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Simultaneous determination of cadaverine and putrescine using a disposable monoamine oxidase based biosensor

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ABSTRACT

The selective and simultaneous amperometric determination of putrescine (Put) and cadaverine (Cad) has been carried out using a novel design of screen-printed carbon electrode (SPCE) with two working electrodes connected in array mode. A mixture of 3% of tetrathiafulvalene (TTF), as mediator, and carbon ink was used for the construction of the screen-printed working electrode. The employment of different amounts of monoamine oxidase (MAO) enzyme on these modified TTF/SPCEs and the use of gold nanoparticles (AuNPs) allowed performing the simultaneous determination of both analytes. The amperometric detection has been performed by measuring the oxidation current of the mediator at a potential of +250 mV vs. screen-printed Ag/AgCl reference electrode. A linear response in the Cad concentration range from 19.6 till 107.1 μM and from 9.9 till 74.1 μM for Put was obtained at the MAO/AuNPs/TTF/SPCE biosensor. This device showed a capability of detection of 9.9 and 19.9 ± 0.9 μM ($n=4$ $\alpha=\beta=0.05$) and a precision of 4.9% and 10.3% in terms of relative standard deviation for Put and Cad, respectively. The developed biosensor was successfully applied to the simultaneous determination of Put and Cad in octopus samples.

1. Introduction

Biogenic amines (BAs) are organic bases with aliphatic (putrescina (Put), cadaverine (Cad), spermine (Spm) and spermidine (Spd)); aromatic (tyramine (Tyr) and phenylethylamine (Pea)) or heterocyclic (histamine (His) and tryptamine (Tryp)) structures [1]. These natural contaminants are synthesized and degraded during normal metabolism of animals, plants and microorganisms [2].

High amounts of certain amines may be present in a wide range of food products including fish, meat, wine, beer, vegetables, fruits, nuts and chocolate [2], as a consequence of microbial contamination and inappropriate conditions during processing and storage. Therefore, the content of BAs, especially Put, Cad, His and Tyr can be considered as freshness markers and could be used as indicator of microbial spoilage [3]. Ingestion of food contaminated with BAs, can lead to several health problems, such as headache, blushing, itching, skin irritation, impaired breathing, tachycardia, hypertension, hypotension and vomit [4]. Moreover, it has been reported that certain types of cancer produce an increase of Put and Cad concentration in some human tissues. In this way, Put is often accumulated in blood, serum and mucous of cancer patients. Thus, Put and Cad are listed as tumor markers and their determination in clinical samples can be important for diagnosis of malignancy and, even for monitoring the efficiency of treatments, such radio or chemotherapy [5]. For these reasons, monitoring of BAs amount present in food and beverages is becoming increasingly demanded by regulatory commissions as the Commission Regulation (EC 2073/2005) [6].

Since BAs is usually present at low levels in complex matrices, the determination of these compounds requires the use of sensitive and selective analytical methods. Traditionally, BAs are determined using chromatographic methods [7,8], which are time-consuming and require special instrumentation. Contrary to the above mentioned methods, biosensors offer simple, rapid and cost-effective solution for the determination of BAs [9]. Amperometric enzymatic sensors hold a leading position among the presently available biosensor systems. These devices combine the selectivity of the enzyme for the recognition of a given target analyte with the direct transduction of the rate of the biocatalytic reaction into a current signal, allowing a rapid, simple and direct determination of numerous compounds [10]. Among the different transducers used, screen-printed electrodes offer additional advantages related to their disposable character and great versatility. This versatility lies in the wide range of possible methods of modification of this kind of electrodes, since the composition of the printing inks may be altered by the addition of different

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Table 1
Biosensors for the detection of biogenic amines.

