Sensors and Actuators B 195 (2014) 28–35

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Molecular imprinted nanosensor based on surface plasmon resonance: Application to the sensitive determination of amoxicillin

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ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form 1 January 2014
Accepted 2 January 2014
Available online 13 January 2014

Keywords:
Amoxicillin
SPR
Molecular imprinting
Validation
Nanosensor

ABSTRACT

In this report, we developed surface plasmon resonance (SPR) sensor for the sensitive determination of amoxicillin (AMOX) in chicken egg and human plasma. Firstly, the modification of gold surface of SPR chip was performed by allyl mercaptane. Then, AMOX-imprinted poly(2-hydroxyethyl methacrylate–methacryloylamidoglutamic acid) [p(HEMAGA)] nanofilm was generated on the allyl mercaptane modified gold surface. The unmodified and imprinted surfaces were characterized by fourier transform infrared (FTIR) spectroscopy, ellipsometry and contact angle measurements. The developed method was validated according to the ICH guideline. The linearity range and the detection limit were obtained as 0.1–2.0 ng/mL and 0.022 ng/mL, respectively. The developed imprinted nanosensor was applied to the chicken egg and human plasma samples for the determination of AMOX. In addition, isotherm models were applied to data to explain adsorption process.

1. Introduction

AMOX (Scheme 1), ((2S,5R,6R)-6-[(R)-(−)-2-amino-2-(p-hydroxyphenyl) acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptanes-2-carboxylic acid trihydrate), is a β-lactam antibiotic of the penicillin group with a thiazolidizine ring connected to a β-lactam ring. It is a semi-synthetic antibiotic, para-hydroxy homologue of ampicillin. AMOX is used extensively in veterinary medicine as a chemotherapeutic, growth promotion and/or prophylactic agent by killing or inhibiting the growth of Gram-positive and Gram-negative microorganisms [1,2]. Various analytical methods have been developed for determination of AMOX including thin layer chromatography (TLC) in pharmaceuticals [3], reverse-phase liquid chromatography (RPLC) in human plasma [4], LC with fluorescence detection in animal tissues [5] and LC/EI/MS/MS in honey and bovine milk [6]. But these methods have some disadvantages such as large material consumption and expensive equipment.

Recently, the various nanosensors have been developed for sensitive determination of biomolecules or drugs [7–29]. SPR sensors have attracted attention for last two decades [30–32]. SPR, an optical phenomenon, is occurred when a p-polarized light goes through a prism; then, hits a metal layer covering the prism surface at a particular angle [33]. SPR was introduced in the early 1990s as the underlying technology in affinity biosensors for biomolecular interaction analysis [34]. SPR sensors have important applications as determination of affinity and binding constants [35,36], monitoring [37], diagnostic [32] and genotype analyzing [38].

Although various methods are used to generate the sensitive SPR sensor, the most effective method is molecular imprinting technique. The method relies on the molecular recognition. It is a kind of polymerization which is formed around the target molecule. Hence this technique forms specific cavities in the cross-linked polymeric matrices [39]. Molecular imprinted polymers (MIPs) have various application such as artificial enzymes [39], solid-phase extraction [40], bioseparation [41–44], affinity detoxification [45,46] and sensor devices [47,48].

In this study, we prepared AMOX-imprinted p(HEMAGA) film on gold surface of SPR chip. In literature, there is no report about determination of AMOX which is performed by AMOX-imprinted p(HEMAGA) film on gold surface of SPR chip. In addition, the developed SPR method shows high sensitivity and high selectivity for determination of AMOX in chicken egg and human plasma samples. The developed non-modified and modified surfaces were characterized by using FTIR, ellipsometry and contact angle measurements. After that, the developed nanosensor was successfully applied to chicken egg and human plasma samples for the determination of AMOX.

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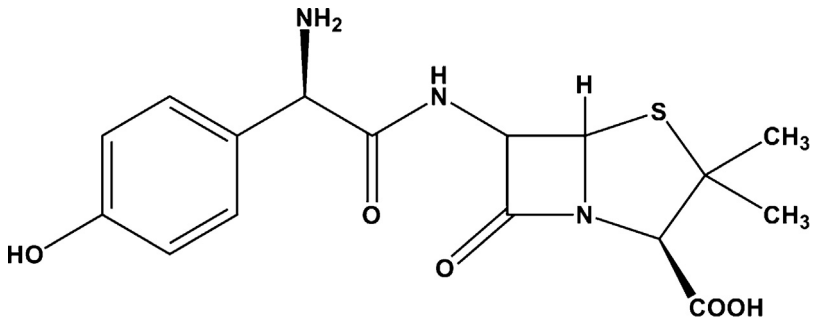
http://dx.doi.org/10.1016/j.snb.2014.01.011



Scheme 1. The chemical structure of AMOX.

