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Tyrosinase based biosensor for the electrochemical determination of sulfamethoxazole

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ABSTRACT

A disposable amperometric biosensor has been developed for the determination of sulfamethoxazole (SMX). Tyrosinase (TYR) has been cross-linked to screen-printed carbon electrodes previously modified by gold nanoparticles. The oxidation current recorded at +500 mV vs Ag/AgCl SPE has been related to SMX concentration. The biosensor showed an acceptable inter and intra immobilization assay, with a RSD of 5.8% ($n = 4$) and 6.7% ($n = 4$), respectively, in the concentration range from 20 μM to 0.2 mM. The capability of detection was 22.6 ± 2.1 μM for a probability of false positive and negative of 0.05. Finally, the developed biosensors have been successfully applied to the determination of SMX in different water samples.

1. Introduction

Aquaculture, practiced for centuries, is today accompanied by the introduction of many chemical substances for therapeutic or prophylactic purposes [1], which could have potential impacts on public health. In this way, sulfonamides have been widely used for preventing bacterial infections in veterinary and human treatments. Undesirable concentrations of these compounds could become part of human food [2], causing resistant bacteria [3]. So that the European Union has fixed the maximum residue limits of total sulfonamides at 100 μg/kg in muscle, liver, kidney, milk and other edible products (Commission Regulation (EU) No 37/2010, 2009) [4,5]. Moreover, aquaculture waters must be analytically controlled in order to decrease the environmental contamination provoked by antibiotic leaching from feces and/or uneaten antibiotic feed [1].

Sulfamethoxazole (SMX) is one of the most frequently used drugs in the sulfonamide family. Their residues in foods of animal origin may lead to thyroid cancer and some other important diseases [6]. Chiavarino et al. developed a gas chromatographic method for SMX detection [7], although this technique has been poorly used due to the laborious processes of derivatization of sulfonamides needed because of their lack of volatility and thermal instability [8,9]. Liquid chromatography [10–17] and capillary electrophoresis [18–28] are the most employed methods to determine these compounds.

As it is well-known, instrumental methods based on chromatography are laborious, time consuming and expensive. Analysis of these compounds in environment and aquaculture requires more simple, rapid and economic methods than the above-mentioned ones [8]. A good alternative for this purpose is electroanalytical methods, which have proved their selectivity, sensitive and economic properties in the analysis of pharmaceutical compounds [29].

Potentiometric [2,30–35], specially ion-selective membrane sensors [2,32–35], and voltammetric [36–44] methods have been mainly developed for SMX determination. In this way, oxidation and reduction mechanisms, based on the primary amino group ($-NH_2$) [36–44] and the sulfonamide group ($-SO_2-$) [36] in that order, have been studied by differential pulse voltammetry (DPV) and square wave voltammetry (SWV) using different modified electrodes (Table 1). The high potentials at which the reaction generally takes place could provoke the interference of other substances in the media, diminishing the selectivity of the procedure. Thus, some authors have attempted the development of analytical methods based on electrochemical immunosensors for SMX detection [5,6]. These immunosensors were based on the competition of SMX and an alkaline phosphatase or a horseradish peroxidase tracers against antibodies immobilized onto screen-printed graphite electrodes [5] or glassy carbon electrodes [6], respectively. In this sense, it would

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Table 1
Voltammetric determinations of SMX based on oxidation mechanisms.

Technique	Electrode	Peak potential	Concentration range (μM)	Reproducibility (RSD)	Repeatability (RSD)	Capability of detection (μM)	Ref.
SWV	Boron-doped diamond (BDD)	+1.1 V vs SCE	6.1 to 60.1	0.7% (250 μM, $n=5$)	0.6% (250 μM, $n=7$)	1.1	[37]
DPV	Hydrogen-terminated BDD (HT-BDD)	+0.9 V vs Ag/AgCl	3.9 to 39.5	–	0.3% (39.5 μM, $n=10$)	14.4×10^{-3}	[38]
DPV	HT-BDD	+0.9 V vs Ag/AgCl	3.9 to 31.6	0.3% ($n=10$)	–	65.1×10^{-3}	[41]
DPV	Metalloporphyrin modified carbon paste (CP)	−0.1 V vs Ag/AgCl	0.01 to 10,000.0	–	–	1.5×10^{-3}	[40]
DPV	Multiwalled carbon nanotubes modified CP	+0.9 V vs Ag/AgCl	1.4 to 118.4	–	2.4% (22.1 μM, $n=10$)	0.4	[42]
SWV	Glassy carbon (GC)	+1.0 V vs Ag/AgCl	55.0 to 395.0	2.3% (200 μM, $n=5$)	1.0% (200 μM, $n=10$)	8.5	[44]
DPV	MWCNT GC	+0.7 V	50.0 to 10,000.0	–	–	10.0	[39]
SWV	Poly (3-methtlthiophene) coated GC	+1.1 V vs Ag/AgCl	6.5×10^{-2} to 35.0	–	–	4.0×10^{-2}	[36]
DPV	Paraffin/MWCNT-SbNPs composite	+0.9 V vs Ag/AgCl	0.1 to 0.7	2.5% (100 μM, $n=5$)	1.8% (100 μM, $n=10$)	24.0×10^{-3}	[43]

be of great interest the development of a generic biosensor to detect residues of a broad sulfonamide group, which would simplify the characteristic tedious measurement procedure of immunosensors.