Enzyme	Working electrode	Immobilization	Potential (mV)	pH	Capability of detection	Biosensor selectivity	Sample	References
DAO	Graphite	Crosslinking (PEGDGE)	−50	7.0	5 μM His	867% Cad, 809% Pea, 686% Put, 215% Spd, 100 His, 40% Tryp, 43% Tyr	Pork, Fish	[3]
MAO			−50	8.0	2 μM Tyr	100% Tyr, 90% Pea, 72% Tryp, 1.8 His, 1.2 Spd		
PUO-HRP			+50	8.0	5 μM Put	100% Put, 11% Cad, 5% Spd, 2% Tyr		
PAO	C-SPE	Crosslinking (GA-BSA)	+260	6.65	2 μM Tyr	100% Cad, 87% Put, 84% Tyr, 74% His, 55% Spm, 36% Spd, 31% Tryp	Cheese	[35]
PUO-HRP	Graphite	Crosslinking (PEGDGE)	+50	8.0	5 μM	100% Put, 10.7% Cad, 5.2% Tryp, 5% Spd, 2% Tyr, 1.7% *His*	Beer	[9]
PSAO	Carbon paste electrodos	membrane NAFION	+400	7.5	10 μg/L Cad 8 μg/L Put	*Put+Cad*	Fish	[12]
DAO	C-SPE	Entrapping polymer	–	–	0.65ppm His	–	Tiger Prawn	[13]
DAO	Pt	Crosslinking (GA-BSA)	−600	7.4	0.01 mg/L	100% His, 39.7% Put, 18.5%	Sausages	[14]
DAO	Nano-Fe_3O_4	Covalent	+400	7.2	0.65 nM	–	–	[15]
DAO	Pt-SPCE Au-SPCE	Crosslinking (BSA-PAP)	+600	7.4	0.2 mg/L	Total BAs	Wine, Beer	[6]
MAO-HRP DAO-HRP	C-SPE	Covalent	+250	9.3	0.18 μM His 0.40 μM His	100% Put, 95% Cad, 80% His, 73% Tyr, Spm, Spd, 23% Tryp	Anchovy	[16]
DAO	C-SPE	Crosslinking (GA-BSA)	−50	7.4	0.1 μM Put	100% Put, 90% Cad, 72% Spd, 70% Spm, 92% His, 85% Pea	Human Saliva	[17]
PSAO	Oxygen sensor	Dissolution	−800	7.0	10 μM (His, Cad, Put)	Total BAs	–	[50]
GPAO-HRP	Graphite	Crosslinking (membrane polymeric)	−50	7.2	500 μM (His, Cad, Put, Spd, Tyr)	Separation in chromatographic column	Cod	[2]
DAO	C-SPE	Photopolymerization (Hydrogel)	+350	7.4	*His* 70 μM	100% His, 5.7% Put and Cad	Tiger prawn	[18]
DAO	Pt	Crosslinking (GA+Gel)	+700	7.0	125 μM His, 250 μM Put, 500 μM Cad	–	Cheese, Anchovy	[19]
HRP	Graphite	Crosslinking (membrane polymeric)	−50	7.0	17 ng/mL	Total BAs	Plasma of rat blood	[20]
PUO	GC (MWCNTS/APTES)	Mixture MWCNTS-PODA-APTES	−450	7.0	5 μM	100% Put, Spd, Spm and Cad < 6.8%	Mamalian plasma	[21]
PUO	GC (MWCNTS/APTES)	Crosslinking (GA-BSA)	−250	8.4	0.5 μM Put	100% Put, 35.5 % Spd, 32.8 % Cad, 2.7% Spm	–	[22]
PUO-HRP	GC	Crosslinking (GA-BSA)	+50	8	5 μM Put	100% Put, 123% Spd, 98% Cad, 2% Spm	Fish	[23]
SPP-AO SSP-GOx SSP-ALCOx	Graphite	Hydrogel (PEGDGE)	−50	7.0	100 μM Put 2 mM Ethanol	–	–	[24]
PUO	Pt – (film photolithography)	Crosslinking (membrane+GA)	+600	8.5	0.5 μM Put	–	Human blood and plasma	[5]
PAO TAO DAO	Pt-SPE	Crosslinking (GA-transglutaminase)	+700	7.5	100 mg/kg His 5 mg/kg Put	100% Tyr, < 1% Put-Cad-His-Spd-Spm-Agm 100 % His, 90 % Tyr, 13% Spd, < 5 % Put-Cad-Spm, < 1% Agm 100% Put, 50% Spd, 8% Spm, 2% His-Cad, < 1% Agm	Salmon, Beer, Cheese, sauerkraut, Wine.	[25]
DAO	GC-Pt GC-Rh/Ru	Crosslinking (membrane+GA) and Electropolymerization	+200	8.0	DAO-GC-Rh/Ru (1 μM Put, Cad, Pea; 10 μM Tryp, 5 μM His and Tyr) DAO-GC-Pt (0.5 μM Put, His, Tyr, Pea; 1 μM Cad, 2 μM Tryp)	Total BAs	Cheese	[26]
PUO	Chitosan porous beads	GA	+500	7.8	0.1 mM Put	Total diamines	Chicken	[27]
AO	Graphite	Crosslinking	+200	7.0	2.2 μM His	Cys>His>Tyr>Agm>Spd>Put>Cad.	–	[28]

