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Prussian blue mediated amplification combined with signal enhancement of ordered mesoporous carbon for ultrasensitive and specific quantification of metolcarb by a three-dimensional molecularly imprinted electrochemical sensor

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ABSTRACT

In this work, we presented a three-dimensional (3D) molecularly imprinted electrochemical sensor (MIECS) with novel strategy for ultrasensitive and specific quantification of metolcarb based on prussian blue (PB) mediated amplification combined with signal enhancement of ordered mesoporous carbon. The molecularly imprinted polymers were synthesized by electrochemically induced redox polymerization of para aminobenzoic acid (*p*-ABA) in the presence of template metolcarb. Ordered mesoporous carbon material (CMK-3) was introduced to enhance the electrochemical response by improving the structure of the modified electrodes and facilitating charge transfer processes of PB which was used as an inherent electrochemical active probe. The modification process for the working electrodes of the MIECS was characterized by scanning electron microscope (SEM) and cyclic voltammetry (CV), and several important parameters controlling the performance of the MIECS were investigated and optimized in detail. The MIECS with 3D structure had the advantages of ease of preparation, high porous surface structure, speedy response, ultrasensitivity, selectivity, reliable stability, good reproducibility and repeatability. Under the optimal conditions, the MIECS offered an excellent current response for metolcarb in the linear response range of 5.0×10^{-10}–1.0×10^{-4} mol L^{-1} and the limit of detection (LOD) was calculated to be 9.3×10^{-11} mol L^{-1} (S/N=3). The proposed MIECS has been successfully applied for the determination of metolcarb in real samples with satisfactory recoveries. Furthermore, the construction route of this ultrasensitive 3D MIECS may provide a guideline for the determination of non-electroactive analytes in environmental control and food safety.

1. Introduction

Molecularly imprinted polymers (MIPs) are cross-linked polymers that exhibit specific binding sites for the template molecule (Stevenson, 1999). Molecularly imprinted electrochemical sensor (MIECS) combined the numerous superiorities of both MIPs and electrochemical sensor, demonstrating high sensitivity and selectivity, chemical and mechanical stability, reusability, ease of preparation, low limit of detection (LOD), easy of miniaturization and automation at low cost (Blanco-López et al., 2004). Due to these special advantages, MIECS have attracted a great deal of attentions in medical detection, biological, environmental analysis and food safety (Chen et al., 2013b; Li et al., 2013; Lian et al., 2013; Tong et al., 2013; Xue et al., 2013; Yang et al., 2013).

Nowadays, carbon materials are extensively used in preparation of modified electrodes because of their chemical inertness, relatively wide potential window, low background current, and suitability for different types of analysis (Pumera et al., 2010). Carbon nanoscaled materials including carbon nanotubes (CNT), graphene and various of their derivatives are extensively used to offer supporting platform, catalyze electrochemical reaction and promote electron transfer reactions (McCreery, 2008). Besides the carbon-based materials mentioned above, there has been significant interest in the development of one such novel nanostructured carbon material, i.e. highly ordered mesoporous carbon (OMC). Since its discovery in 1999 (Ryoo et al., 1999), OMC has attracted considerable interest both in fundamental and practical field

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owing to its fascinating properties such as extremely high specific surface area, large pore volume, excellent mechanical stability, good thermal stability and electrical conductivity, endowing the OMCs ideal candidates as carrier of mass and electron transfer which is suitable for applications in catalysis and sensing (Jia et al., 2007; Joo et al., 2001). The CMK-3 mesoporous carbon, the very example of OMC, comes into widespread use and is a promising host for advanced nanoparticles owing to its unique physical and chemical features with 3D nanostructure.

Prussian blue (PB) with the merits of high stability, excellent electrocatalytic activity, low-cost and scalability of mass production is arguably representatives of idealized electrocatalysts (Zhu et al., 2013). PB thin films, often prepared by electrochemical methods, have been extensively used as electronic mediator in electrochemical sensors and biosensors (Chiu et al., 2009; Keihan and Sajjadi, 2013; Li et al., 2013; Oliveira et al., 2013). Studies have shown that the performance of carbon materials as transducers can be significantly enhanced upon modification with PB film (Bo et al., 2011; Jiang et al., 2011). More importantly, as an inorganic conductive film, PB film can directly produce electrochemical signals to improve sensitivity (Li et al., 2013). Thus, the PB-CMK-3 hybrid composite with 3D structure can act as both the low-potential redox mediator and electron transfer carrier, which combined the desirable hosting structure and efficient facilitation effect on electron transfer of CMK-3 and the effective low-potential electron transfer mediation effect of PB. In molecular imprinting process on sensing transducer, the low density of imprinted recognition sites requires relatively thick sensing matrices that introduce, in turn, slow diffusion of the analyte to the binding sites, and consequently, long response time of the sensors. Furthermore, the thick MIP matrices associated with electrodes, usually prevent electrical communication between remote binding sites and the electrodes (Riskin et al., 2007). Thus, construction of a 3D structure on transducer surface which exhibits large surface area, high electrical conductivity and the electron transfer ability is of great significance (El-Said et al., 2010; Yamauchi et al., 2012; Yang et al., 2014).

