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Fabrication of an electrochemical sensor based on computationally designed molecularly imprinted polymer for the determination of mesalamine in real samples

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ABSTRACT

A novel electrochemical sensor based on mesalamine molecularly imprinted polymer (MIP) film on a glassy carbon electrode was fabricated. Density functional theory (DFT) in gas and solution phases was developed to study the intermolecular interactions in the pre-polymerization mixture and to find the suitable functional monomers in MIP preparation. On the basis of computational results, o-phenylenediamine (OP), gallic acid (GA) and p-aminobenzoic acid (ABA) were selected as functional monomers. The MIP film was cast on glassy carbon electrode by electropolymerization of solution containing ternary monomers and then followed by Ag dendrites (AgDs) with nanobranch deposition. The surface feature of the modified electrode (AgDs/MIP/GCE) was characterized by scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS). Under the optimal experimental conditions, the peak current was proportional to the concentration of mesalamine ranging from 0.05 to 100 μM, with the detection limit of 0.015 μM. The proposed sensor was applied successfully for mesalamine determination in real samples.

1. Introduction

Mesalamine (mesalazine, MES), chemically known as 5-aminosalicylic acid, is an important non-steroid anti-inflammatory drug (NSAID) used in the treatment of Crohn's disease and ulcerative colitis, which may provide protection against the development of colorectal cancer in patients suffering from inflammatory bowel diseases (IBD) [1]. On the other hand MES is an amino salicylate drug that acts locally in the gut and has its predominant actions, thereby having few systemic side effects. As a derivative of salicylic acid, MES is also thought to be an antioxidant that traps free radicals, which are potentially damaging by products of metabolism [2]. MES is considered as the active moiety of sulfasalazine, which is metabolized to Sulfapyridine and MES [3]. Therefore, the need for clinical and pharmacological studies requires fast and sensitive analytical techniques for drug determination especially in several biological fluids.

Up to now, most common procedures for the determination of MES in pharmaceutical dosage forms and biological fluids were based on chromatographic techniques [4–7], spectrophotometry [8] and colorimetry [9]. MES is an electroactive compound, thus the electrochemical techniques which are known as selective methods offer another possibility for the estimation of this compound without any pre-separation treatment. Hence the development of electrochemical determination assumes importance. In the recent decades, there have been several reports on the electroanalytical determination of MES [10–13]. These reports suffer from some drawbacks such as low sensitivity and or short linear range. Thus, there is still a need to develop selective and sensitive electrodes that can detect MES in pharmaceutical forms as well as analysis in biological fluids.

As a new class of materials which possesses high selectivity and affinity for the target molecule, MIPs have been widely applied for sensor development [14–16]. Molecular imprinting involves positioning functional monomers around the target molecules (template) by covalent or noncovalent interaction, followed by polymerization [17]. After removing the template, MIPs possess surface cavities complementary to the template and it can be used as a selective binding medium for the imprinting molecules. The application of MIPs in electrochemistry is directed to combining the great characteristics of electrochemical method and molecular imprinting techniques and offering high selectivity, simple operation, and low cost [18–20]. The method of electropolymerization is one of the efficient ways to prepare MIP membranes with spatial selectivity to the analytes [21–24]. However, in order to prevent the synthesis of MIP by trial and error which it is accompanied with consumption of large amount of unfriendly environment organic solvents, the computational method was used as an alternative approach for the rational design of MIPs [25–27]. In this regard the density functional theory (DFT) method was widely used to select functional monomers by calculating the energy difference (ΔE) [25,28,29].

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The detection sensitivity of the imprinted sensor is based on the amount of effective sites on the surface of the sensor. To further increase the amount of effective sites, various nanomaterials have been introduced at the surface of electrodes [30,31]. By incorporation with nanoparticles (NPs), electrochemical sensors have shown great promise for diagnosis of trace molecules and enhanced the intensity of the electrochemical signal. On the other hand, NPs can be used as carriers to load a large amount of electroactive species for amplifying detection of molecules [32,33]. Here, silver dendrites (AgDs) are chosen to amplify the electrochemical response because they are quite stable in aqueous environment.

In this work a selective and sensitive sensor based on terpolymer of o-phenylenediamine-co-gallic acid-co-p-aminobenzoic acid functional monomers was constructed by electropolymerization and subsequently, electrochemical deposition of Ag dendrites (AgDs) with nanobranch for the first time. DFT-based computational approach was used as the rational design of MIPs for MES as template molecule. The experimental parameters that affect the performance of the imprinted sensor were investigated and optimized. Further investigation was carried out on selectivity, reproducibility, and stability of the resulted sensor. The molecularly imprinted terpolymer decorated by Ag dendrites was characterized with scanning electron microscopy (SEM), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). The results of the study confirm strong adsorption ability of MIP, high specific area and subtle electronic properties of the modified electrode. The detection procedure is based on the oxidation of MES after its selective extraction in the modified electrode using square wave voltammetry. The developed sensor has been successfully applied to determine MES in real samples.

