Bioelectrochemistry 120 (2018) 150–156

Contents lists available at ScienceDirect

Bioelectrochemistry

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

Rose petal and P123 dual-templated macro-mesoporous TiO_2 for a hydrogen peroxide biosensor

Xu Wu [a], Huanhuan Zhang [b], Kejing Huang [c,*], Yan Zeng [d], Zhihong Zhu [d,*]

[a] College of Physics and Electronic Engineering, Xinyang Normal University, Xinyang 464000, PR China
[b] Collaborative Innovation Center of Henan Province for Energy-Saving Building Materials, Xinyang Normal University, Xinyang 464000, PR China
[c] College of Chemistry and Chemical Engineering, Xinyang Normal University, Xinyang 464000, PR China
[d] Institute of Nano-science and Nano-technology, College of Physical Science and Technology, Central China Normal University, Wuhan, 430079, China

ARTICLE INFO

Article history:
Received 11 September 2017
Received in revised form 16 December 2017
Accepted 16 December 2017
Available online 19 December 2017

Keywords:
Electrochemical biosensor
Rose petal templates
Mesoporous TiO_2
Horseradish peroxidase
H_2O_2 detection

ABSTRACT

In this work, highly ordered macro-mesoporous TiO_2 has been successfully synthesized using fresh rose petals and P123 ($EO_{20}PO_{70}EO_{20}$) as dual templates through a simple soaking and calcining process. Characterization of the as-prepared TiO_2 indicated that the mesoporous structure of the TiO_2 was highly ordered, with a pore diameter of approximately 3 nm. After electrodeposition of Pt nanoparticles onto the TiO_2 as an electron transfer enhancer and the immobilization of horseradish peroxidase (HRP) onto the TiO_2-modified electrode, a biosensor for detecting hydrogen peroxide (H_2O_2) was realized. This biosensor showed a wide linear detection range from 5 μM to 8 mM and a low detection limit of 1.65 μM with good stability and high selectivity, suggesting that the sensor is well-suited for the detection of H_2O_2.

1. Introduction

TiO_2, an n-type semiconductor, is widely applied in catalysis [1], molecular adsorption [2], biosensing [3] and energy storage [4] due to its advantages such as high photocatalysis, nontoxicity, good biocompatibility and excellent chemical/physical stability. For sensing applications in particular, TiO_2 has been extensively studied based on its ability to exist in various morphologies, such as sol — gel matrices [5], nanotubes [6,7], nanowires [8,9], nanorods [10] and hollow microspheres [11]. Among these architectures, the mesoporous structure [12–14] has shown much promise due to its possession of a high surface area that provides more active sites for the immobilization of noble metals and biomolecules.

Recently, much attention has been directed towards the development of biotemplates, based on their low cost, nontoxicity, environmentally friendliness and extensive existence in nature. Accordingly, biotemplates have been widely used to prepare hierarchical nanomaterials for use in photocatalysis applications, supercapacitors, lithium ion batteries and biosensors. For example, morpho butterfly wings were used as biotemplates to fabricate PMAA photonic crystals with hierarchical structures [15]. Collagen fiber was also utilized to synthesize cerium-doped TiO_2 mesoporous nanofibres for photocatalysis [16]. Additionally, eggshell membrane [17], microalgae [18], regenerated cellulose membrane [19], pollen grains [20], cotton [21] and cellulose [22] have also been used as biotemplates. However, almost all biotemplates need to be pretreated under heavy acidic or alkali conditions.

