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A new Approach to Tricyclic Antidepressants Detection Based on Graphite Microsensors Fabricated by an Innovative Method

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Abstract

In this study a novel low-cost method of amperometric sensors' fabrication based on thick-film technique on solid support and their analytical application are presented. Complete graphite/silver cell sensors were fabricated with the new technology using a desk-top XYZ-dispensing robot and printing pastes. The amperometric sensors were applied for the detection of three selected tricyclic antidepressant drugs (TCAs): imipramine (IMI), amitriptyline (AMI) and doxepin (DOX). The monitoring of TAC concentration in patients' body fluids is need, because these drugs can cause side or even toxic effect when overdosed. The possibility of simultaneous and individual amperometric determination of these compounds in media of different pH is demonstrated. The highest sensitivity was obtained for imipramine (213 nA/μM), while for doxepin and amitriptyline sensitivity was similar 32 and 34 nA/μM, respectively. Moreover, electrochemical determination of imipramine was characterized by the lowest potential of peak current (+0.77 V).

Key words: sensors fabrication, amperometric sensors, tricyclic antidepressant drugs, imipramine, amitriptyline, doxepin.

Introduction

Tricyclic antidepressant compounds belong to the group of psychotropic drugs, which are widely applied for affective or 'mood' disorders treatment (depression, neurosis, bulimia nervosa, etc.). The mechanism of TCAs action is attributed to binding to 5 HT and noradrenaline re-uptake transporters in brain [1]. Despite their therapeutic action, TACs can cause serious side-effects including: chest pain, blurred vision and panic attacks. Toxic-effects can be induced especially when overdosed and/or drugs high-dose is combined with alcohol [2]. Therefore, the detection of TAC concentration in patients' body fluids became an important analytical task.

Nowadays, chromatography [3-5], electrophoresis [6-8], spectrophotometery [9-11] and electrochemistry [12-14] are the most frequently used methods of TACs determination. In this study, we propose a simple method for quantitative tricyclic antidepressants detection using amperometric microsensors fabricated in a new technology.

The Fabrication of Electrochemical Sensors

A variety of substances that might be determined using electrochemical sensors forces ongoing research concerning electrodes materials and methods of their deposition. In our laboratory a novel method based on a desk-top XYZ-dispensing robot (325 Ultra TT, EFD, USA) for deposition of different materials in a liquid form was developed. The robot allows printing lines down to 0.2 mm width and ca. 0.05 mm thick as well as complex geometric patterns for electrical contact pads, conductive paths, sensing areas and insulation layers.

In this experiment, we set our equipment for production of up to 96 sensors in one automatic dispensing cycle. The sensors structures were obtained by the deposition of 3 layers, consecutively: silver (5000 DuPont), graphite (L 951, ITME, Poland) and insulation (7165 DuPont) onto a polyester foil (Autostat CT7). Each layer was dried at temperature 120 - 130°C for 15 min. A full compatibility of commercially available materials designed for screen-printing with this equipment was demonstrated.

Main advantage of a novel deposition method over screen-printing is reduction of deposited material lost. The printing pastes are transferred from a disposable applicator thru a dispensing tip onto substrate accordingly to prior designed pattern. The excessive material can be returned from the applicator to the storage container and laborious and solvent-consuming cleaning of the equipment (screens, drivers) becomes unnecessary. The sensor design can be easily modified by means of software without generation of additional costs of photographic masks, screens etc. This technique can be used for deposition of various materials such as: pastes, inks, emulsions, epoxy and silicone resins and organic solutions on different types of substrates e.g. polymeric foils, glass, ceramics and many others. Additional advantages of desk-top XYZ-dispensing robot utilization are flexibility in design and pattering applicable for both rapid prototyping of test samples and mass production. The same equipment configuration can be used independently of production scale.

Reagents and Methods

Tricyclic antidepressant drugs: imipramine, amitriptyline and doxepin as hydrochlorides were obtained from Sigma. Drugs stock solutions (0.1 M) were prepared in water and stored at +5°C in the dark. Calibration standards were prepared just before experiments by the dilution of stock with running buffer to desired concentrations daily prior to use. All other reagents were of analytical grade. All solutions used throughout the experiments were prepared using ultrapure water (resistivity of 18 MΩ) made with Milipore system. Before use the solutions were deoxidized with gaseous nitrogen.

Measurements were carried out using 3-electrode sensors with graphite layers as working and counter electrodes and silver chloride layer as a reference electrode. The silver chloride electrode was formed by electrochemical chlorination of silver path. Voltamperometric measurements were performed using PalmSens potentiostat (Palm Instruments BV, The Netherlands) employing: cyclic voltammetry (CV) and linear sweep voltammetry (LSV). All measurements were carried out at room temperature.

Results

As mentioned above, the fabricated amperometric sensors were employed for electrochemical quantification of three selected TCAs, namely: imipramine (IMI), amitriptyline (AMI) and doxepin (DOX). The difference between those three substances consists in heterocyclic rings.

The first attempts were made to choose the best chemical and electrochemical conditions for performing quantification of individual TCAs. The detection of each TAC was investigated in wide concentration range using cyclic voltammetry. 50 mM phosphate buffer pH 7 (0.1 M KCl) was chosen as an optimal solution for electrochemical detection for all determined TCAs. Cyclic voltammograms were recorded in a potential range from 0 to 1.2 V with the scan rate of 100 mV/s. Exemplary CV curves for different IMI concentrations and corresponding calibration curve are presented in Figure 1. It was found that the potential of peaks current for IMI, AMI and DOX were +0.77 V, +0.97 V and +0.98 V, respectively. Analytical parameters which specify the determination of selected TACs are compiled in table 1. Parameters were defined for drugs over the concentration range from 0.1 to 10 mM, which include plasma TACs level [15, 16]. The highest sensitivity of the amperometric graphite sensors was obtained for imipramine – 213.0 nA/μM, while for doxepin and amitriptyline was comparable, namely 32.0 nA/μM and 34.2 nA/μM, respectively.

