Biosensors and Bioelectronics 105 (2018) 226–235

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Novel tungsten phosphide embedded nitrogen-doped carbon nanotubes: A portable and renewable monitoring platform for anticancer drug in whole blood

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ARTICLE INFO

Keywords:
Biosensor
Anticancer drug
Tungsten phosphide
Nitrogen doped carbon nanotubes
Point-of-care testing

ABSTRACT

Biosensors based on converting the concentration of analytes in complex samples into single electrochemical signals are attractive candidates as low cost, high-throughput, portable and renewable sensor platforms. Here, we describe a simple but practical analytical device for sensing an anticancer drug in whole blood, using the detection of methotrexate (MTX) as a model system. In this biosensor, a novel carbon-based composite, tungsten phosphide embedded nitrogen-doped carbon nanotubes (WP/N-CNT), was fixed to the electrode surface that supported redox cycling. The electronic transmission channel in nitrogen doped carbon nanotubes (N-CNT) and the synergistic effect of uniform distribution tungsten phosphide (WP) ensured that the electrode materials have outstanding electrical conductivity and catalytic performance. Meanwhile, the surface electronic structure also endows its surprisingly reproducible performance. To demonstrate portable operation for MTX sensing, screen printing electrodes (SPE) was modified with WP/N-CNT. The sensor exhibited low detection limits (45 nM), wide detection range (0.01–540 μM), good selectivity and long-term stability for the determination of MTX. In addition, the technique was successfully applied for the determination of MTX in whole blood.

1. Introduction

The rapid development of highly electrocatalytic materials that are able to meet on-site fast response monitoring and inexpensive detection costs have prompted researchers to improve or redesign biosensors (Kimmel et al., 2012; Turner, 2013). For example, the field of clinical diagnostics and treatment especially requires sensors for monitoring drug levels in patients (McKeating et al., 2016). Hence, there is an urgent need to obtain accurate test results in a short time for a large number of samples to assist the doctor to decide on the course of medical treatments (Ronkainen et al., 2010). In addition, in vivo and real-time monitoring of anticancer drug concentration in plasma of patients will improve treatments, provided the availability of sensors. More importantly, frequent monitoring can avoid toxic effects and damage to organs (Cohen, 2000). Developing sensors for drug monitoring is important in the sensing community.

Methotrexate (MTX) is used worldwide in the treatment of a number of cancers and belongs to the class of antifolates. In addition, it is also frequently used to treat some autoimmune diseases, such as rheumatoid arthritis, psoriasis and lupus, because it has effective anti-inflammatory and immunosuppressive properties (Asadian et al., 2017). However, excessive use of MTX in clinical treatment can cause serious toxic side effects such as lung and liver disease, ulcerative stomatitis and low white blood cell counts (Rozenszajn and Radnay, 1974). Therefore, monitoring the concentration of MTX in blood is necessary in order to create an optimized dosage and thereby minimize the toxicity.

Current MTX detection methods focus on the use of modern instruments such as high performance liquid chromatography (HPLC), (Merás et al., 2005) fluorimetry, (Chen et al., 2007) UV–vis spectrophotometry, (Sastry and Lingeswara Rao, 1996) electrospray ionization tandem mass spectrometry (ESI-MS), (Barbieri et al., 2006) surface plasmon resonance (SPR) sensing (Zhao et al., 2015) and capillary electrophoresis (CE) (Szakács and Noszál, 2006). These techniques have the advantages of sensitivity and accuracy, meanwhile, they share some common drawbacks, such as high cost and tedious sample preparation method, which make them not suitable for continuous monitoring. In this regard, electrochemical methods with respect to their advantages such as simplicity, sensitivity, accuracy and ease of on-site applications have received considerable attention in recent years (Asadian et al., 2017). However, to the best of our knowledge, most of the research is

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https://doi.org/10.1016/j.bios.2018.01.045
Received 15 November 2017; Received in revised form 31 December 2017; Accepted 21 January 2018

focused on the development of high-performance electrode materials or the improvement of the electrochemical sensors property. There is still a need for the miniaturization of MTX sensors for portable, reusable, and point-of-care testing.

Tungsten phosphide (WP_2 and WP) and its composite materials, an important family of catalysts, are beneficial for hydroprocessing catalysts (Clark et al., 2002; Li et al., 2017) and hydrogen evolution reaction (HER) (McEnaney et al., 2014; Xing et al., 2014). Obviously, WP_2 and WP have such a wide range of applications mainly due to their high electrical conductivity (Xing et al., 2014). In addition, support materials exhibit great influence on the cost, performance, and durability of electrocatalysts (Shao et al., 2009). Micro-nanostructured carbon materials, such as carbon black (Shu and Oyama, 2005), nitrogen-doped carbon matrix (Pu et al., 2016), and carbon cloth (Pu et al., 2014) among others, are important classes of novel support materials for WP mainly because their superior conductivity can significantly promote electrocatalytic performance. Furthermore, carbon nanotubes (CNT) and nitrogen-doped carbon nanotubes (N-CNT) are extremely important carbon-based supporting materials. Their unique structural, electrical, and mechanical properties were employed as novel composite materials with superior performance (Collins et al., 1997; Kang et al., 2009). Unfortunately, high-performance electrode materials that reasonably combine these two excellent species, WP and N-CNT, have not yet been reported. Meanwhile, these unique electron conduction rates and high electrocatalytic activity using for electrochemical sensors have also not been reported. Therefore, exploring the simple and reliable method of preparation WP modified N-CNT (WP/N-CNT) and developing high-performance electrochemical sensors based on this material is of great significance.