Herein, fresh rose petals and P123 were used as templates to synthesize highly ordered macro-mesoporous TiO_2 by a dual-template method for use in the development of an electrochemical enzyme biosensor. Horseradish peroxidase (HRP) was also used in the construction of the biosensor. The developed method is a simple, facile and controllable synthetic process that involves only soaking and calcining, along with the use of fresh rose petals without any pretreatment beyond washing in deionized water. The macroporous structure of the as-prepared highly ordered macro-mesoporous TiO_2 was obtained from the papillae on the surface of the rose petal, which had an average diameter of 10 μm, and the mesopores were oriented by the structure-directing agent P123. These two templating agents worked together to generate the unique hierarchical macro-mesoporous structure of the product. The high biocompatibility of TiO_2 is very beneficial in maintaining the bioactivity of the HRP after its immobilization onto the biosensor. However, TiO_2 does not have good conductivity; therefore, platinum nanoparticles were introduced onto the TiO_2 electrode by electrodeposition to further enhance the direct electron transfer speed [23]. After the electrodeposition, the Pt/TiO_2-modified electrode was reacted with HRP to form a hydrogen peroxide (H_2O_2) biosensor.

* Corresponding authors.
E-mail addresses: kejinghuang@xynu.edu.cn (K. Huang), zhzhu@mail.ccnu.edu.cn (Z. Zhu).

https://doi.org/10.1016/j.bioelechem.2017.12.009
1567-5394/

Cyclic voltammetry and chronoamperometry methods were used to investigate the direct electrochemical and electrocatalytic behaviours of the prepared biosensor. The results of these tests demonstrated that the fabricated biosensor possessed a wide linear detection range from 5 μM to 8 mM and a low detection limit of 1.65 μM. The sensor also displayed good stability and high selectivity. These attributes suggested great potential for use of the developed biosensor in the detection of H_2O_2. Two primary biological factors played key roles in this study. First, the natural morphological features of rose petals were explored for use as hard templates in the synthesis of the nanomaterial. Then, the properties of the obtained nanomaterial were exploited to develop a biosensor through the immobilization of an enzyme (in this case HRP, though other enzymes could also be used) onto the macro-mesoporous TiO_2.

2. Experimental

2.1. Reagents and instrumentation

Horseradish peroxidase (>250 U mg^{-1}) and P123 (M 5800) were obtained from Sigma-Aldrich. Tetrabutyl titanate and $H_2PtCl_6 \cdot 6H_2O$ were purchased from Sinopharm Chemical Reagent Co., Ltd. Acetylacetone was purchased from Tianjin Fuchen Chemicals Reagent Factory. The supporting electrolyte was a 0.1 M phosphate buffer solution (PBS, pH 7.0). All chemicals used in this work were of analytical grade.

All electrochemical measurements were carried out on a CHI electrochemical workstation (Shanghai CH Instrument, CHI 440A, China) with a three-electrode system consisting of an HRP-modified electrode as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. Ultraviolet-visible (UV–vis) light absorption spectra were collected on a Shimadzu spectrophotometer (Shimadzu UV-1700, Japan). Fourier transform infrared (FT-IR) spectroscopy was performed on a Nicolet spectrophotometer (Nicolet Company, 5700 FT-IR, America). Scanning electron microscopy (SEM) was performed on a JSM-6700F instrument (JEOL, Japan), with all samples metalized by coating with Au and immobilized onto dark conducting resin prior to analysis. X-ray powder diffraction (XRD) patterns were collected on an X'Pert PRO MRD (PANalytical, Netherlands).

2.2. Synthesis of macro-mesoporous TiO_2

Macro-mesoporous TiO_2 was synthesized by a dual-template method using fresh rose petals and P123 [24]. First, P123, ethyl alcohol and hydrochloric acid were added to deionized (DI) water with aggressive magnetic stirring for 1 h, after which acetylacetone was added dropwise into the solution. After stirring for another 20 min, tetrabutyl titanate was injected to produce a solution with a molar ratio of tetrabutyl titanate:P123:HCl:ethyl alcohol:H_2O:acetylacetone = 1:0.01:0.79:12.86:4.89:0.10. After allowing the solution to stand for 24 h, fresh rose petals were added individually into the obtained solution and held for 20 s, then lifted. The fresh rose petals were kept at room temperature for 7 days, then heated to 90 °C for 24 h to strengthen the interaction between the inorganic components. Finally, macro-mesoporous TiO_2 was obtained after calcination at 450 °C in air for 3 h.