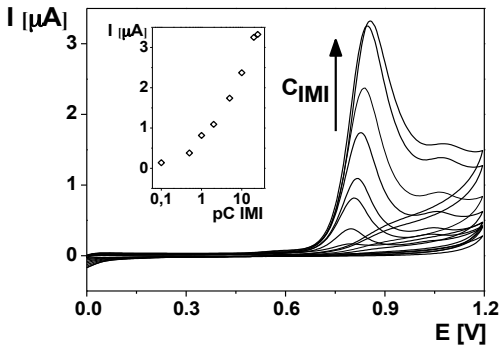

Fig. 1. Typical cyclic voltamperograms for IMI and corresponding calibration graph (inset). Potential range: 0 - 1.2 V, scan rate: 100 mV/s, incubation time: 2 min.

The proposed approach under optimal conditions was adapted for the simultaneous detection of IMI, AMI and DOX in mixture. Linear sweep voltammograms for mixtures of drugs in different concentration recorded in 50 mM phosphate buffer pH 7, 0.1 M KCl are shown in Figure 3. As can be seen the potential of imipramine oxidation stays unchanged, whereas for AMI and DOX one peak consisting of two merged peaks current is recorded. It is worth noticing, that the peak current for IMI

is amplified in mixture comparing to individual drug determination (compare Figures 2 and 3).

Tab. 1: Analytical characteristic of proposed detection method for three selected TACs in concentration ranged from 0.1 to 10 µM. Optimal conditions: 50 mM phosphate buffer pH 7, 0.1 M KCl. Cyclic voltammetry, potential range: 0.0 V – 1.2 V, scan rate 100 mV/s, Estep 5mV. Drug incubation time: 2 min.

Analytical parameter	Tricyclic Antidepressant Drug		
	IMI	AMI	DOX
Sensitivity [nA/µM]	213.0	34.2	32.0
Oxidation Potential [mV]	770	970	980
R^2	0.9161	0.9598	0.9853

Next, a linear sweep voltammetry for each drug was performed (Figure 2).

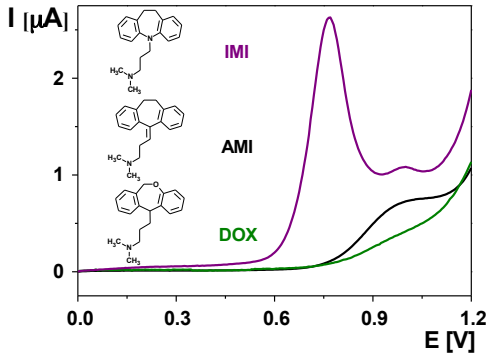

Fig. 2. Typical linear sweep voltamperograms for selected TACs (depicted in the graph). Linear sweep voltammetric response recorded for drugs of 10 µM concentration in 50 mM phosphate buffer pH 7, 0.1 M KCl.

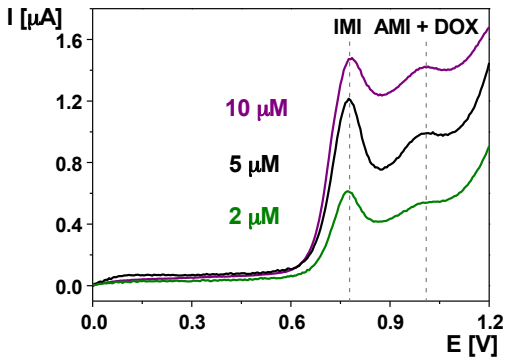

Fig. 3. Simultaneous detection of IMI, AMI, DOX of different concentrations (2, 5 and 10 µM of each drug) in mixture.

Finally, it was proved that selectivity for IMI in TCAs mixture can be improved by use of a buffer solution of appropriate pH. For comparison, in figure 4, linear sweep voltammograms for the mixtures of the three drugs in solutions at pH 2 and 7 are presented. We found that electrochemical oxidation takes place also in buffer solutions of pH 2 for imipramine only. However, the determination of imipramine in solutions of pH 2 is characterized by lower sensitivity.

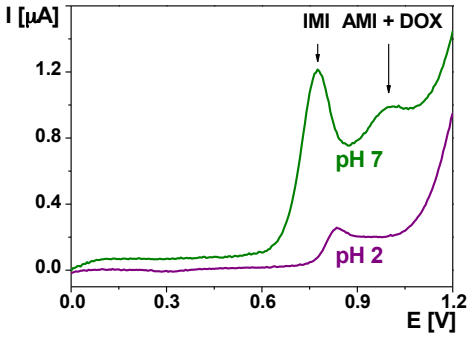

Fig. 4. Simultaneous detection of IMI, AMI, DOX of 5 µM concentration in mixture in buffers of different pH (depicted in the graph).

Conclusions

In this study, we presented an innovative method for the fabrication of thick-film sensors on solid support using a desk-top XYZ-dispensing robot. The advantages of proposed method were discussed.

The complete electrochemical cells consisting of graphite working and counter electrodes and silver/silver chloride reference electrode were shown to be suitable for the detection of tricyclic antidepressant drugs. The highest sensitivity (213 nA/µM) and the lowest potential of peak current (+0.77 V) were obtained for imipramine. In addition, the possibility of imipramine, amitriptyline and doxepin quantification independently as well as in the mixtures was presented.

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