Analytica Chimica Acta 946 (2016) 40–47

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Preparation of TiO_2 nanosheet-carbon nanotube composite as immobilization platform for both primary and secondary antibodies in electrochemical immunoassay

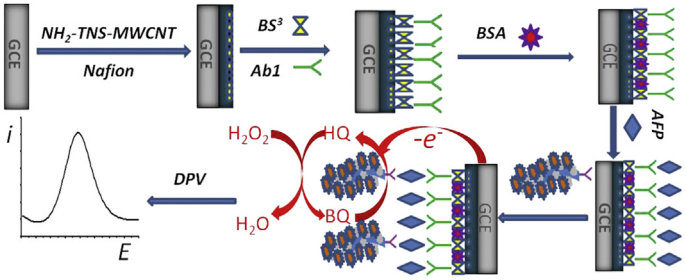
Xiaoqiang Liu*, Pepipei Liu, Xiaohe Huo, Xiuhua Liu, Jin Liu

Institute of Environmental and Analytical Sciences, College of Chemistry and Chemical Engineering, Henan University, Kaifeng, Henan Province, 475004, PR China

HIGHLIGHTS

- TiO_2 nanosheet-carbon nanotube composite was synthesized by hydrothermal method.
- The composite exhibits improved properties compared to the individuals.
- The composite is designed as electrode scaffold for immobilizing primary antibody.
- Secondary antibody and horseradish peroxidase are immobilized on the composite as a label.
- The tracing label exhibits great amplification effect for immunosensing.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 August 2016
Accepted 16 October 2016
Available online 24 October 2016

Keywords:
TiO_2 nanosheets
Multi-wall carbon nanotubes
Sandwich-type immunoreaction
α-Fetoprotein
Nano-composite

ABSTRACT

TiO_2 nanosheets (TNSs) were synthesized and deposited on multi-wall carbon nanotubes (MWCNTs) to form a nano-composite through a hydrothermal method, followed by the characterization with various spectroscopic and microscopic techniques. The TNS-MWCNT composite was then applied as not only an electrode scaffold to immobilize primary antibody, but also as a carrier to load secondary antibody and horseradish peroxidase (HRP). In both cases, bis(sulfosuccinimidyl) suberate sodium salt acted as an amino cross-linker to covalently bind the biomolecules on TNS-MWCNT composite through their surface primary amino groups. After the sandwich-type immunoreaction, HPR was quantitatively captured on the electrode surface via the binding between secondary antibody and antigen, and electrochemical response of the immunosensor was then amplified by a H_2O_2 mediated HRP catalytic reaction. Using α-Fetoprotein as a model analyte, a linear range between 0.005 and 320 ng mL^{-1} with a detection limit of 2.0 pg mL^{-1} was achieved by differential pulse voltammetry. The improved immunosensor performance could be attributed to the biocompatibility and high specific surface area of TNS, and excellent electrical conductivity of MWCNTs, which accelerated the electron transfer at the electrode surface.

1. Introduction

Various immunoassay techniques including chemoluminescence immunoassay [1], enzyme-linked immunosorbent

* Corresponding author. Tel.: +86 13781157777.
E-mail addresses: 13781157777@163.com, liuxiaoqiang@henu.edu.cn (X. Liu).

http://dx.doi.org/10.1016/j.aca.2016.10.020
0003-2670/

assay (ELISA) [2], fluorescence immunoassay [3,4] and radioimmunoassay [5] have been extensively applied in the medical field such as cancer screening, early disease diagnosis, therapeutic efficacy and so on [6]. As a powerful supplement to the above techniques, electrochemical immunosensors exhibited certain advantages including operation convenience, simple preparation, rapid analysis, high sensitivity, and uncomplicated instrumentation [7,8]. For example, they are more sensitive than spectrometric or optical immunoassays because the latter necessitate bulky and power-intensive light sources, monochromator and optical detectors. Moreover, turbid and coloured samples may produce false positive signals when the optical methods are used to detect such samples.

In recent years, different nanomaterials such as magnetic particles [9–11], noble metal nanomaterials [12,13], carbon nanomaterials [14–16] and oxide nanomaterials [17,18] have been extensively applied for the development of electrochemical sensors to improve their performance including dynamic range, detection of limit, selectivity, stability, precision and etc. Among them, carbon nanomaterials became the most excellent electrode materials due to their outstanding electrical conductivity, good chemical stability, considerable mechanical strength and chemically modifiable surfaces [19]. For example, Malhotra et al. [20] have prepared an electrochemical immunosensor for detecting cancer-related levels of interleukin-6 in squamous cell carcinomas of head and neck cells. The immunosensor was constructed on an electrically conductive, high surface area and densely upright packed single wall carbon nanotube (SWCNT) forest with primary antibody attached to their terminals. The antigen would bind to the primary antibody, followed by the horseradish peroxidase (HRP)-labelled secondary antibody to facilitate a sandwiched assay. High sensitivity was achieved through enzymatic catalysis amplification on SWCNT forest, resulting in a low detection limit of 30 pg mL^{-1} for human interleukin-6 in calf serum.