Here, a facile method to fabricate WP embedded nitrogen-doped carbon nanotubes via a two-step reaction was designed, and we report a sensor suited for on-site detection for the anticancer drug MTX. The uniform distribution of WP and the synergistic effect of N-CNT as electronic transmission channel ensured that the proposed sensor had superior sensing performance with a short response time, wide detection range, low detection limits, satisfactory selectivity and reproducibility for detection of MTX in whole blood. Finally, good performance and ease of use were obtained with the combination of WP/N-CNT with SPE, demonstrating its applicability to analyze small sample volumes and point-of-care testing (Fig. 1a).

2. Experimental section

2.1. Materials and Chemicals

Dopamine hydrochloride, multi-walled carbon nanotubes (CNT), tris(hydroxymethyl)aminomethane (Tris), ethanol, sodium hypophosphite, and tungsten chloride were purchased from Aladdin (Shanghai, China). Nitric acid (65%), Na_2HPO_4, and NaH_2PO_4 were purchased from Sinopharm Chemistry Reagent Co., Ltd. (Shanghai, China). Nafion (5%) and methotrexate were obtained from Sigma-Aldrich. All of the chemicals were analytical grade and used directly without further purification. Human blood was provided by the Affiliated Hospital of Jiangnan University and the 4th People's Hospital of Wuxi (Jiangsu, China). A SPE consisting of a carbon working electrode, a carbon counter electrode, and an Ag/AgCl reference electrode (Zensor R&D, Taiwan) was used for designing of portable electrochemical sensors. The deionized water was purified by a Millipore-Q system (Millipore Co., USA) and used for all aqueous solution preparation. As the supporting electrolyte, phosphate buffer (PB, 0.1 M) solution was prepared with NaH_2PO_4 and Na_2HPO_4.

2.2. Fabrication of WP/N-CNT modified GCE and SPE

Prior to modification, the bare GCE was successively polished to a mirror finish with 1, 0.3, and 0.05 μm alumina slurry, then sonicated successively with anhydrous alcohol and double-distilled deionized water, and dried with a N_2 stream. An amount of 2 mg WP/N-CNT (Detailed synthesis process can be seen in Supporting Information) was dispersed in a solution containing 100 μL water, 100 μL ethanol, and 0.05 wt% nafion. Then, the as-prepared WP/N-CNT solution (0.01 mg mL^{-1}, 10 μL) was cast onto the pretreated bare GCE or SPE surface and dried at room temperature. The electrode was noted as WP/N-CNT/GCE or WP/N-CNT/SPE.

2.3. Electrochemical measurement

Differential pulse voltammetry (DPV), cyclic voltammetry (CV) and chronoamperometric (CA) experiments were performed with an Electrochemical Analyzer (Model: 120C, S/N: uEA120C10001, Homiangz LLC) at room temperature. The GCE or modified GCE, platinum wire, and Ag/AgCl (KCl saturated) were served as working, counter and reference electrodes, respectively. For other experiments, a SPE was fixed on the electrode holder, and 25–30 μL of the sample was deposited on the surface of the three electrodes for detection. The DPV, and CV measurements were conducted in a PB solution (0.1 M, pH 4.0–9.0), and the voltage scanned from 0.4 to 1.0 V.

3. Results and discussion

3.1. Characterization of WP/N-CNT

The structure of the WP/N-CNT samples were observed with SEM. As shown in Fig. 1b, the CNT was coated with polydopamine (PDA), which is used as a unique supporting material for the adsorption and reduction of tungsten ions, while maintaining good dispersion of CNT. Then, high magnification SEM images further revealed that the surface of the WP/N-CNT was very rough and the diameter increased significantly (Fig. 1c). The TEM images of WP/N-CNT are shown in Fig. 1d and e. The multilayer structures was clearly observed, which proved that the PDA layer, approximately 12.18 nm, was successfully coated on the surface of the CNT. Meanwhile, tungsten trioxide was self-reduced and oxidized by the PDA layer. The formation of tungsten phosphide embedded nitrogen doped carbon was then realized through the high temperature calcination and phosphating treatment. Fig. 1f shows the TEM and corresponding EDX elemental mapping images, further indicating both P, W, N, C and O elements are uniformly distributed throughout the whole nanotubes. All these observations provide strong evidence to support the successful chemical conversion of CNT-PDA-WO_3 into WP/N-CNT after phosphating reaction. Especially, in-situ nitrogen doped carbon nanotubes (N-CNT) as an excellent electron transport channel greatly improved the electrochemical performance of electrode materials (Hueso et al., 2007). The introduction of WP on the surface layer could further enhance electrical conductivity and electrocatalytic properties.

The X-ray diffraction (XRD) patterns for CNT-PDA-WO_3 and WP/N-CNT were shown in Fig. 2a. As can be seen, the CNT-PDA-WO_3 shows the characteristic diffraction peaks of hexagonal-phase WO_3 (JCPDS No. 01–0486). After phosphating, the XRD pattern of WP/N-CNT could be clearly distinguished from the starting material as a series of diffraction peaks appeared as (110), (111), (121), (221) and (311) for WP, as well as diffraction peaks ascribed to (001), (200), (202), (002), (110), (201) and (311) for WP_2 according to PDF card (JCPDS No. 01-089-4778 and 01-076-2365). The diffraction peaks were sharp and intense, indicating that the WP/N-CNT was obtained with high purity and good crystallinity, which was also in agreement with the results of TEM and corresponding EDX elemental mapping. The morphological and structural characterization of the WP/N-CNT prompted us to make a structural model to reveal electrochemical properties derived from the structure and constituent. As shown in Fig. 2b, two characteristic peaks (D and G peaks) of graphitized carbon (1346 and 1575 cm^{-1}) were observed in the Raman spectrum of WP/N-CNT (I_D/I_G = 0.91) and