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A novel sensitive electrochemical sensor based on in-situ polymerized molecularly imprinted membranes at graphene modified electrode for artemisinin determination

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ABSTRACT

To develop a rapid and simple method for sensitive determination of artemisinin (ART) in complicated matrices, a novel electrochemical sensor was constructed by in-situ polymerization of ART-imprinted membranes (ART-MIMs) on the surface of graphene (G) modified glassy carbon electrode (GCE) using acrylamide (AM) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as cross-linking agent after the experimental parameters for the preparation of ART-MIMs such as functional monomer, molar ratio of template, monomer and cross-linking agent together with extraction condition were optimized. Under the optimal conditions, the sensor named as ART-MIM/G/GCE exhibited a good selectivity, high sensitivity and considerably better resistance against some analogs of artemisinin such as dihydroartemisinin (DHA), artemether (ARM) and artesunate (ARTS). The calibration graph for the determination of artemisinin by the sensor was linear in the range of 1.0×10^{-8} mol L^{-1} to 4.0×10^{-5} mol L^{-1} with the detection limit of 2.0×10^{-9} mol L^{-1}. Meanwhile, this sensor possessed of good regeneration, stability and practicability. It could retain more than 94% of its original response after used at least 80 times or stored in water at room temperature for 60 days. The obtained sensor was successfully applied to determine the contents of artemisinin in the extract of *Artemisia annua* L. with the relative standard deviation (RSD) of less than 3.5% ($n=5$).

1. Introduction

Artemisinin (ART) is a sesquiterpene-lactone endoperoxide isolated from *Artemisia annua* L., a plant with a long history of medical use against malaria in China (Dost and Davidson, 2003). As the main active component of *A. annua* L., artemisinin and its derivatives are considered to be the most promising compounds in the search for treatment of drug-resistant malaria. They not only have become increasingly popular in Asia over the last 20 years (Kohler et al., 1997), but also are regarded as part of the ideal strategy for malaria in Africa by WHO nowadays (Yao and Li, 2005). The growing application of artemisinin in clinical medicine has attracted the interest of analysts in developing simple, selective, and sensitive methods for its determination.

Due to the absence of appropriate UV absorbance, the determination of artemisinin is very difficult. At present, the most effective methods for the determination of artemisinin are high performance or ultra-performance liquid chromatography with mass spectrometry (HPLC–MS) (Fu et al., 2012) and tandem mass spectrometry detection (HPLC–MS/MS) (Suberu et al., 2013; Carr et al., 2014; Li et al., 2008). But MS and MS/MS detectors both are expensive and require high cost of maintenance and skilled operation technique. Moreover, some other methods, like thin layer chromatography (TLC) (Quennoz et al., 2010), HPLC with evaporative light scattering detection (HPLC-ELSD) (Liu et al., 2007) and refractive index (HPLC-RI) (Lapkin et al., 2009), gas chromatography with electron capture detection (GC-ECD) (Liu et al., 2008) and high performance capillary electrophoresis with field amplified sample accumulation technique and photo-diode array detection (FASA-HPCE-PDA) (Yang, 2012), have also been proposed to detect and quantify artemisinin. Among these methods, TLC is not a reliable technique to quantify artemisinin (Quennoz et al., 2010), and HPLC-ELSD (Liu et al., 2007), HPLC-RI (Lapkin et al., 2009), GC-ECD (Liu et al., 2008) as well as FASA-HPCE-PDA (Yang, 2012) lack of required sensitivity for determination of artemisinin in biological samples. Compared with the methods mentioned above, electrochemical analysis possesses of

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excellent characteristics like convenience in performance and simplicity in apparatus. But due to the fact that the electrochemical activity of artemisinin is very poor, there is few reports related to the determination of artemisinin by electrochemical method. Phukon et al. (2014) fabricated a PHA/AuNPs/HRP/ITO based biosensor for electrochemical determination of artemisinin. However, the method developed with this biosensor is unsatisfactory in the determination range and some common disadvantages of biosensor like unpredictable shelf-life and stability, poor inter-batch reproducibility and availability, environmental intolerance (e.g. pH, temperature, ionic strength, organic solvents) and poor engineering characteristics (Hillberg et al., 2005).

Recently, molecular imprinting technology, which is known as a method for preparation of polymeric materials, like molecularly imprinted polymers (MIPs) and molecularly imprinted membranes (MIMs), has become a well-established analytical tool (Tokonami et al., 2009; Xing et al., 2012). Because the molecular imprinting process can introduce the recognition properties into synthetic materials by using appropriate template (Yan and Ramstrom, 2005; Garcia-Calzon et al., 2007), MIPs/MIMs own the surface cavities complementary to the template molecule and have high selective recognition to the template molecules over its structurally related compounds (Zhang et al., 2010; Kan et al., 2012). This advantage together with its other advantages over the biological recognition element, like stability, reusability, simplicity and low cost in preparation, make MIPs/MIMs become ideal candidates as recognition elements for sensors (Merkoci and Alegret, 2002; Fuchs et al., 2012). So far, several studies have been published with respect to the preparation of sensor based on MIP/MIM for electrochemical determination of various substances, including cancer marker (Viswanathan et al., 2012), oxytetracycline (Li et al., 2014a), chrysoidine (Wang et al., 2014), dopamine (Qian et al., 2014), 3-indoleacetic acid (Li et al., 2014b) and quinoxaline-2-carboxylic acid (Yang et al., 2013).