PSAO-HRP	GC	Crosslinking (GA+Gel)	0.0	7.4	25 μM Put	100 % Put 58 % Pea 56.5 % His 36.5% Tyr	–	[29]
DAO PUO	GC-Pt	Pore glass beads	+600	7.0	DAO 0.5 μM His PUO 10 μM *Put*	100% His, 37% Agm, 28% Put, 14% Cad, 1% Tyr 100% Put, 12% Cad, 10% Agm and Tyr, 9% Spm, 8% Tryp, 1%>His	Fish	[30]
DAO PAO	Pt	Covalently immobilized polymeric membrane	+650	8.0 5.5	50 μM	1 μM Put, Spd, Spm 1 μM Spd, Spm	Fruits	[31]
DAO	Pt	Crosslinking (membrane+GA)	+650	8.0	0.5 μM (Put-Cad-His-Spd-Spm-Tyr), 0.1 μM Tryp	Total BAs	Anchovy	[32]
DAO	Pt	Copolymerization (GA-membrane)	+700	7.4	25 μM His, Put, Cad	Total BAs	–	[33]
PUO	Au-Pt	Crosslinking	+500	8.5	25 μM Put	100% Put, 40% Cad, 20% Spm,	Vaginal epithelial surface	[34]

ALCOx: Alcohol oxidase; AO: Amine oxidase; DAO: Diamine oxidase; GOx: Glucose oxidase; GPAO: Amine oxidase from grass pea; HRP: Horseradish peroxidase; PAO: Plasma amine oxidase; PSAO: Pea seedlings amine oxidase; PUO: Putrescine oxidase; SPP: sweet potato peroxidase; TAO: Tyramine oxidase.
PEGDGE: Polyethyleneglycol diglycidyl ether. Cad: Cadaverine; His: Histamine; Pea: Phenylethylamine; Put: Putrescine; Spd: Spermidine; Spm: Spermine; Tyr: Tyramine; Tryp: Tryptamine;

substances such as metals, enzymes, polymers or mediators [11]. Therefore, fast determination of BAs with different enzymatic disposable biosensors has become increasingly popular. Recently, electrochemical biosensors [2,3,5,6,9,12–35] or bioreactors [36,37] based on commercial or home-purified amine oxidases have been proposed in the literature for the detection of BAs. An overview of different biosensors used for the detection of BAs is given in Table 1. The main problems of the described biosensors come from the low selectivity of enzymes and the high working potentials needed, leading to an increase in interferences [8]. The complexity of food matrix, the presence of potential interferences and the simultaneous occurrence of several BAs are therefore typical problems encountered in the analysis of BAs in food [38] when using this kind of biosensors.

At present, efforts in the development of biosensors for BAs are mainly focused on the improvement of their selectivity and sensitivity as well as their miniaturization, driven by the growing need for rapid in situ analyses to secure the imposed safety standards of food [8].

Within this context, a new method has been developed in this work for the simultaneous and selective determination of Put and Cad in food samples. The novelty of this work lies in the use of an innovative electrochemical disposable screen-printed device, which includes two different modified working electrodes connected in array mode.

2. Experimental

2.1. Reagents

Different inks were used in the fabrication of screen-printed carbon electrodes (SPCEs), namely C10903P14 (carbon ink) and D2071120D1 (dielectric ink) (Gwent Electronic Materials, Torfaen, UK), Electrodag 418 (Ag ink) and Electrodag 6037 SS (Ag/AgCl ink) (Acheson Colloiden, Scheemda, The Netherlands). All solutions were prepared using Milli-Q water (Millipore, Bedford, USA). All reagents used were of analytical grade. The supporting electrolyte used for the measurements was a 100 mM KH_2PO_4 buffer solution (Fluka, Steinheim, Germany), containing KCl 100 mM (Merck, Darmstadt, Germany). A 1 M NaOH solution (J.T. Baker, Deventer, The Netherlands) was used to adjust the pH. Tetrathiafulvalene (TTF) was provided by Sigma-Aldrich (Steinheim, Germany). A 1 mM hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4.3H_2O$, Sigma-Aldrich, Steinheim, Germany) and 0.5 M sulfuric acid (Merck, Darmstadt, Germany) solution was used for the electrodeposition of gold nanoparticles (AuNPs).

Monoamine oxidase A human recombinant (MAO, EC 1.4.3.4; activity, 92 U mg^{-1}) was obtained from Sigma-Aldrich, (Steinheim, Germany). Bovine serum albumin (BSA, Sigma-Aldrich, Steinheim, Germany) solution, which was prepared by dissolving the appropriate amount of this reagent in a 50 mM KH_2PO_4 buffer (pH 6) solution (Fluka, Steinheim, Germany), and glutaraldehyde (GA, Sigma-Aldrich, Steinheim, Germany) aqueous solution were used in the enzyme immobilization process.

