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An electrochemical sensor based on multiwall carbon nanotubes and molecular imprinting strategy for warfarin recognition and determination

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ABSTRACT

A sensitive electrochemical sensor for warfarin was prepared based on molecular imprinting strategy by electropolymerization qof o-phenylenediamine (o-PD) on a glassy carbon electrode via cyclic voltammetry (CV). In order to enhance the electrode sensitivity and electronic transmission, multiwall carbon nanotubes (MWCNT) containing carboxylic functional group (f-MWCNTs) were introduced on glassy carbon electrode (GCE). Thin film of molecularly imprinted polymer (MIP) with specific binding sites for warfarin was cast on the modified electrode using electrochemical deposition. In order to form a double layer with MIP layer as an insulating electrolyte, Au nanoparticles (AuNPs) was introduced at the MIP surface to form final modified electrode (AuNP/MIP/f-MWCNT/GCE). The properties of AuNP/MIP/f-MWCNT/GCE were studied in the presence of $K_3Fe(CN)_6$ as a probe for signal transduction and also by the use of electrochemical impedance spectroscopy (EIS). AuNP/MIP/f-MWCNT/GCE exhibits fast binding kinetics and good selectivity to template due to their high ratio of surface imprinted sites, large surface-to-volume ratios and large affinity to template. The modified electrode was used to detect the concentration of warfarin with a linear range and detection limit ($S/N = 3$) of 0.031–0.616 ng mL^{-1} and 0.024 ng mL^{-1}, respectively. Finally, the modified electrode was successfully applied to determine warfarin in human serum sample.

1. Introduction

Warfarin is widely used as an oral anticoagulant in the prevention and treatment of venous and arterial thromboembolism. It is most likely to be the drug popularly referred to as a "blood thinner," yet this is a misnomer, since it does not affect the thickness or viscosity of blood. Instead, it acts on the liver to decrease the quantity of a few key proteins in blood that allow blood to clot [1]. Therapeutic concentration of warfarin is about 2.0–5.0 μg mL^{-1}. Warfarin has a long half-life. The half-life is 20–60 h (mean 40 h) [2].

Several methods have already been described to determine warfarin such as high performance liquid chromatography (HPLC) with fluorescence detection [3,4], LC and HPLC with UV detection [5–8], HPLC-MS/MS [9–11], multi-mode UPLC (ultra performance liquid chromatography)-MS/MS method [12,13], LC-MS/MS [10], chiral capillary electrophoresis with spectrophotometric detection [14], chiral stationary-phase liquid chromatography-fluorescence system coupled with on-line circular dichroism detector [15] and time-correlated single-photon counting [16]. However, there may be several disadvantages for these methods. For example some of them are time consuming and tedious, while others use large biological fluid volumes and need expensive instruments and toxic solvents, plus time consuming procedures.

As a capable element for specific recognition, molecularly imprinted polymers (MIPs) have paying attention in recent years [17]. Molecular imprinting technique is an approach to synthesizing a polymer matrix with molecular recognition sites, which are specific in shape and size to the target molecular, exhibiting specific high binding behaviors to the target molecules. Due to low chemical and physical stability of the antibodies or enzymes avoids their use in harsh circumstances and at high temperatures. MIPs have some specific advantages such as low cost of preparation, high affinity to the template, robustness and long-term stability [18,19]. MIPs have been used for molecular recognition variety from tiny molecules [20–24] to large molecules [25–28]. To improve some limitation of MIPs, such as low density of imprinted sites, slow binding time,

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heterogeneous distribution of imprinted sites and insulting polymer in electrochemically application of MIPs, several studies suggested the formation of thin MIP layer on nanomaterials surface [29–32]. Own to their unique structure, high chemical stability and high surface-to-volume ratio, carbon nanotubes, as new class nanomaterials, are extremely attractive in electrochemical sensors [33]. In order to enhance the electrode sensitivity and electronic transmission, MWCNTs and AuNPs were introduced in both of insulating MIP layer. Electrochemical synthesis of MIP is one of the most promising techniques with which to develop electrochemical sensors [34]. On the other hand, the incorporation of electrochemical devices and MIPs, which exhibit good sensitivity and selectivity, is an attractive approach for the development of biochemical sensors [34–43]. Some of the previous works using electropolymerization method for the fabrication of imprinted sensing system were reported [20,34–44].

Here in we attempt to investigate the feasibility of fabricating a MIP sensor. The sensor, AuNP/MIP/f-MWCNT/GCE, was fabricated by electrochemical deposition of o-PD at the surface of glassy carbon electrode (GCE), surface modified with MWCNTs containing carboxylic functional group (f-MWCNTs) and followed by deposition of AuNPs using a potentiostatic method. The electrochemical deposition method has many benefits such as simple preparation, easy control of the film thickness, and good reproducibility of identical polymer films [45]. f-MWCNTs and AuNPs were used to enhance the electrode sensitivity and electronic transmission. Electrochemical impedance spectroscopy, cyclic voltammetry were used to study of the sensor. Both of the nanoparticles were introduced in both sides of the MIP layer to form a double layer state. $K_3Fe(CN)_6$ was used an electrochemical probe for signal transduction.