2. Experimental

2.1. Reagents and apparatuses

Voltammetric measurements were carried out with an Autolab (Eco Chemie B.V., Netherlands) PGSTAT30 potentiostat/galvanostat. The electrochemical cell consisted of unmodified and modified glassy carbon electrode (GCE) as working electrodes, Ag/AgCl and platinum wire as a reference and counter electrodes. A Metrohm pH-meter (model 691) was also applied for pH adjustment. Scanning electron microscopy (SEM) images were obtained with a field emission gun scanning electron microscope (Philips XL 30, USA). O-phenylenediamine (OP), gallic acid (GA) and p-aminobenzoic acid (ABA) were purchased from Sigma-Aldrich (Munich, Germany). Mesalamine, phenylephrine, warfarin and minoxidil were obtained from Merck (Darmstadt, Germany). A 1.0 mM of methanolic solution of warfarin and aqueous solutions of the mesalamine, phenylephrine and minoxidil were prepared. All drug solutions were protected from light and stored at 4 °C. Ethanol–water solution (2:1, v/v) was used to extract the template from the MIP matrix. Phosphate buffered solutions (PBS, 0.01 M) at various pH values were prepared from 0.01 M H_3PO_4, 0.01 M KH_2PO_4 and 0.01 M K_2HPO_4.

2.2. Preparation of the MIP modified electrode

General procedure to prepare imprinted polymer was as previous report [34]. The surface of the bare GCE was carefully hand-polished with 0.3 and 0.05 μm alumina–water slurry using a polishing cloth in sequence, and thoroughly ultrasonically rinsed with HNO_3, ethanol, and doubly distilled water for 10 min in turn. The MIP modified GC electrode was prepared by electropolymerization of ternary monomer mixture (OP–GA–ABA) in the presence of MES as a template using cyclic voltammetry. Fifteen consecutive cyclic scans were used in the potential range of −0.4 and +0.8 V and at a scan rate of 50 mV s^{-1}. The electropolymerization was carried out in a 10 mL of 0.01 M phosphate buffer solution (PBS, pH = 2) containing 0.2 M KCl, 40 μmol OP, 20 μmol GA, 20 μmol ABA and 10 μmol MES and resulted a MES–MIP/GC electrode. For comparison, a non-imprinted polymer/GCE (NIP/GCE) was fabricated following the same procedure in the absence of the template. The electrode was washed with distilled water for several times and then immersed in a solution containing $NaNO_3$ (0.1 M) and $AgNO_3$ (2.5 mM). AgDs were deposited on the imprinted and non-imprinted electrodes by potentiostatic electrodeposition at potential of −0.4 V for 10 min [35]. The removal of template from the AgDs/MES–MIP/GCE was carried out by its immersion in ethanol–water (2:1 v/v) solution as extractant for 5 min. The complete removal of the template was checked by recording the cyclic voltammogram of the resulted electrode in phosphate buffer solution (pH = 2). Finally the prepared electrode was washed with doubly distilled water for several times and stored for further uses. Furthermore, in order to prevent from memory effect, after each measurement, the electrode was immersed in the extractant solution (ethanol–water (2:1 v/v)).

2.3. General analytical procedure

A 10 mL phosphate buffer solution (PBS) with pH = 2 was introduced into the electrochemical cell and then an accumulation potential of −0.6 V was applied to the electrode for 120 s, while the solution was stirred at 400 rpm. After 10 s rest period, its square wave voltammogram (SWV) was recorded in the potential range of 0.2 to 1.0 V and was used for background correction. Then, appropriate volumes of sample solution were added to the voltammetric cell, after 120 s accumulation at −0.6 V, their voltammograms were recorded.

2.4. Preparation of samples

Serum samples, obtained from healthy volunteers, were collected and stored frozen until assay. An aliquot serum sample was fortified with the appropriate amount of MES to achieve certain concentration, and treated with 1.0 mL methanol as serum protein precipitating agent. After adjusting the sample volume and vortexing for 30 s, the precipitated protein was separated out by centrifugation for 10 min at 14,000 rpm. The clear supernatant layer was filtrated through a 0.45 μm milli-pore filter to produce a protein-free human serum.

The urine sample was centrifuged and diluted with distilled water without any further pretreatment. An aliquot urine sample was fortified with the appropriate amount of MES to achieve certain concentration. The MES content of both real samples was determined by the proposed sensor by using standard addition method.

3. Results and discussion

3.1. Theoretical study of template–monomer interactions

The density functional approach has been used for the calculation of electronic ground state energy of MES and its binding energy with individual, binary and ternary mixture of monomers (GA, OP and ABA) in gas and solution phases and the results are presented in supplementary information file. To examine the applicability computational designing approach in the synthesis of MIP, four polymers for MES were prepared by electropolymerization using OP-co-ABA (MIP_1), OP-co-GA (MIP_2), GA-co-ABA (MIP_3) and OP-co-GA-co-ABA (MIP_4) as functional monomers. These sensors were applied for monitoring MES using cyclic voltammetric technique. The most sensitivity was achieved when OP-co-GA-co-ABA was used as functional monomers (Fig. 1). Also three MIPs with individual monomer were also synthesized and used for monitoring of the drug. No significant response was obtained when single monomer MIPs were used. Therefore, MIP_4 was selected for the preparation of the MES sensor. Furthermore, by comparison of the theoretical and experimental results it was revealed that, the experimental results are consistent with those obtained with PCM model.