Colloids and Surfaces B: Biointerfaces 118 (2014) 188–193

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Preparation of graphene oxide doped eggshell membrane bioplatform modified Prussian blue nanoparticles as a sensitive hydrogen peroxide sensor

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ARTICLE INFO

Article history:
Received 2 June 2013
Received in revised form 13 January 2014
Accepted 16 January 2014
Available online 25 January 2014

Keywords:
Eggshell membrane
Graphene oxide
Prussian blue
Bioplatform

ABSTRACT

This study describes the preparation and characterization of graphene oxide doped eggshell membrane (GO-ESM) as a novel electrochemical bioplatform for electroanalytical purposes. The GO-ESM bioplatform was prepared by incorporation of GO nano-sheets into the ESM via a facile sonication procedure. Field emission scanning electron microscopy and X-ray diffraction powder techniques were used to characterize the developed bioplatform. The electrochemistry of GO-ESM was investigated by decorating it on the surface of carbon ceramic electrode (CCE) by an O-ring. The GO-ESM platform was modified with Prussian blue (PB) via a facile dip-coating method. Then the resulted modified electrode (PB|GO-ESM|CCE) was used as a novel hydrogen peroxide electrochemical sensor. The fabricated electrode responds efficiently to H_2O_2 over the concentration range 125 nM–195 μM with a detection limit of 31 nM (S/N = 3) and sensitivity 8.8 μA μM^{-1} cm^{-2}. The PB|GO-ESM|CCE has been successfully applied to determination of H_2O_2 content in spiked milk samples. Due to good stability, environmental friendly, cheapness, nontoxic, well behaved electrochemical properties, and biocompatibility, the fabricated bioplatform has the promising future for practical applications.

1. Introduction

During the last years, natural polymers have attracted significant research interests for application in tissue engineering, fuel cell, drug delivery, separation, and electrochemical sensors [1]. Some natural polymers such as chitosan [2], silk [3], and eggshell membrane [4] have been reported as an appropriate substrate for electrode materials. Eggshell membrane (ESM) is a light pink double-layered membrane inside the eggshell, which is composed of biological molecules and highly cross-linked protein fibers [5]. It possesses excellent water-permeability, biocompatibility, nontoxicity, cheapness, and stability. Recently ESM was successfully used to design electrochemical sensors and biosensor [6–13]. However, ESM suffers from low conductivity which decreases its efficiency in electrochemical applications.

Graphene oxide is a typical pseudo-two-dimensional of carbon atoms having unique electrical, optical and structural properties [14]. Recently it has been used vastly in bioimaging, catalysis, photovoltaic, and electrochemical sciences [15]. Due to good biocompatibility, high electron transfer ability, and high surface to volume ratio, GO was composited with various polymers to improve their special properties [16,17].

This study describes the preparation of GO doped ESM (GO-ESM) as a new flexible electrode material for electrochemical applications. The conductivity of ESM bioplatform was enhanced after doping with GO nano-sheets. In order to survey the efficiency of GO-ESM in electron transfer process and electrocatalytic activity, it was modified with Prussian blue (PB) as an electron transfer mediator via a facile dip-coating method. Due to high conductivity, good porosity, and cheapness, carbon ceramic electrode (CCE) was used as a substrate for the developed bioplatform. Finally the constructed electrode (PB|GO-ESM|CCE) was used for the electrocatalytic determination of hydrogen peroxide in milk samples.

Facile incorporation of GO into the ESM, effective modification of GO-ESM bioplatform with PB nanoparticles, good stability and high catalytic activity of the fabricated electrode, are the important advantages of the developed hydrogen peroxide sensor.

2. Experimental

2.1. Reagents

Fresh hen eggs were purchased from the local supermarket. The eggs albumen and yolk were removed and the broken eggs were immersed in 1% acetic acid solution for 30 min in order to obtain

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http://dx.doi.org/10.1016/j.colsurfb.2014.01.020

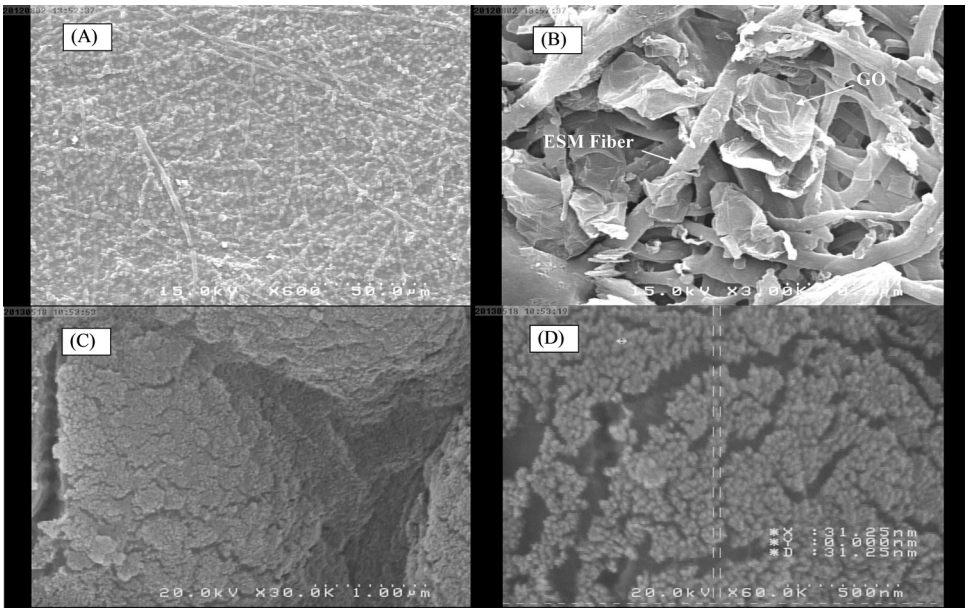

Fig. 1. (A) FESEM images of ESM, (B) GO-ESM, (C) PB|GO-ESM (magnification 30,000), (D) PB|GO-ESM (magnification 60,000).