2. Experimental

2.1. Chemicals

Warfarin, hydrochlorothiazide (HCT), and phenazopyridine (PAP) were supplied from Sigma–Aldrich. Dimethylformamide (DMF), o-PD, phosphoric acid, sodium acetate, acetic acid, and methanol were purchased from Merck. All chemicals and reagents used were of analytical grade and doubly distilled water was used throughout.

MWCNTs were brought from Iran's Research Institute of Petroleum Industry (synthesized by chemical vapor deposition (CVD) with the purity of 95% and the average thickness of wall and length of the MWCNT were about 40 nm and several micrometers, respectively). Trisodium citrate and gold chloride ($HAuCl_4$) were supplied from Aldrich. Phosphate buffer solution (PBS, 0.03 mol L^{-1}) with different pH values was used to study of pH effect. pH values were measured using a Metrohm (Model 827 pH-lab) pH meter.

2.2. Electrochemical measurements

Electrochemical measurements were performed with a potentiostat–galvanostat μAutoLab (Echo Chemie, B.V., Netherlands, NOVA software), and a conventional three-electrode configuration, containing 50 mL glass cell, AuNP/MIP/f-MWCNT/GCE or AuNP/NIP/f-MWCNT/GCE as the working electrode, and platinum wire as the auxiliary electrode was used. Potentials were measured versus saturated calomel electrode (SCE). Electrochemical impedance spectroscopic (EIS) measurement was performed with the Autolab system (PGSTAT 12, Eco Chemie B.V., Utrecht, Netherlands). The system was run on a PC using GPES and FRA 4.9 software. In each measurement, the modified electrode was immersed into the solution of warfarin in PBS (pH 6.5) for 90 s and then the electrochemical measurements were done in a solution containing 5.0 mmol L^{-1} $K_3Fe(CN)_6$ in 0.1 mol L^{-1} KCl as a probe, and completed to the mark with PBS at pH 6.8 [20]. The cyclic voltammetric curve obtained under a potential range of −0.10 V to 0.50 V and at a scan rate of 50 mV s^{-1}. After each analysis, the MIPCNT or NIPCNT electrode was recovered with methanol to remove the adsorbed molecules. Then, the recovered electrode was employed to the subsequent rebinding. For impedance measurements, a frequency range of 100 kHz to 5.0 MHz was employed. The AC voltage amplitude and the potential were 5.0 mV and 0.20 V, respectively. All of electrochemical experiments were carried out at room temperature.

2.3. Preparation of AuNPs

AuNPs were prepared by following reported procedure [46]. Briefly, 4.0 mL 1.0% (w/v) sodium citrate was added rapidly to the solution obtained by dissolving 1.0 mL 1.0% (w/v) $HAuCl_4$ and 99 mL water and then, keeping the mixture in an oven for 90 min at 60 °C. The color solution changed from pale yellow to blue and finally to red–violet. The prepared AuNPs were stored in a dark bottle at 4 °C until further use.

2.4. Preparation of the modified-imprinted sensor

MWCNTs containing carboxylic functional group (f-MWCNTs) was prepared as follow; 500 mg of crude MWCNTs were added into a glass reactor containing 60 mL of HNO_3. The mixture was kept under ultrasonicating for 15 min. This mixture was refluxed for 22 h at 80 °C and then cooled, filtered and washed by passing through about 5 L of distilled water. The prepared f-MWCNTs were used as a supporting material for the formation of the polymer on the surface of GCE.

Prior to use, the surface of GCE was polished carefully to a mirror-finished with slurry alumina (0.05 μm) at polishing cloth for 2 min. Then, the electrode was rinsed with distilled water and cleaned ultrasonically into a mixture of water:methanol (1:1) for 3 min to remove particles that might be trapped at the surface. A 5.0 mg of f-MWCNTs were added into 2.5 mL DMF and f-MWCNTs were dispersed by ultrasonic treatment for 6 min. Then, 3.0 μL of the dispersed mixture was poured on the GCE surface and allowed to evaporate solvent and formed f-MWCNTs on the electrode surface.o-PD functionalized on the electrode surface modified with f-MWCNTs were prepared firstly by immersing the f-MWCNTs electrode into a acetic acid/acetate buffer solution (ABS, pH 5.2) containing 5.0 mmol L^{-1} o-PD and 1.7 mmol L^{-1} warfarin, for 13 h. The MIP/f-MWCNT/GCE sensor was fabricated by electrochemical deposition via cyclic voltammetry in the potential range of −0.40 V to 1.00 V at a scan rate 50 mV s^{-1} for 20 cycles. Then, potentiostatic deposition method was applied to prepare the Au-nanoparticles modified onto MIP/f-MWCNT/GCE (AuNP/MIP/f-MWCNT/GCE) by immersing of MIP/f-MWCNT/GCE into 1.5×10^{-4} mol L^{-1} $HAuCl_4$ and 0.1 mol L^{-1} KNO_3 solution, using a potential of −0.20 V for the duration of 180 s [47]. The template was extracted from the imprinted film by immersing the obtained electrode under gentle stir in methanol for 90 min. The completely removal of warfarin was checked electrochemically, so that after 90 min as an incubation time, the redox peak of the probe was reached to maximum value and stayed constant. Therefore, 90 min was chosen as an incubation time for complete removing the template by methanol. The non-imprinted polymer (NIP) sensor was also prepared by using the same method without addition of warfarin. The entire schematic representation is illustrated in Fig. 1.