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Carbon paste electrode modified with duplex molecularly imprinted polymer hybrid film for metronidazole detection

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ABSTRACT

A novel electrochemical sensor based on duplex molecularly imprinted polymer (DMIP) hybrid film modified carbon paste electrode (CPE) has been developed for highly sensitive and selective determination of metronidazole (MNZ). A conductive poly(anilinomethyltriethoxysilane) film is firstly electrodeposited on the surface of a CPE, and then a molecularly imprinted polysiloxane (MIPS) membrane is covalently covered on the film via sol-gel process. The as-constructed DMIP hybrid film, combining the advantages of MIPS and conducting MIP, can make feasible the direct and efficient signal transformation between the target analyte and the transducer, as well as enhance the imprinting recognition capability, mass transfer efficiency and the detection sensitivity. Under optimized conditions, the reduction peak currents of MNZ are linear to MNZ concentrations in the range from 4.0×10^{-7} to 2.0×10^{-4} mol L^{-1} with a detection limit of 9.1×10^{-8} mol L^{-1}. The RSD values vary from 2.9% to 4.7% for intra-day and from 3.4% to 4.2% for inter-day precision. The DMIP-based sensor has been successfully applied for the determination of MNZ in biological and pharmaceutical samples. The accuracy and reliability of the method is further confirmed by high performance liquid chromatography.

1. Introduction

Metronidazole (MNZ) is widely used for treating diseases caused by protozoa or anaerobic bacteria in human being (Müller, 1986; Mahugo-Santana et al., 2010; Peng et al., 2012). It has also been used to promote the growth of cattle, pigs and poultry, and to improve feed efficiency in breeding industry (Chen et al., 2013). However, a growing number of studies have shown that when the accumulated dose of MNZ exceeds a certain value, some toxic effects, for instance, seizures and peripheral neuropathy, will be caused to hazard the health of both humans and wildlife (Han et al., 2014; Liu et al., 2015). Therefore, accurate and reliable determination of MNZ in biological fluids and pharmaceuticals is of great importance for the assurance of consumers' health (Jafari et al., 2009; Peng et al., 2012).

Several methods have been reported to detect MNZ in different matrices, such as high-performance liquid chromatography (HPLC) (do Nascimento et al., 2005; Maher et al., 2008; Wang et al., 2012), thin-layer chromatography (TLC) (Gaugain and Abjean, 1996), immunoassay (Thompson et al., 2009), supercritical fluid chromatography (Bari et al., 1998), spectrophotometry (Mahrouse and Elkady, 2011; Saffaj et al., 2006; Zheltvai et al., 2013), and electrochemical sensor (Chen et al., 2013; Gholivand and Torkashvand, 2011; Gu et al., 2015; Liu et al., 2013; Nejati and Asadpour-Zeynali, 2014; Peng et al., 2012; Yang et al., 2014). Among these methods, electrochemical approaches, especially molecularly imprinted polymers (MIPs) based electrochemical sensors, have attracted more attention in recent years for reasons of their good recognition capability, high sensitivity and selectivity, fast response and real time detection nature.

Molecular imprinting is a powerful approach to design smart electrode materials to mimic molecular recognition by natural receptors (Alizadeh et al., 2010; Zhang et al., 2006). Traditionally, the preparation of MIPs is based on radical copolymerization of functional monomer with cross-linker in the presence of template (Zhao et al., 2014; Lakshmi et al., 2009). The MIPs prepared in this way have a molecular memory and are able to specifically recognize and rebind the template molecules. However, they suffer from some disadvantages in certain applications, such as exhausting template removal, low-efficiency of mass transfer, solvent swelling, and the reported difficulties related to the integration of the MIP with the transducer (Rezaei et al., 2014; Wang et al., 2014).

An ideal solution to these problems is the development of

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surface molecular imprinting technique with a sol-gel process. The MIPs prepared by the sol-gel method have been confirmed to possess much more effective recognition sites situated at the surface and be much faster for mass transfer (Fang et al., 2005; Liang et al., 2011). Recently, surface molecularly imprinted polysiloxane (MIPS) modified electrodes have been used to detect a variety of interested substances (Chen et al., 2013; Hu et al., 2015; Leite et al., 2014; Lu et al., 2007; Santos et al., 2011; Xu et al., 2014; Zhang et al., 2010). The MIPS-based sensors exhibit excellent physical rigidity, chemical stability (Santos et al., 2011), and negligible swelling in organic solvents (Walcarius and Collinson, 2009). Most importantly, the enhanced hydrophilicity makes the rebinding ability of the MIPS in aqueous solution impregnable. However, the insulativity of the MIPS might limit the efficiency of the signal transduction (Lu et al., 2007).

Electropolymerization (Yan et al., 2012; Zheng et al., 2014) allows the generation of a rigid and uniform MIP layer with good adherence to a transducer surface of any shape and size, and the thickness and density of the polymer layer can be commendably controlled as well (Liu et al., 2011). Additionally, it is possible to implement a direct effective interaction between the MIP membrane and the transducer. Our group successfully developed two MIP-based electrochemical transducers for melamine (Liu et al., 2011) and isocarbophos detection (Yan et al., 2012), respectively, via electropolymerization of the optimized functional monomers on the surface of glassy carbon electrodes. Generally, this method works well for the detection of non-electroactive analytes using non conductive MIP-based sensor (Deng et al., 2015; Liu et al., 2011; Yan et al., 2012). On one hand, if the analyte is electroactive within the potential window for electrodeposition of the MIP film, the template molecules might react electrochemically, producing new compound with the structure different from that of the original template. On the other hand, if the MIP film is conductive, the electrochemical reaction might occur not only at the bulk electrode after the analyte molecules or the active probes pass through the imprinted cavities and reach the electrode surface, but also on the MIP layer without taking advantage of molecular recognition by the imprinting effect. This is because the electroactive MIP can transduce signals directly between the MIP membrane and the bulk electrode, which could deteriorate the selectivity of the MIP-based sensor.