2. Experimental

2.1. Materials

AMOX, Ampicillin (AMP) and Cephalexin (CEP) were purchased from Fargem Company (Düzce, Turkey) and used as received. The stock solution of AMOX (1.0 mM) was prepared by dissolving it in 20 mL of ultra pure quality water and then diluting it with ultra pure quality water to 50 mL. The working solutions were prepared by diluting the stock solution with 0.10 M phosphate buffer (pH 7.0). Allyl mercaptane (CH_2CHCH_2SH), 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), N,N′-azobisisobutyronitrile (AIBN), sodium chloride (NaCl) were obtained from Sigma-Aldrich. MAGA was obtained Nanoreg Ltd. Şti., Ankara, Turkey. The other chemicals were used as received.

2.2. Surface modification of the SPR chip

2.2.1. Allyl mercaptane modification of SPR chip

To modify gold surface of the SPR chip with CH_2CHCH_2SH, the chip was washed with 20 mL of acidic piranha solution (3:1 H_2SO_4:H_2O_2, v/v). After the SPR chip was dipped in cleaning solution for 5 min, it was washed with ethyl alcohol and dried in vacuum oven (200 mmHg, 35 °C) for 2 h. To let vinyl groups into the gold surface, the chip was dipped in an ethanol/water (4:1, v/v) solution containing 3.0 M CH_2CHCH_2SH allowed to form self-assembled monolayer for 24 h. Then, it was cleaned with ethyl alcohol and dried under nitrogen atmosphere.

2.2.2. Polymer preparation on SPR chip surface

AMOX-imprinted p(HEMAGA) film on CH_2CHCH_2SH modified SPR chip was prepared according to this protocol: Firstly, AMOX and MAGA monomer were mixed with 500 μL of phosphate buffer (pH 7.0) at room temperature for 3 h. MAGA-AMOX molar ratio was 2:1. After that, 5.0 mg of AIBN as initiator was dissolved in 1250 μL of HEMA and 500 μL of EGDMA and 200 μL of MAGA-AMOX complex was added into this solution to prepare stock monomer solution. The solutions were passed with nitrogen gas for 15 min. Then, 20 μL of aliquot was taken from the stock monomer solution and dropped onto the SPR chip surface by using *spin coating method*. The method is used to deposit uniform thin films to SPR surface. After 10 seconds, The SPR chip was removed from spin coater and polymerization was started by UV light (100 W, 365 nm). After 60 minutes, polymer coated SPR chip was washed with ethanol three times, then and dried in vacuum oven.

2.3. AMOX removal from SPR chip surface

There are the electrostatic interactions and hydrogen bonding between the carboxylic acid groups of MAGA monomer and polar groups of AMOX molecules. In order to break the interactions, we used 1.0 M NaCl solution in water as a desorption agent. Firstly, the removal study of AMOX was performed via batch system. AMOX-imprinted p(HEMAGA) surface was dipped into 25 mL of desorption agent. The SPR chip was swinged in bath (200 rpm) at room temperature. After AMOX removal, the SPR chip was washed with ultra pure quality water and dried with nitrogen gas under vacuum (200 mmHg, 25 °C).

2.4. Characterization methods

Contact angle of the surfaces was obtained with KRUSS DSA100 (Hamburg, Germany) instrument. Contact angles were measured with Sessile Drop method by dropping one water drop. The ten photos were obtained from the different parts of SPR chips. The calculated values for the SPR surfaces were the mean of the ten measurements.

Ellipsometer measurements were also performed by using an auto-nulling imaging ellipsometer (Nanofilm EP3, Germany) to characterize the surface of SPR chips. The measurements have been carried out at a wavelength of 532 nm with an angle of incidence of 72º. In the layer thickness analysis, a four-zone auto-nulling procedure integrating over a sample area of ~50 μm × 50 μm followed by a fitting algorithm has been performed. The measurements were performed at six different points of SPR chip and the results were obtained as mean value.

For FTIR measurements, AMOX-imprinted p(HEMAGA) nanosensor was put into sample holder of FTIR spectrophotometer (Thermo Fisher Scientific, Nicolet iS10, Waltham, MA, USA). The spectra were obtained in the wave number range of 650–4000 cm^{-1} with 2 cm^{-1} resolution.

2.5. Sample preparation

The chicken egg samples were bought from local supermarket. The extraction and dilution procedures of the samples were as follows: Chicken egg white was efficiently stored at −20 °C. 1.0 g samples and 10 mL of phosphate buffer (pH 7.0) were mixed for 2 min and centrifuged at 4500 rpm for 15 min. The supernatant liquid was diluted with phosphate buffer (pH 7.0) for analysis.

1.2 mL methanol were added to an aliquot of 0.4 mL human plasma sample in a 2.0 mL plastic centrifuge tube and vortex-mixed for 1 min, followed by centrifugation at 20,000 rpm for 15 min. The upper clear layer solution was diluted with phosphate buffer (pH 7.0) for analysis.

2.6. Procedure of the analysis

The real time determination of AMOX was performed using SPR system (GenOptics, SPRi-Lab, Orsay, France). The SPR nanosensor was washed with deionized water (50 mL, 2.0 mL min^{-1} flow-rate) and equilibration buffer (pH: 7.0, phosphate, 50 mL, 2.0 mL min^{-1} flow-rate). After the AMOX-imprinted p(HEMAGA) surface was washed with ultra pure quality water (5.0 mL) (2.0 mL min^{-1} of