2.3. Preparation of the modified electrode

A glassy carbon electrode (GCE, d = 4 mm) was sequentially polished with 2000 mesh metallographic sandpaper and 0.05 μm alumina slurry. It was then washed with ethanol and DI water and dried under a purified nitrogen stream. The prepared TiO_2 was re-suspended in DI water before drop-coating onto the electrode. For this process, 7 μL of a suspension containing 0.1 mg mL^{-1} TiO_2 was applied dropwise onto the electrode to obtain a mesoporous TiO_2-modified electrode (TiO_2/GCE). Electrodeposition of Pt onto the TiO_2/GCE was performed in 5 mM H_2PtCl_6 for 50 s at −0.3 V. After rinsing with DI water, the resulting Pt/TiO_2/GCE

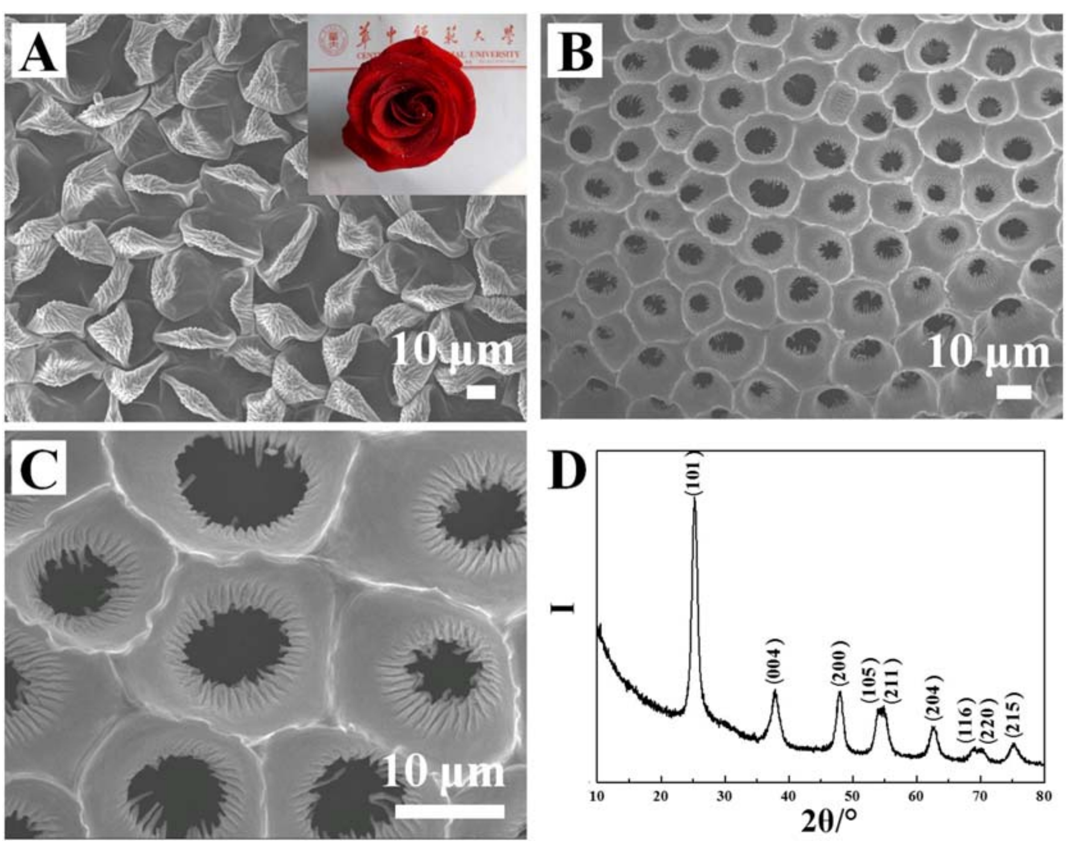

Fig. 1. SEM (A) and optical (inset) images of rose petal; SEM (B and C) images and XRD spectrum (D) of as- prepared macro-mesoporous TiO_2.