The aim of this investigation is to fabricate a sensor based on molecularly imprinted membranes for simple, sensitive and selective determination of artemisinin. To increase the sensitivity of the sensor, the glassy carbon electrode (GCE) was firstly modified with graphene (G) to enhance the electrical conductivity and specific surface area, and then assembled with artemisinin imprinted membrane formed by in-situ polymerization technique using ART as template and acrylamide (AM) as functional monomer (Scheme 1). The experimental parameters affecting the response sensitivity of the sensor for artemisinin were optimized in detail. The analytical application and selectivity behavior of the sensor were also described and discussed. To our knowledge, artemisinin imprinted sensor has not been reported till now.

2. Experimental

2.1. Reagents and chemicals

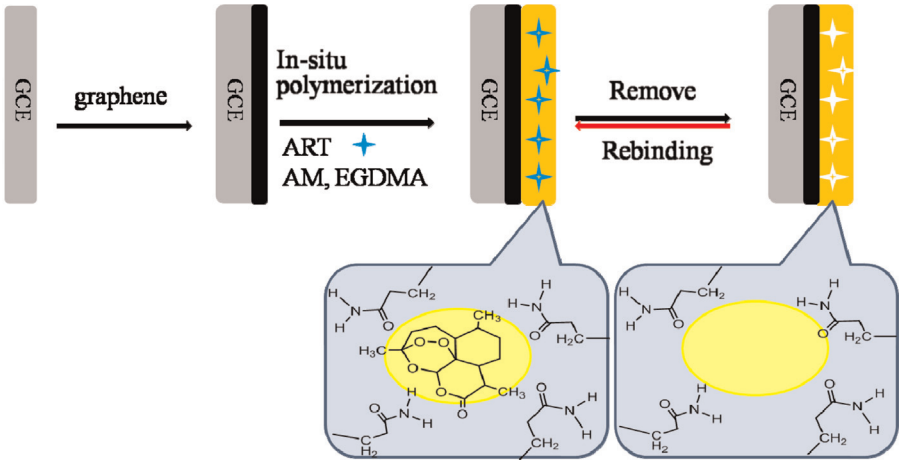
Artemisinin (ART), dihydroartemisinin (DHA), artemether (ARM) and artesunate (ARTS) were purchased from Nanjing Zelang Phytochemistry Technology Co., Ltd. (Nanjing, China). Acrylamide (AM), methacrylic acid (MAA), 2-Vinylpyridine (2-VP) and 4-Vinylpyridine (4-VP) were purchased from Sigma-Aldrich (USA). Ethylene glycol dimethacrylate (EGDMA) was purchased from Suzhou Anli Chemical Factory (Jiangsu, China) and distilled under vacuum to remove the stabilizers prior to use. Azobisisobutyronitrile (AIBN) was purchased from Shanghai Reagent Factory (Shanghai, China) and purified by recrystallization from ethanol before used. Graphite powder was purchased from Alfa Aesar (Tianjin, China). The other reagents and solvents were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China).

2.2. Apparatus

Electrochemical data were performed using a CHI660D electrochemical workstation (Shanghai Chenhua Co., China), connected to a three electrode cell. The bare glassy carbon electrode (GCE, 3 mm diameter) or a modified GCE, a saturated calomel electrode (SCE) and a platinum wire electrode were employed as a working, auxiliary and reference electrode, respectively. Capillary electrophoresis analysis was performed using a Beckman P/ACETM MDQ capillary electrophoresis system (Beckman Coulter, USA) equipped with a photodiode array detector (Beckman Coulter, USA) and a 57 cm (50 cm effective length) × 75 μm I.D. uncoated fused-silica capillary (Yongnian Optical Fiber Factory, Hebei, China).

2.3. Preparation of the sample solution

1.0000 g pulverized *A. annua* L. (obtained from the pharmaceutical store of Kunming, Kunming, China) was extracted by refluxing for 6 h at 60 °C with 100 mL petroleum ether and then ultrasonic vibration for 30 min. After cooling to room temperature, the extract was filtered and washed five times with 20 mL of ethanol. Subsequently, the filtrate was combined and dried by evaporation and the residue was dissolved to 25 mL with ethanol as stock solution. This solution was analyzed directly by high performance capillary electrophoresis (Yang, 2012) or by electroanalytical method after it was diluted 20 times with ethanol.



Scheme 1. Detailed procedure diagram for fabrication of the ART-MIM/G/GCE sensor.