However, the direct formation of the bioconjugate between secondary antibody and signal enzymes involves many complicated steps, which may lead to denaturation of the biomolecules. Accordingly, carbon nanomaterials were also used as the support for immobilizing both signal enzymes and secondary antibody. For example, Zhao et al. [21] fabricated an electrochemical immunosensor involving carbon nanospheres for microcystins-LR (MCLR) detection. In their work, HRP and secondary antibody were co-immobilized on carbon nanospheres to form signal-amplifying labels. Due to the three-dimensional porous structure and high conductivity of carbon nanospheres, this approach yielded a linear range from 0.05 to 15 μg L^{-1} MCLR with a detection limit of 0.016 μg L^{-1}.

Although carbon nanomaterials behaved well as both the immobilization platform and the support for secondary antibody/enzymes, they still suffer from certain disadvantages as described below. In the first place, the electrochemical signals resulted from CNT surface carboxyl groups may bring interference to the immunosensor test and affect the detection accuracy. Furthermore, the biocompatibility of carbon materials is not as good as that of semiconductors, therefore, carbon materials have shown weaker capability of retaining bioactivity than semiconductors.

In this work, TiO_2 nanosheets with high surface area, negligible electrochemical interference and excellent biocompatibility were deposited on highly conductive carbon nanotube surface to form a composite, which retained the advantages of both materials and minimize their shortages [22,23]. For the first time, this composite was applied as both the immobilization scaffold for primary antibody (Ab1) and the carrier for secondary antibody (Ab2) and HRP. This novel strategy will consume less time compared with the traditional electrochemical immunoassay, which has to prepare Ab1 scaffold and Ab2 support separately. The obtained immunosensor was applied to detect a model analyte of α-Fetoprotein (AFP) with differential pulse voltammetry (DPV) technique to demonstrate the superiority of TNS-CNT composite over CNT.

2. Experimental

2.1. Materials and reagents

Short carboxyl multi-wall carbon nanotubes (MWCNTs) (outside diameter: 20–30 nm, length: 0.5–1 μm) were purchased from Chengdu Organic Chemical Co., Ltd., Chinese Academy of Sciences, China. Titanium (IV) isopropoxide (TIP; 97%) and diethylenetriamine (DETA; 99%) were purchased from J&K Scientific Ltd., Beijing, China. Mouse monoclonal primary and secondary anti-AFP antibodies (clone no. A14C11 and A46C9) and AFP (≥96%) (Product no. A0101) were purchased from Shuangliu Zhenglong Biochem. Lab (Chengdu, China) and immediately diluted to the required concentrations with 0.02 M phosphate buffer (pH 7.4) before use. 3-aminopropyltriethoxysilane (APTES), suberic acid bis(3-sulfo-*N*-hydroxysuccinimide ester) sodium salt (BS^3), HRP, bovine serum albumin (BSA), 30% H_2O_2 (v/v) and Nafion were obtained from Sigma-Aldrich (Shanghai, China). Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all the assays. All the other reagents were of analytical grade and used as received. The phosphate buffer solutions (PBS, pH 7.4) were prepared by mixing the stock solutions of 0.05 M KH_2PO_4 and 0.05 M K_2HPO_4 containing 0.10 M KCl as the supporting electrolyte. The washing buffer (PBST) consists of PBS (0.05 M, pH 7.4) and 0.05% (w/v) Tween 20 and the blocking solution was PBS (0.05 M, pH 7.4) containing 5% (w/v) BSA.

2.2. Apparatus

All electrochemical measurements were conducted at a CHI630C electrochemical workstation (CH Instruments, Shanghai, China) in a conventional three-electrode cell. Glassy carbon electrode (GCE), platinum wire and Ag|AgCl (3.0 M KCl) electrode were used as working electrode, counter electrode and reference electrode respectively. All the electrodes were purchased from Gaoshiruilian Co., Ltd., Wuhan, China. Electrochemical impedance spectroscopy was collected at an IM6ex electrochemical station (ZAHNER, Germany). The morphology and chemical composition of the nanocomposite were studied by transmission electron microscope (TEM, Tecnai G2 20, USA), scanning electron microscope (SEM, JSM-7500F, JEOL., Japan), X-ray diffraction (XRD, Bruker D8 Advance, Germany) with Cu Kα radiation and Fourier transform infrared spectra (FT-IR, Nicolet 170, USA). X-ray photoelectron spectra (XPS) were collected on an X-ray photoelectron spectrometer (ESCALAB 250Xi, USA) with a monochromated Al Kα source ($h\nu = 1486.6$ eV), 150 W Power and 500 μm beam spot. The spectra were calibrated on the C1s peak (284.8 eV) and analyzed using XPSPEAK41 software.

2.3. Preparation of TNS-MWCNT composite and its amination

The TNS-MWCNT composite was prepared as follows: 50 mg of MWCNTs was dispersed in 40 mL isopropyl alcohol by 20 min of ultrasonication, followed by addition of 50 μL DETA. Then 3.6 mL TIP was added to the above dispersion under proper stirring. The mixed solution was subsequently transferred into a Teflon-lined stainless steel autoclave and the autoclave was kept in an electric oven at 200 °C for 24 h TiO_2 nanosheets are formed through the hydrolysis of TIP catalyzed by DETA, which also aids in assembling TiO_2 nanosheets on the surface of carboxyl modified MWCNTs.