This work reports a tyrosinase (TYR) based biosensor using screen-printed carbon electrodes (SPCEs) for the amperometric detection and quantification of SMX. Apart from its natural substrates (monophenols and *o*-diphenols), TYR is also capable of oxidizing a variety of aromatic amines and *o*-aminophenols [45–47]: amines are generally hydroxylated to the corresponding *o*-aminophenols, and *o*-aminophenols are oxidized to the corresponding *o*-quinoneimines [45]. The experimental conditions for TYR immobilization on the SPCEs, as well as the main variables that can influence the amperometric response, have been optimized with respect to SMX using the experimental design methodology. The registered oxidation current has been enhanced by the use of gold nanoparticles (AuNPs), taking into account their good catalytic effect, wide surface and increase of electronic transfer. The performance of the developed biosensor has been studied in terms of reproducibility, repeatability, capability of detection, as well as by its application to the quantification of sulfonamides in different water samples.

2. Material and methods

2.1. Reagents

C2000802P2 carbon ink and D2071120D1 dielectric ink (Gwent Electronic Materials, Torfaen, UK), as well as Electrodag 6037 SS Ag/AgCl ink (Acheson Colloiden, Scheemda, The Netherlands), were used in the fabrication of SPCEs.

All reagents used were of analytical-reagent grade. Milli-Q water (Millipore, Bedford, MA, USA) was used for preparing aqueous solutions.

TYR from mushroom (CAS: 9002-10-2; 5771 U/mg solid), $HAuCl_4{\cdot}3H_2O$, glutaraldehyde (GA) and bovine serum albumin (BSA) were obtained from Sigma (Sigma–Aldrich, Steinheim, Germany).

50 mM phosphate buffer solutions (Merck, Darmstadt, Germany), containing 50 mM of KCl (Merck, Darmstadt, Germany), were used as supporting electrolyte, except for the optimization process. 1 M NaOH solutions (J.T. Baker, Deventer, The Netherlands) were used to adjust the pH value to 8.

Stock standard solutions of SMX (Fluka Analytical, Sigma–Aldrich, Steinheim, Germany) were prepared by dissolving the adequate amount in buffer solution.

2.2. Apparatus

SPCEs were produced on a DEK 248 printing machine (DEK, Weymouth, UK). pH of solutions was measured with a HI 221 pH meter (Hanna instruments, USA). A PalmSens® portable electrochemical potentiostat with the PS Trace program (PalmSens® Instruments BV, Houten, The Netherlands) was used for electrochemical measurements.

2.3. Biosensors manufacturing

SPCEs (Working area, 12.56 mm^2) based on configurations of three electrodes (working, Ag/AgCl reference and counter electrode) were home-produced by sequential layer deposition on 0.5 mm thickness polyester films (HiFi Industrial Film, Dardilly, France). A carbon conductive ink was first used to define conductive paths, counter and working electrodes. The reference electrode (Ag/AgCl SPE) was next design by screen-printing the Ag/AgCl ink. In order to define the final geometry of the three electrodes and prevent the conducting paths from the solution, the dielectric ink was finally screen-printed. The different inks were processed and cured according to the manufacturer's specifications.

The electrochemical deposition of AuNPs onto the carbon working electrode was performed in a 100 μL drop of 1 mM $HAuCl_4$, prepared in 500 mM H_2SO_4, applying a potential of +180 mV vs Ag/AgCl SPE during 10 s, except for the optimization process [48]. The AuNPs modified SPCEs (AuNPs-SPCEs) were then washed with Milli-Q water.

SPCEs and AuNPs-SPCEs were then enzymatically functionalized by cross-linking (TYR-SPCEs and TYR-AuNPs-SPCEs). 3.7 μL of 3% (w/v) TYR solution and 2.3 μL of 2.5% (v/v) GA solution, was dropped onto the carbon working electrode surface, except for the optimization process, and left to react at 4 °C for 90 min.

2.4. Measuring amperometric procedure

The detection of SMX was carried at room temperature in a cell containing 5 mL of the supporting electrolyte solution. Amperometric measurements were performed at a working potential of