Sensors and Actuators B 241 (2017) 560–566

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

"In-situ" antimony film electrode for the determination of tetracyclines in Argentinean honey samples

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ARTICLE INFO

Article history:
Received 12 July 2016
Received in revised form 18 October 2016
Accepted 27 October 2016
Available online 28 October 2016

Keywords:
"In-situ" antimony film electrode
Square wave cathodic stripping voltammetry
Tetracyclines
Honey

ABSTRACT

The main goal of this work is the application of an antimony film electrode (SbFE) prepared for the first time "in-situ" to determine organic compound as tetracyclines commonly used in Argentine for hive treatment. The SbFE was prepared on a glassy carbon electrode (GCE) and square wave cathodic stripping voltammetry (SWCSV) technique was used. The SWCSV parameters were optimized based on a Draper Lin small composite design. The SWCSV response was linear in the TCs concentration range from 0.40 to 3.00 μM with a limit of detection (LOD) of 0.15 μM. The proposed method is a good and simple alternative to the determination of tetracyclines in Argentinean honeys samples. Recovery experiments were performed using spiked honey samples with standard deviation values from 0.75% and 9.69%.

1. Introduction

Tetracyclines, a family of antibiotics with broad-spectrum activity, are frequently used to treat bacterial infections [1,2]. The TCs are widely used in many areas of veterinary, such as animal food production [3,4], apiculture [5–7], etc. Its use as veterinary drug is banned in the European Union, but is still widely used in countries like United States, Canada, Australia and India [8]. In Argentina, is common the use of tetracycline and oxitetracycline (TCs) in activities such as beef cattle raising and beekeeping. The community apiary employs these antibiotics due to its action against bacterial diseases (American and European foulbrood), which severely affect bee larvae and bee products production [9]. Commonly in beekeeping, the application of antibiotics over the hive must take place when the honey harvest was finished. Otherwise, antibiotics can persist mostly in honey and others bee products like propolis, pollen and royal jelly. These antibiotics have a significant residual effect on human foodstuff and it can cause allergic reactions in susceptible persons, chronic toxicity and antimicrobial resistant [10–12].

Currently there are several methods to determine tetracycline in honey. The most common method is high performance liquid chromatography (HPLC) with mass spectrometry, chemiluminescence, UV–vis or fluorescence detection. These methods require expensive equipment, extensive sample preparation, experimental analysis skills and consume high amounts of reactive, not compatible with the green chemistry philosophy [13–16]. Capillary electrophoresis is also applied to determine tetracyclines in honey samples, nevertheless requires overpriced equipment and a sample pretreatment process [17]. Other methodologies as inmuno-sorbent assay (ELISA) used for this purpose, have drawbacks such as the presence of organic and inorganic interference in honey samples, which may be reduced by a biotin–avidin mediated ELISA method [18].

Voltammetric techniques such as differential pulse, square wave and adsorptive stripping voltammetry are used as a simple and low cost alternative for tetracycline determination in sewage, river water, artificial urine, pharmaceutical and milk samples [19–21]. Therefore, few works determine tetracyclines in a complex matrix as honey. Lian et al. modified a gold electrode with cyclodextrin-multiwalled carbon nanotube composites, gold nanoparticles-polyamide amine dendrimer nanocomposites and chitosan derivative, in order to improve the analytical signal of chlortetracycline in these samples [22]. Furthermore, to determine tetracycline in the same matrix, a gold electrode modified with gold nanoparticles and molecularly imprinted polymers was performed [23]. A commercial graphene screen-printed carbon electrodes has been employed to quantify tetracycline in honey [24].The disadvantages of these methods is that surface electrodes modifications requires laborious steps and employs expensive electrodes and reactives. This is a drawback for a routine quality control.

Bismuth and antimony films electrodes were developed as an alternative to the use of mercury drop and mercury film electrodes since 2000 and 2007, respectively [25,26]. These films electrodes have the advantage of being prepared "in-situ" or "ex-situ" on

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http://dx.doi.org/10.1016/j.snb.2016.10.125
0925-4005/

many electrodes surfaces, such as glassy carbon, carbon paste, carbon fiber or boron-doped diamond [27–30]. Moreover, they have a wide potential window, no effect of dissolved oxygen and fast and simple film preparation steps, especially on the "in-situ" way. Sattayasamitsathit et al. employed an "ex-situ" bismuth film electrode as an alternative to the use of mercury drop and mercury film electrodes to determine tetracycline in commercial capsules [31]. Unlike bismuth electrode, antimony film electrode (SbFE) allows the use of acidic buffers with pH ≤ 2.0 with a wide potential window. This electrode was employed successfully in electrochemical stripping analysis to determine metals [28–30]. However, there were only a few works that determine organic compounds with SbFE. In 2011 a SbFE prepared "ex-situ" was used for the first-time for the determination of sulfasalazine, a pharmaceutical compound, in commercial delayed-release tablets [32]. Few years later, Nigovic and Hocevar reported the determination of pantoprazole in pharmaceutical preparations, with successful analytical results using this electrode [33].

It is important to highlight that to the best of our knowledge, the use of SbFE prepared "in-situ" into organic compounds it has not been even employed. Some of the advantages of "in-situ" film preparation, is that improves sensitivity, selectivity and reproducibility for stripping voltammetry technique. Furthermore, this procedure consists of adding directly Sb (III) ions into the sample solution and a new reproducible surface is generated in the electrochemical cell at each analysis.

