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An azithromycin electrochemical sensor based on an aniline MIP film electropolymerized on a gold nano urchins/graphene oxide modified glassy carbon electrode

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ABSTRACT

In this study, a selective and sensitive procedure was developed based on a molecular imprinted polymer to detect and determine Azithromycin (AZT) antibiotic. AZT-aniline MIP films were electropolymerized (to form polyaniline) on the surface of gold nanourchin/graphene oxide modified glassy carbon electrodes (GNU/GO/GCE), with aniline serving as a monomer and AZT as a template. The morphological and analytical properties of the fabricated nanosensor were characterized by field emission scanning electron microscopy, cyclic voltammetry and electrochemical impedance spectroscopy. The selectivity, linear range, limit of detection, sensitivity and repeatability of the proposed procedure were also evaluated. The analytical performance of the presented sensor that was assessed through differential pulse voltammetry proved to be in the linear range of 0.3 nM to 920.0 nM with the detection of limit 0.1 nM for AZT. In addition, the average current and precision in terms of the relative standard deviation for eight repetitive measurements in 60.0 nM AZT were found to be 0.293 ± 0.07 μA and 2.5% respectively. The proposed sensor was successfully applied for the detection of AZT in human serum.

1. Introduction

Azithromycin (AZT) is a novel macrolide antibiotic used to treat or prevent certain bacterial infections such as ear infections, strep throat, pneumonia, typhoid, bronchitis, and sinusitis [1]. This drug prevents the survival and growth of bacteria by creating a series of disorders in them [2]. A major part of the consumed azithromycin is excreted unchanged through urine. Excessive use of antibiotics leads to the resistance of bacteria strains to them, allergic reactions, liver damage, yellow teeth, and digestive disorders. It also results in the transfer of genes resistant to antibiotics and can potentially cause cancers in those who consume animal source foods contaminated with antibiotics [3].

So far, several analytical methods have been reported for determination of antibiotics and pharmaceuticals, such as spectrophotometry [4], HPLC [5–7], atomic-spectroscopic [8], electrochemiluminescence [9], capillary electrophoresis [10], radio receptor assay [11], and electrochemistry [12–15]. Although these methods are sensitive and accurate enough to determine antibiotics, there exist some problems in that regard. For instance, the methods are time-consuming, sample preparation is expensive, and large amounts of specimens are needed. Due to the complexity of foods and the presence of low levels of analytes (about ng/L), purification and pre-concentration must be done before any assessment [16–18].

Among different sensing methods, electrochemical technique has received considerable attention in analytical and bioanalytical chemistry. This is due to its being fast, simple, and cost-effective as well as its high selectivity and reproducibility [19–22]. In recent years, many authors have confirmed that a combination of molecular imprinted polymers and electrochemical determination makes a promising method for detection of various electro-inactive compounds [23,24]. In particular, electropolymerization of MIP films on electrodes has been found to have some advantages such as simplicity, tunability of the preparation procedure, and formation of a thin film on the electrode surface. In addition, the reaction initiator in this method is an electrical

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source which does not need any chemical or inorganic catalyst [25]. The sensors can be applied for ultra-trace determination of antibiotics; they are particularly responsive in the case of electro-inactive antibiotics [26]. MIP-based electrochemical sensors may be of use in the quality control of pharmaceutical industries and/or detection of food fraud [27]. Polyaniline (PANI) is a conductive polymer with suitable electrochemical properties which can be easily synthesized and functionalized. The PANI-based MIPs have been reported electrochemical detection methods previously in combination with other materials/nanostructures [28–32].

This paper aims at the procedure of fabricating an electrochemical sensor based on a porous composite that contains recognized sites for azithromycin (AZT). This composite was prepared through electropolymerization of aniline on a glassy carbon electrode modified with graphene oxide and gold nanourchin in the presence of AZT as a template. Graphene oxide along with some other carbon based nanomaterials have been used in different electrochemical studies [33,34]. The gold nanourchines poses higher surface area than regular spherical gold nanoparticles. This feature along with the other advantages of gold nanomaterials and also graphene oxide could be a way to enhance the sensitivity of the proposed sensing method [20,35–37].

To optimize the pre-concentration condition and to reduce non-specific interactions, the effects of such parameters as pH, temperature, and incubation time of the electrode in a pre-concentration solution were carefully examined.

2. Experimental

2.1. Materials and instruments

Azithromycin (AZT) (with a purity of 90%) was purchased from Tehran Shimi Co. Aniline (98%), sulfuric acid (98%), and nitric acid (65%) were obtained from MERCK (Germany). Also, potassium ferrocyanide, ethanol, graphene oxide (4.0 mg/mL, dispersion in H_2O) and gold nano-urchin (with a diameter of 70 nm) suspension in 0.1 mM PBS were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Electrochemical measurements and electropolymerization tests were performed on potentiostat–golvanostat microautolab III (Netherlands). A three-electrode system was used, where a platinum flake and a saturated calomel electrode (SCE) served as the counter and reference electrodes respectively. Also, a modified glassy carbon electrode (Azar electrode Co. Urmia, Iran) was used as the working electrode. The field emission scanning electron microscopic (FESEM) characterization of the MIP films electrode was performed using an MIRA3 TESCAN-XMU scanning electron microscope.

