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# Molecular imprinted polypyrrole modified glassy carbon electrode for the determination of tobramycin

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ABSTRACT

Over the past two decades, molecular imprinted polymers have attracted a broad interest from scientists in sensor development. In the preparation of molecular imprinted polymers the desired molecule (template) induces the creation of specific recognition sites in the polymer. In this study, the glassy carbon electrode (GCE) based on molecularly imprinted polypyrrole (PPy) was fabricated for the determination of tobramycin (TOB). The developed electrode was prepared by incorporation of a template molecule (TOB) during the electropolymerization of pyrrole on GCE in aqueous solution using cyclic voltammetry (CV) method. The performance of the imprinted and non-imprinted electrodes was evaluated by square wave voltammetry (SWV). The effect of pH, monomer and template concentrations, electropolymerization cycles on the performance of the imprinted and non-imprinted electrodes was investigated and optimized. The non-modified and TOB-imprinted surfaces were characterized by using atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), electrochemical impedance spectroscopy (EIS) and CV. The linearity range of TOB was $5.0 \times 10^{-10}$–$1.0 \times 10^{-8}$ M with the detection limit of $1.4 \times 10^{-10}$ M. The developed nanosensor was applied successfully for the determination of TOB in egg and milk.



## 1. Introduction

Tobramycin is aminoglycoside antibiotics which compose of two or more amino-sugars linked by glycosidic bonds to an aminocyclitol named 2-desoxystreptamine [1]. The aminoglycoside drugs are used to treat the infections caused by aerobic Gram-negative and some Gram-positive microorganisms [2]. However, the use of large amount may cause the effects of ototoxicity and nephrotoxicity. Hence, the monitoring of the drug in patients is important. Tobramycin is obtained from the fermentation of the actinomycete *Streptomyces tenebrarius* and used in a variety of pharmaceuticals such as TobraDex® and TOBI®. Various analytical methods such as chromatography have been developed for determination of aminoglycoside antibiotics [3], including thin-layer chromatography [4], gas chromatography [5], liquid chromatography [6], liquid chromatography–mass spectrometry [7], capillary zone electrophoresis [8]. But these methods have some disadvantages such as large material consumption and expensive equipment. Hence, it needs sensitive and reliable analytical methods to qualitatively and quantitatively determine tobramycin in animal-origin foods.

The electrochemical sensors have been developed for a long time. Many studies have been reported about sensor applications [9–22] and drug determination [23–26] via electrochemical methods. In recent years, one of the most efficient approaches is the using of the modified electrodes prepared with molecularly imprinted technology (MIT) that promises to produce recognition elements for sensors. MIT has widely been used as sensitive components in chemical/biological sensors for many compounds because it provides advantages in the construction of the sensor including low cost, simplicity, mechanical/chemical stability, reliability and a wide choice of templates and functional polymers [27–30]. Recently, molecularly imprinted polymers thin films have been prepared in situ at glassy carbon electrode, Au nanoparticles modified glassy carbon electrode, $TiO_2$ nanotubes modified electrodes and Au nanoparticles/$TiO_2$ nanotubes [31,32]. For

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example, a chlorpyrifos (CPF) templated molecularly imprinted film was electrochemically synthesized on a pencil graphite electrode by electropolymerization of pyrrole in the presence of CPF. The fabricated modified electrode was used as a novel impedimetric sensor for the determination of an important organophosphorus pesticide [28]. In addition, a molecularly imprinted polypyrrole based film was fabricated for the determination of ascorbic acid. The film was prepared by incorporation of ascorbic acid during the electropolymerization of pyrrole onto a pencil graphite electrode in aqueous solution using cyclic voltammetry method [29].

In this study, molecularly imprinted film was prepared by in-situ electropolymerization of Py in the presence of tobramycin on the glassy carbon electrode surface and tobramycin was linked to the cavities constructed by binding the sites of the molecularly imprinted polymer film. The non-modified and tobramycin-imprinted surfaces were characterized by using atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry. The proposed nanosensor was applied successfully for the determination of tobramycin in egg and milk.

## 2. Experimental

### 2.1. Chemicals and materials

TOB, KANAMYCIN A (KAN-A), AMIKACIN (AMIK) and GENTAMYCIN (GEN) were purchased from Fargem Company (Düzce, Turkey) and used as received. The stock solution of TOB (1.0 mM) was prepared by dissolving it in 5 mL of ultra pure quality water and then diluting it with ultra pure quality water to 25 mL. The working solutions were prepared by diluting the stock solution with 0.10 M phosphate buffer (pH 7.0). Pyrrole (Sigma-Aldrich), HPLC grade acetonitrile (MeCN) (Sigma-Aldrich), isopropyl alcohol (IPA) (Sigma-Aldrich), activated carbon (Sigma-Aldrich), tetrabutylamonium tetrafluoroborate (TBATFB) (Fluka), potassium ferricyanide ($K_3[Fe(CN)_6]$) (Sigma-Aldrich), potassium ferrocyanide ($K_4[Fe(CN)_6]$) (Merck), potassium chloride (KCl) (Merck), trichloroacetic acid (TCA) (Merck) and sodium chloride (NaCl) (Merck) were reagent grade quality and were used as received. The preparation of the aqueous solutions was carried out using ultra pure quality of water with a resistance of 18.3 MΩ $cm^{-1}$ (Human Power $1^+$ Scholar purification system).