In order to identify the best variables operating conditions, is commonly and usefully used the response surface methodology as Draper Lin small composite design [34,35]. This method allows the simultaneous analysis of more than one factor at the same time and their statistical significance study to reduce the number of experiments, minimize reagent consumption and assess the interaction between the factors.

Then, in this paper an antimony film electrode, prepared "in-situ" to determine organic compounds as tetracyclines (tetracycline and oxitetracycline) is used for the first-time. The SbFE is prepared on a glassy carbon electrode and square wave cathodic stripping voltammetry to perform the antibiotic determination in Argentinean honey samples, was employed. A Draper Lin small composite design has been used for the first time to optimize the SWCSV parameters.

2. Material and methods

2.1. Honey samples preparation

Honey samples were obtained from beekeepers at different localities of Buenos Aires province, Argentina such as: Coronel Dorrego (M1), 30 de Agosto (M2), Sierra de la Ventana (M3), Pedro Luro (M4), Coronel Suarez (M5) and Villarino (M6). In these localities, *Diplotaxis tenuifolia* (L.) *DC.* (commonly known as "Flor amarilla", Brassicaceae family), *Centaurea* sp. (Asteraceae family) and Eucalyptus sp (Myrtaceae family) are the predominant species [36]. Tetracyclines were extracted from honey samples by liquid extraction with acetone [37]. For this purpose, 1.0 g of honey was mixed with 6.0 mL of acetone and immerse in an ultrasonic bath for 30 min. This step was performed twice. Then, the solvent was evaporated and the dry extract suspended with supporting electrolyte solution (10.0 mL).

2.2. Reagents and solutions

All reagents were of analytical grade and solutions were prepared using ultra-pure water (18 MΩ). Antimony working solutions were prepared by a proper dilution of the atomic absorption standard solution (1.001 mg mL^{-1}, Merck). The waste, which contains antimony, is placed into a hermetically sealed recipient for further treatment. Hydrochloridric acid (Merck) solution 0.01 M was adjusted to pH 2.5 with sodium hydroxide (Merck) and was used as supporting electrolyte 0.01 M HCl/NaCl. Tetracycline and Oxytetracycline (≥98.0% w/w Sigma-Aldrich) stock solutions (500 μg mL^{-1}) were prepared in methanol and kept refrigerated in dark. These solutions are stable for a month [38]. All the experiments were carried out at room temperature and without removing oxygen.

2.3. Instrumentation

All voltammetric measurements were performed using an Epsilon potentiostat (BASi-Bioanalytical System, USA) and controlled by electrochemical analysis software. The RDE-2 Rotating Disk Electrode (BASi-Bioanalytical System, USA) module was employed for experimental measurements with rotating control.

A standard three-electrodes configuration was used with an antimony film prepared "in-situ" on a glassy carbon electrode as a working electrode (0.0707 cm^2 exposed area). The reference and counter electrodes were Ag/AgCl (3 M NaCl) and a platinum wire respectively. The glassy carbon surface was polished manually with 0.3 μm and 0.05 μm alumina, sonicated and thoroughly rinsed with ultra-pure water. All electrodes were provided from BASi-Bioanalytical System. Scanning Electron Microscopy (SEM) images were performed on a LEO EVO-40 XVP microscope (detector SE1).

2.4. Square wave cathodic stripping voltammetry measurements (SWCSV)

The square wave cathodic stripping voltammetry (SWCSV) were performed with "in-situ" deposition of antimony prepared on a glassy carbon electrode (GCE). The voltagramms were carried out in an electrochemical cell of 25.0 mL with the three electrodes immerse in 0.01 M HCl/NaCl solution (pH 2.5) as supporting electrolyte and 2000 μg L^{-1} of Sb(III). The experiments were performed with a deposition potential of −0.600 V for 7 s and the working electrode rotation rate was kept at 6000 rpm. After 10 s of quiet time, voltagramms were recorded from −0.600 V to −1.000 V. In order to remove unwanted compounds from the electrode surface, a clean step was carried out by applying 0.20 V during 30 s under stirring conditions (6000 rpm) and polished after each measurement. Prior each experiment, cyclic voltagramms were performed at 100 mV s^{-1} between −1.000 and −0.600 V in the electrolyte solution to stabilize the surface of the working electrode. The optimized square wave voltammetry parameters were: step potential (ΔE_s) 4 mV, frequency (f) 130 Hz and amplitude of the square wave (ΔE_{SW}) 160 mV. All experiments were performed in the presence of oxygen due it have no effect on the antimony film formation [30,39,40].

3. Results and discussion

The reduction response of tetracycline and oxitetracycline were studied with SbFE as working electrode. For this purpose, 10 μM of each antibiotic solution was used to scan cyclic voltammetry at 100 mV s^{-1} from −1.10 to −0.600 V in 0.01 M HCl/NaCl (pH 2.5). As can be seen in Fig. 1a), the electrochemical reduction process is irreversible for both cases and presents a reduction peak at (−0.980 ± 0.05) V and the peak current intensities are similar. For this fact tetracycline is used for further studies. In order to evaluate the adsorption behavior of tetracycline on the electrode surface, the electrode was submerged in a 10 μM tetracycline solution 0.01 M HCl/NaCl (pH 2.5) and after one minute (under stirring condition) four successive cyclic voltammograms