enzymes in living cells. Graphene, as the key material, has attracted tremendous research interest since its discovery because of its extraordinary chemical, optical, electrical, and mechanical properties [13–17]. Particularly, its excellent electrical conductivity, electron mobility, small band gap, and ultrahigh surface area make it widely applicable in the area of biosensors [1,18]. These advantages of graphene would bestow good conductivity to the capsule film, further facilitate fast electron transfer between enzyme and basal electrode, and enhance the sensitivity and detection limit of biosensors [19]. In addition, the ultrathin film of graphene is found to possess unique permeability properties similar to cellular membrane, which can permit the small molecules to pass through and prevent the big molecules from getting through [16,20]. Therefore, microorganisms would be

* Corresponding author. Tel.: +86 931 4968076.
E-mail address: jqwang@licp.cas.cn (J. Wang).

http://dx.doi.org/10.1016/j.electacta.2014.11.022
0013-4686/

blocked in the outer environment of capsule, preventing enzyme deactivation caused by microorganisms. Furthermore, several studies have found that graphene possesses sterilizing ability, thus offering further protection [21,22]. At present, however, studies about GRCAPS are only focused on drug delivery systems and oil absorption [23,24]. So far, to the best of our knowledge, GRCAPS used in biosensors have not been reported, and this new biomimetic GRCAPS is believed to have similar function to the cell, which can protect the enzyme, permit H_2O_2 to pass through, and freely allow electron transfer.

In this study, we report a new H_2O_2 biosensor based on biomimetic GRCAPS for H_2O_2 determination. For the preparation of GRCAPS, porous calcium carbonate ($CaCO_3$) microspheres were used as sacrificial templates and temporary HRP immobilization carriers, and graphene oxide (GO) as building block of the capsule. After the templates were removed, GRCAPS with encapsulated HRP can be obtained. This mimics the existing form of bio-enzymes in organisms, and this special structure would provide good protection for enzyme bioactivity. The biosensor was constructed by alternatively assembled GRCAPS and graphene-poly(sodium 4-styrenesulfonate) (GS-PSS) onto the substrate of indium tin oxide (ITO) glass sheet, aiming at resolving the problems of enzyme loss and inactivation, and attaining a novel biosensor with high sensitivity, wide linear range, low detection limit, as well as good selectivity and long-time stability.

2. Experimental

2.1. Apparatus

The morphologies of the synthesized samples were characterized by transmission electron microscopy (TEM, JEOL JEM–2010) and field-emission scanning electron microscopy (FESEM, JEOL JSM–6701 F). The thickness of GO sheets was quantified by atomic force microscopy (AFM, Nanoscope III, Veeco). The surface area was determined from the linear part of the BET equation ($P/P0 = 0.05$–0.35) according to the N_2 adsorption-desorption isotherm (Micromeritics ASAP 2020 analyzer). The Electrochemical measurements were performed on a CHI660C electrochemical analyzer (Shanghai, China).

2.2. Reagents

Graphite, KCl, and $NaH_2PO_4 \cdot 2H_2O$ were purchased from Sinopharm Chemical Regent Co., Ltd. Na_2CO_3 was obtained from Shanghai Sanyou Reagent Factory. $Na_2HPO_4 \cdot 12H_2O$ were obtained from Tianjin Guangfu Chemical Reagent Factory. Ascorbic acid (AA) and ethylenediamine tetraacetic acid disodium salt (EDTA) were purchased from Tianjin Regent Factory. HRP, (3-aminopropyl) triethoxysilane (APTES), uric acid (UA), PSS (Mw = 70 000), and polyethylenimine (PEI, average Mw = 2500) were obtained from Sigma. All chemicals are of analytical grade and used as received. Ultrapure water (>18 MΩ · cm) was used for rinsing and the solvent.

2.3. Preparation of GRCAPS with HRP

As illustrated in Fig. 1, the preparation process of GRCAPS can be divided into two stages, which involve the initial preparation of porous $CaCO_3$ template and the subsequent construction of GRCAPS. GO colloid solution was obtained from graphite according to the modified Hummers method [25]. Porous $CaCO_3$ was prepared by the modified Volodkin method [26]. In detail, 0.33 mol l^{-1} Na_2CO_3 solution was added into 0.33 mol l^{-1} $CaCl_2$ aqueous solution under vigorous stirring by using a magnetic stirrer for 30 s, and then the mixed solution was left without being stirred for 10 min. Afterwards, the solution was centrifuged at 3000 rpm for 5 min. The obtained precipitate was dried at 40 °C overnight. To prepare GRCAPS, HRP was added into the 3 mg/ml of $CaCO_3$ solution to get a final concentration of 2 mg/ml. This mixed solution was placed on an ice bag, and shaken at 200 rpm for 24 h on a vibration table, ensuring full absorption of HRP in the porous of $CaCO_3$. This solution was centrifuged at 3000 rpm for 5 min, and washed away the HRP without adhesion to $CaCO_3$ with phosphate

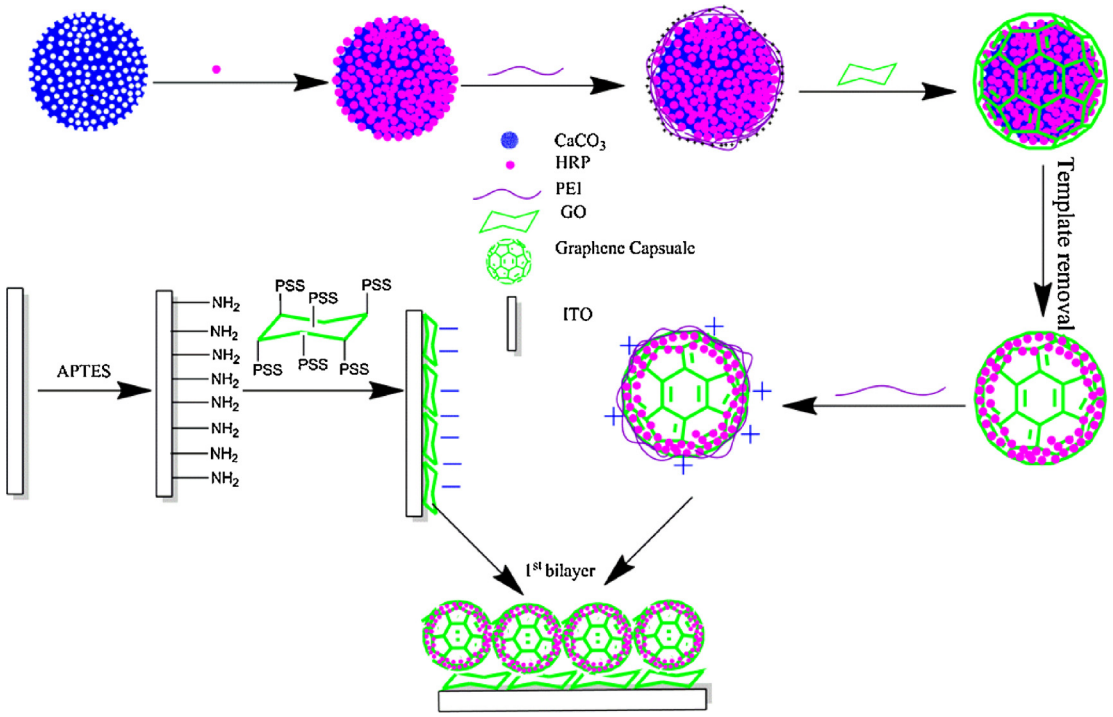

Fig. 1. Schematic view for constructing GRCAPS and electrode assembly.