### 2.2. Electrochemical system and measurements

Before electrochemical experiments, the prepared solutions (pyrole, TOB stock solutions and supernatant solutions of egg and milk samples) were purged with pure argon gas (99.999%) at least for 10 min and an argon atmosphere was maintained over the solution during experiments. All electrochemical experiments were performed using Gamry Reference 600 work-station (Gamry, USA) and BAS-100B electrochemical analyzer (Bioanalytical System Inc., Lafayette, IL, USA) equipped with C3 cell stand.

Working electrode was a bare or modified glassy carbon disk (BAS) with a geometric area of 0.027 $cm^2$. The reference electrode was either a $Ag/AgCl/KCl_{(sat)}$ used in aqueous media or a $Ag/Ag^+$ (0.01 M) used in MeCN. To prepare $Ag/Ag^+$ (0.01 M) reference electrode, pure $AgNO_3$ was dissolved in 0.1 M TBATFB in MeCN to obtain a 0.01 M $Ag^+$ inner solution. The counter electrode was a Pt wire.

Electrochemical impedance spectroscopic experiments were carried out with a Gamry Reference 600 workstation equipped with a PCI4/300 potentiostat in conjunction with EIS 300 software. The imprinted and non-imprinted electrodes were characterized in 1.0 mM ferrocyanide/1.0 mM ferricyanide redox couple via EIS methods. EIS data were measured at 100 kHz to 0.1 Hz at 10 mV wave amplitude and at an electrode potential of 0.030 V, the formal potential of ferrocyanide/ferricyanide redox couple.

### 2.3. Surface characterization methods and processes

Infrared spectra were recorded directly from the film deposited on glassy carbon surface in a Bruker-Tensor 27 FTIR spectrometer (Bruker Optics Inc., Ettlingen, Germany).

In order to characterize the glassy carbon surfaces, tapping mode AFM was used (Nano Magnetics Instruments, Oxford, UK). The surfaces were installed on sample holder. 2 μm × 2 μm sample area was displayed with a 128 × 128 pixels resolution. The scan rate was 2 μm $s^{-1}$. The studies were performed in air atmosphere.

The glassy carbon electrodes were cleaned and prepared by polishing to a mirror-like finish with fine wet emery paper (grain size 4000). They were polished successively in 0.1 μm and 0.05 μm alumina slurries (Baikowski Int. Corp. USA) on microcloth pads (Buehler, Lake Bluff, IL, USA). The electrodes were sonicated first in ultra pure water two times and in 50:50 (v/v) isopropyl alcohol and acetonitrile (IPA + MeCN) solution purified over activated carbon. After removal of trace alumina from the surface by rinsing with water and brief cleaning in an ultrasonic bath (Bandelin RK 100, Germany) with water then IPA + MeCN mixture purified over the activated carbon, GCE was rinsed with MeCN to remove any physisorbed, unreacted materials from the electrode surface. Before electropolimerization, the electrodes were dried with an argon gas stream. GCE was immersed into the phosphate supporting electrolyte including 60 mM pyrrole and 25 mM TOB. The imprinting procedure was started by CV in a potential range between −0.6 V and +1.8 V at a scan rate of 50 mV $s^{-1}$ and 5 cycles in pH 7.0 of phosphate supporting electrolyte. TOB imprinted PPy/GCE was soaked in pH 7.0 of phosphate supporting electrolyte to remove non polymeric pyrrole from the electrode surface. In order to extract TOB from the imprinted polymer, the TOB imprinted PPy/GCE was immersed in 1.0 M NaCl solution in water and stirred in at constant speed for 10 min. A control experiment, non-imprinted polymer modified electrode (NIP), was prepared under the same experimental conditions but without adding TOB, to check the reliability of the measurements.

### 2.4. Sample preparation

The two different foods such as egg and milk were purchased from local supermarkets. The extraction and dilution procedures of the samples are described as follows: 5.0 mL of milk sample was mixed with 1.0 mL of TCA (10% m/v) by a vortex mixer for 25 s and centrifuged at 4500 rpm for 15 min. The supernatant was transferred to another centrifuge tube and the precipitate was treated twice as mentioned above. The collected supernatant was centrifuged again at 4500 rpm for 5 min and filtrated by a 0.50-μm syringe filter. The filtrate was directly used as the sample solution. The filtrate was diluted with phosphate buffer (pH 7.0) for analysis. Yolk and egg white were mixed adequately and stored at -20 °C. 1.0 g samples and 10 mL phosphate buffer (pH 7.0) were mixed for 1 min and centrifuged at 4500 rpm for 15 min. The supernatant liquid was diluted with phosphate buffer (pH 7.0) for analysis with imprinted-PPy/GCE nanosensor. All of the diluted supernatants were again filtered before analysis.

## 3. Results and discussion

### 3.1. Electropolymerization of molecularly imprinted polypyrrole

The TOB imprinted PPy/GCE (MIP) was obtained by electrodeposition on the surface of GCE using CV in potential range between −0.6 V and +1.8 V during 5 cycles (scan rate 50 mV $s^{-1}$) (Fig. 1) in