the ESM easily [18]. Then it was washed with adequate ultra-pure water. Graphene oxide was purchased from Azar Kimia Nanotechnology Co., Tabriz, Iran, which was synthesized according to the modified Hummers method [19]. Methyltrimethoxysilane, KNO_3, $K_3[Fe(CN)_6]$, $Fe(NO_3)_3{\cdot}9H_2O$ and other reagents were of analytical grade and were purchased from Merck or Aldrich. The acetate buffer solution (0.1 M) was prepared from CH_3COOH, and NaOH. All solutions were prepared with doubly distilled water. Also all the experiments were carried out at room temperature ($20 \pm 2\,^{\circ}C$).

2.2. Apparatus

The electrochemical experiments were carried out using an AUTOLAB PGSTAT-100 (potentiostat/galvanostat) equipped with an USB electrochemical interface and driven GPES software in conjunction with a three-electrode system and a personal computer for data storage and processing. The utilized three-electrode system was composed of a saturated (KCl) calomel electrode (SCE) as the reference electrode, a platinum wire as the auxiliary electrode and PB|GO-ESM|CCE as a working electrode (prepared as follows). An ultrasonic bath (model LBS2, Falc Instruments, Treviglio (BG), Italy) was used during the doping of GO to ESM structure. The morphology of the GO-ESM was studied by field emission scanning electron microscopy (FESEM) (Hitachi, model S-4160). X-ray diffraction (XRD) was performed by using a Brucker AXF (D8 Advance) X-ray power diffractometer with a Cu Kα radiation source ($\lambda = 0.154056$ nm) generated at 40 kV and 35 mA.

2.3. Preparation of graphene oxide doped eggshell membrane

Firstly, the broken fresh eggshell was incubated in diluted 1% acetic acid at 22 °C for 1 h. Afterward the ESM was easily separated and cut into small pieces (1 cm^2) and was cleaned with copious amount of twice distilled water. Then, the ESM was immersed in graphene oxide solution (1 mg mL^{-1}) which was prepared by oxidizing nature graphite powder according to the reported method in the literature [19], and then placed in ultrasonic bath for 3 h. After changing the color of ESM from purple to brown in the presence of GO, the constructed bioplatform (GO-ESM) was immersed in ultra-pure water for 1 h to remove the excess of GO from the membrane.

2.4. Preparation of Prussian blue modified GO-ESM bioplatform

Due to some unique properties of carbon ceramic electrodes (CCE) involving high porosity, renewable surface, good conductivity, and economy of fabrication, it was used as the electrode substrate. CCE was prepared according to the procedure described by Lev and co-workers [20]. 0.3 mL methyltrimethoxysilane, 0.45 mL methanol, and 10 μL hydrochloric acid (0.1 M) were mixed for 3 min. afterward, 0.3 g carbon powder was added and the resultant mixture was shaken for about 1 min. A piece of Teflon tube was filled with the sol–gel mixture, and was dried overnight under ambient conditions. A small piece (0.5 cm × 0.5 cm) of GO-ESM bioplatform was put on the surface of CCE (0.119 cm^{-2}) and was fit by an O-ring. The resulting electrode is donated as GO-ESM|CCE.

The surface of GO-ESM|CCE was modified with Prussian blue (PB) according to our previous report [21]. The surface of GO-ESM|CCE was immersed in 2 mM aqueous solution of $K_3[Fe(CN)_6]$, 2 mM $Fe(NO_3)_3{\cdot}9H_2O$, and 0.5 M KNO_3 as derivatizing reagent (8 min), resulting in a stable film of PB on the surface of the electrode. The modified electrode was rinsed with twice distilled water, and used for electrochemical studies. Finally, electrical contact was made with a copper wire through the back of the electrode.

3. Results and discussion

3.1. Characterization of GO doped ESM|CCE

The FESEM images of ESM, GO|ESM, and PB|GO-ESM were shown in Fig. 1. As can be seen, the structure of the ESM (Fig. 1A) is an intricate lattice network composed of highly cross-linked protein fibers. This membrane is composed of interlinked and coalescing fibers ranging from 1 to 3 μm in diameter, and micropores about 5–10 μm in size [22]. This structure allows facile incorporation of GO nanosheets into the semipermeable ESM which results a homogeneous bionanocomposite throughout the whole membrane. Fig. 1B shows the FESEM image of GO-ESM, representing that graphene oxide sheets appropriately doped in ESM protein fibers. The ESM contains an abundance of amine, amide and carboxylate functional groups [23], which causes appropriate interaction with graphene oxide containing hydroxyl, epoxy, and carboxylic acid functional groups [24]. Fig. 1C and D shows the FESEM images of