2.2. Fabrication of an AZT nanosensor and the AZT measurement procedure

An AZT nanosensor was prepared by modifying a glassy carbon electrode using a porous composite. The fabrication steps of this sensor were as follows. Firstly, a GCE was cleaned with ethanol and rinsed thoroughly with distilled water. Then, a drop of GO with the volume of 2.0 μL (2.25 mg/mL in H_2O) was placed on the GCE. After the GCE was dried at an ambient temperature, 3.0 μL of gold nanourchins (GNU) (175 $\mu g\,mL^{-1}$ in H_2O) was dropped on the GO/GCE. The modified electrode was incubated in a wet environment until completely dried. All the steps of the GCE modification with GO and GNU were characterized using cyclic voltammetry, electrochemical impedance spectroscopy and scanning electron microscopy.

To continue, in order to form an MIP film, GNU/GO/GCE was inserted into a solution containing 0.075 M HNO_3, 0.025 M H_2SO_4 and 0.1 M aniline. In the presence of 5.0 mM AZT, aniline was electropolymerized on the GCE by cyclic voltammetry from −0.5 V to +1.0 V at the scan rate of 20 mV/s for five cycles. The modified electrode was removed from the solution and immersed in an ethanol/water mixture as a solvent. AZT molecules could be extracted from a polymer matrix due to the solubility of AZT in aqueous mediums. After AZT was removed from the polymer, pores, known as molecular imprints, were created at the surface.

To rebind AZT and to determine it, the sensor was dipped into AZT solutions with different concentrations for 20 min. After they were washed with water, AZT free or AZT-rebinding MIP film electrodes were tested by CV in a 0.1 M phosphate buffer at pH 7.0.

The PANI films without AZT were electropolymerized on GNU/GO/GCE with the same method as for the MIP films but in the absence of the AZT template in solution, which served as a non-molecularly imprinted polymer (NIP).

To characterize the developed modified electrode and also MIP and NIP we have used a field emission scanning electron microscope (FE-SEM) instrument model TESCAN MIRA3-XMU. We also performed Infrared spectroscopy using Perkin-Elmer Fourier transform infrared (FT-IR) (KBr disks) for characterization of MIP and NIP and also AZT.

2.3. Optimization of the conditions for AZT detection

The influences of effective factors, including solution pH (in phosphate buffer solution with pH from 3 to 10), incubation time (1–120 min) and temperature (20–55 °C), on the analytical signal of the AZT accumulated on the MIP film electrodes were optimized by the central composite design (CCD) methodology. The levels of the selected variables are presented in Table 1. In fact, using this statistical method with the lowest number of experiments, an investigation was performed

Table 1
Experimental range and levels of the independent variables.

Parameters	Unit	Symbol	Levels				
			−α	−1	0	1	α
pH	–	A	3	4.75	6.5	8.25	10
Incubation time	min	B	1	30.75	60.50	90.25	120
Temperature	°C	C	20	28.75	37.50	46.25	55

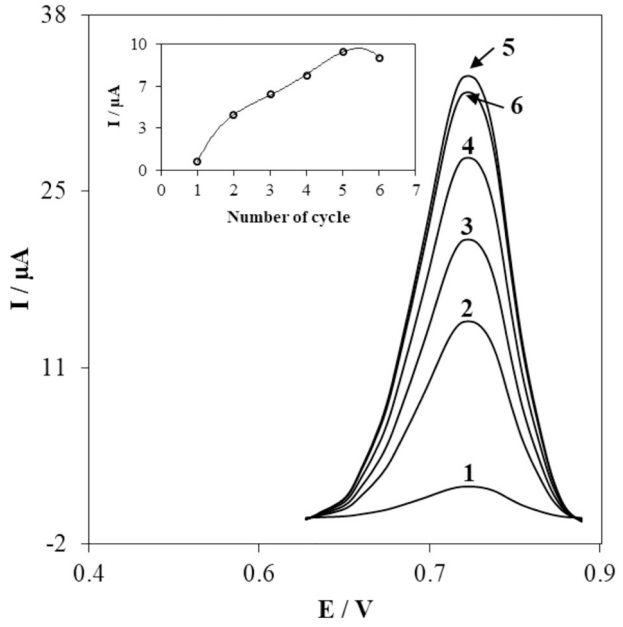

Fig. 1. The effect of the number of cycles of PANI electropolymerization on GNU/GO/GCE and on the analytical performance of the azithromycin nanosensor. (Numbers 1–6 represent the cycle number). Inset: the oxidation current of AZT as a function of the number of cycles of aniline electropolymerization.