Metolcarb, an N-methylcarbamate pesticide, has been widely employed in agricultural production to control rice leafhoppers, plant hoppers and fruit flies. Its pesticidal mechanism was the inhibition of acetylcholinesterase transmission at nerve endings (Jin et al., 2004; Ma et al., 2006), which also makes it potentially hazardous to human health. Therefore, it is necessary to develop sensitive and selective methods for the determination of metolcarb in agricultural products.

Herein, we present a novel strategy for the preparation of MIECS with 3D structure in which PB was used as an inherent electroactive signal and CMK-3 was introduced to enhance the signal response and improve the surface structure of the modified electrodes. The metolcarb adsorbed selectively on the MIP membrane was detected by electrochemical signal reduction of PB due to electrochemical inert impeding of PB electron transfer through imprinted cavities at the electrode surface. To the best of our knowledge, there has been lots of researches using PB as redox mediator and electron transfer facilitator, but PB-based molecular imprinting technique using PB as an electroactive signal for the determination of non-electroactive analytes in environmental control and food safety is rarely reported. Meanwhile, there is no report combining the merits of OMC and PB using CMK-3 as a carrier to construct a 3D structure recognition element of MIECS.

2. Experimental

2.1. Instruments and reagents

Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) were performed using a LK-2006 electrochemical workstation (Tianjin Lanlike Chemical and Electronic High Technology Co., Ltd., China). EIS experiments were performed on PARSTAT 2273 (Princeton Applied Research, USA). A conventional three-electrode system was employed, consisting of a bare or modified glassy carbon electrode (GCE, 4 mm diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum column electrode as the auxiliary electrode. Scanning electron microscopy (SEM, SU1510, HITACHI, Japan) was employed to observe the surface morphology of various modified electrodes. HPLC system consisted of two LC-10ATVP pumps and a Shimadzu SPD-10AVP UV detector (Shimadzu, Kyoto, Japan).

CMK-3 was purchased from XFNANO Materials Tech Co., Ltd. (Nanjing, China). Metolcarb were purchased from Sigma-Aldrich (St. Louis, USA). Structural analogs including propoxur, carbaryl, isoprocarb were obtained from Fluka (Germany). All chemicals were at least analytical grade. Milli-Q purified water was used for all experiments.

2.2. Preparation of PB-CMK-3 modified GCE

Prior to modification, bare GCE was polished with 0.3 and 0.05 μm alumina slurry successively followed by rinsing thoroughly with doubly distilled water (DDW) between each polishing step until a mirror-like surface was obtained. The GCE was scanned by CV from −0.2 to +0.6 V in aqueous solution consisting of 1 mmol L^{-1} $K_3[Fe(CN)_6]$ and 0.1 mol L^{-1} KCl until standard cyclic voltammograms of $K_3[Fe(CN)_6]$ appeared. The electrodes were washed with DDW and dried in air before use. A layer of CMK-3 modified GCE (CMK-3/GCE) was obtained as follows: CMK-3 (5 mg) and DMF/H_2O solution (1:1, *v*:*v*, 10 mL) were mixed in a glass tube with the help of ultrasonic treatment to form a homogeneous CMK-3 suspension (0.5 mg mL^{-1}). Then, the GCE was coated with 8.0 μL of the resulting CMK-3 suspension solution and allowed to dry at room temperature (RT).

The CMK-3/GCE was inserted into an aqueous mixture consisting of 3.0 mmol L^{-1} $K_3[Fe(CN)_6]$, 3.0 mmol L^{-1} $FeCl_3$, 0.1 mol L^{-1} KCl and 0.1 mol L^{-1} HCl, then a constant potential of −0.40 V was applied for 500 s according to the literatures with a little modification (Chen et al., 2012a, 2012b). Subsequently, the obtained PB-CMK-3/GCE was rinsed with DDW and immersed into a aqueous solution containing 0.1 mol L^{-1} KCl and 0.1 mol L^{-1} HCl for cyclic voltammetric sweep in the potential range from 0 to 0.35 V at a scan rate of 50 mV s^{-1} until a stable response was obtained.

2.3. Preparation of MIP and non-MIP (NIP) membrane modified electrodes

The MIP based on poly(*p*-ABA) membrane modified PB-CMK-3/GCE was electrochemically synthesized by CV between 0 and +1.6 V for 10 cycles in a hydrochloric acid aqueous solution (0.01 mol L^{-1}) containing 5 mmol L^{-1} *p*-ABA and 1.67 mmol L^{-1} metolcarb. High-purity nitrogen was bubbled at least 10 min to remove dissolved oxygen before electro-polymerization. After electro-polymerization, the resulting GCE was suspended in 40 mL of methanol/acetic acid solution (8:2, *v*:*v*) with stirring magnetically for 20 min to remove the template. Then the modified electrode was rinsed with DDW and air-dried at RT for further experiments. Similarly, the NIP membrane was also fabricated under the same conditions in the absence of metolcarb. The