To settle this problem, in this study for the first time, we have developed a smart electrochemical sensor to determine MNZ, which is based on a novel type of MIP, called duplex molecularly imprinted polymer (DMIP), modified carbon paste electrode (CPE). The DMIP hybrid film is composed of a conductive polymer layer and an imprinted polysiloxane layer. Firstly, the conductive poly (anilinomethyltriethoxysilane) (poly(AMTEOS)) film is electrodeposited on the surface of CPE, and then, the pendant-like triethoxysilyl groups ($-Si(OC_2H_5)_3$) attached to the polymer matrix are hydrolyzed with 3-aminopropyltriethoxysilane (APTMS) (as functional monomer) and tetraethyl orthosilicate (TEOS) (as crosslinker) in the presence of template MNZ, to form a MIPS film over the conductive polyaniline layer. The DMIP modified CPE (DMIP/CPE) has been applied for determining MNZ in biological and pharmaceutical samples via differential pulse voltammetry (DPV) with high selectivity and accuracy, and the results are further verified by HPLC.

2. Experimental

2.1. Chemicals and reagents

AMTEOS and MNZ were obtained from Aladdin Reagent Company (Shanghai, China). Other chemicals and reagents used are presented in Supplementary Material (SM). Double distilled water (DDW) was used throughout this study.

2.2. Instruments

The scanning electron microscope (SEM) images were collected by a Nova nanoSEM230 scanning electron microscope equipped with an energy dispersed spectrum (EDS) unit (Czech Republic). A Delta-320 pH meter (Mettler Toledo Instruments Co. Ltd. China) was employed for the pH adjustments.

Electrochemical measurements were performed on a CHI660C Electrochemical Workstation (CH Instruments, Chenhua Co. Ltd. China). A conventional three-electrode cell assembly consisting of a bare or a modified CPE (2.5 mm in diameter) serving as the working electrode, a saturated calomel electrode (SCE, 0.2415 V vs SHE) as the reference electrode and a platinum wire as the counter electrode. All measurements were carried out at room temperature (RT). All solutions were deaerated with high-purity nitrogen for 10 min before each electrochemical experiment.

2.3. Fabrication of the DMIP modified CPE

The traditional CPE was prepared by hand-mixing of graphite powder with paraffin oil at a ratio of 83/17 (w/w) in an agate mortar. The paste was then tightly pressed into a polypropylene tube with inner diameter of 2.5 mm. A copper wire was inserted into the carbon paste to provide the electrical contact. The external electrode surface was smoothed with weighing paper. The poly (AMTEOS) modified CPE (poly(AMTEOS)/CPE) was prepared via electrodepositing the conducting poly(AMTEOS) layer on a bare CPE in 0.1 mol L^{-1} AMTEOS solution containing 0.2 mol L^{-1} H_2SO_4 by cyclic potential scanning from 0.9 to −0.5 V at a scan rate of 120 mV s^{-1} for 15 circles, and then rinsed with ethanol for the preparation of DMIP/CPE soon after.

The DMIP/CPE was prepared according to the following procedures: Firstly, 100 μL of APTMS, 300 μL of TEOS, 3 mL of 2-ethoxyethanol, and 770 μL of 0.03 mol L^{-1} MNZ solution in 2-ethoxyethanol were mixed and stirred for 10 min at 35 °C. Then, 200 μL of 0.1 mol L^{-1} NaOH and 90 μL DDW were added into the above mixture sequentially and stirred for another 20 min to obtain a homogeneous sol. Secondly, the DMIP/CPE was prepared by simply dropping 10 μL of the sol onto the surface of the poly (AMTEOS)/CPE and aged at room temperature overnight to form the DMIP hybrid film via a condensation process and to evaporate residual solvent as well. The obtained DMIP/CPE was immersed in 5 mL of methanol/acetic acid (8:2, v/v) eluant with stirring magnetically for several times to remove the template MNZ. The complete removal of MNZ was verified by DPV. Then the template-free DMIP/CPE was rinsed with DDW and air-dried at RT for further experiments. The non imprinted CPE (DNIP/CPE) was prepared in the same way as that for DMIP/CPE but without the presence of template MNZ. For comparison, a CPE that was only modified with the imprinted sol was also fabricated (denoted as MIPS/CPE) in the absence of AMTEOS. The schematic representation of the fabrication process for the DMIP/CPE is shown in Scheme 1A.

2.4. Electrochemical measurements

A standard three-electrode cell connected to the CHI660C was used for electrochemical measurements. After being immersed in 5.0 mL of test solution for 10 min to accumulate MNZ, the working electrode was washed carefully with DDW to remove the possible physical adsorbates and dried under nitrogen. Then, the electrode was transferred to an electrochemical cell containing 5.0 mL background solution, composed of 0.04 mol L^{-1} Britton-Robinson