Put, Cad (Sigma-Aldrich, Steinheim, Germany), Tyr, His (Fluka, Steinheim, Germany), Spd, Spm and Tryp (Across Organics, Geel, Belgium) solutions were used.

$HClO_4$ (Panreac, Barcelona, Spain), ethyl acetate, NaCl and butanol (Fluka, Steinheim, Germany), ammonia solution, acetone and Na_2SO_4 (Merck, Darmstadt, Germany) were used for Put extraction in real samples.

Fresh octopus samples were obtained from a local market.

2.2. *Apparatus*

The different screen-printed electrodic systems were produced on a DEK 248 printing machine (DEK, Weymouth, UK), using several screen polyester mesh and polyurethane squeegees.

Electrochemical measurements were made with a PalmSens Bipotentiostat electrochemical system (Palm Instrument BV, Houten, The Netherlands). The pH of the buffer solutions was measured with a HI 221 pH meter (HANNA Instruments, USA).

2.3. *Electrodic systems*

Two different home-made electrodic systems, including one or two working electrodes, have been used. The first one served for the optimization of experimental variables in the separate determination of Cad and Put, and has been constructed according to our previous works [39–41]. This system consisted of three electrodes: a Ag/AgCl reference electrode, a carbon counter electrode and a working electrode modified with TTF (TTF/SPCEs). The working electrode was modified with MAO (MAO/TTF/SPCEs) for determination of Put, and with MAO and AuNPs (MAO/AuNPs/TTF/SPCEs) for both Put and Cad determination.

The second one represented a novel device (array-TTF/SPCEs) for the simultaneous analysis of both analytes, Put and Cad. A home-made screen-printed electrochemical design with two different working electrodes connected in array mode has been designed (Fig. 1(a)). The manufacturing of the electrodic system was carried out in five steps, through which the different electrodes were printed following a similar procedure as described in previous works [39–41]. Fig. 1(b) shows a schematic diagram of these steps. In this case TTF in a 3% proportion has been incorporated directly into the carbon ink for printing the working electrode (step 3).

2.3.1. *Deposition of gold nanoparticles onto the SPCEs*

The modification of a working electrode with AuNPs was carried out by electrochemical deposition. A volume of 100 μL of a 1 mM $HAuCl_4$, prepared in 0.5 M H_2SO_4 solution, was deposited onto the electrodic system and a potential of +0.18 V vs. screen-printed Ag/AgCl reference electrode was applied during 10 s [39]. The electrodic system was finally washed with Milli-Q water.

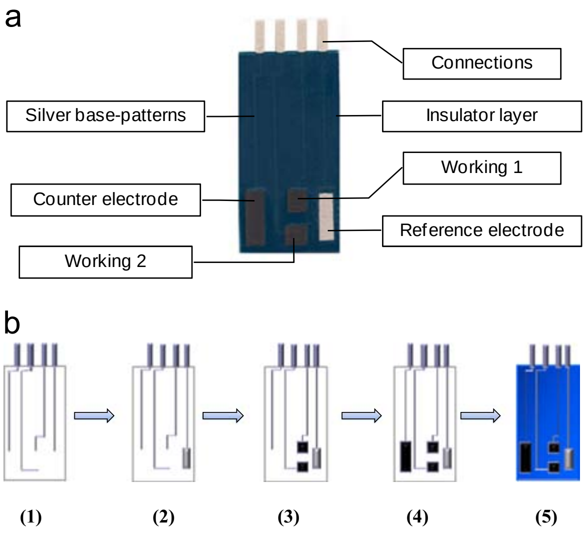


Fig. 1. (a) Homemade array-TTF/SPCE (2.8 cm × 1.8 cm); (b) Schematic diagram of the array-TTF/SPCEs preparation procedure: (1) Silver base-patterns, (2) reference electrode (Ag/AgCl), (3) working electrode (carbon ink+TTF 3% with respect to the mass of the carbon ink), (4) counter electrode (carbon ink) and (5) insulator layer.

2.3.2. *Immobilization of MAO onto the screen-printed working electrode*

MAO enzyme was immobilized on the working electrodes surface using a crosslinking process with GA and BSA. First, a mixture of 1 μL of a 6% (w/v) BSA solution prepared in 50 mM phosphate buffer pH 6, and 2 μL of a 2.5% (v/v) GA aqueous solution was deposited onto the working electrode. Next, 0.46 or 0.92 units of MAO solution were deposited onto the surface of working electrodes by adding 1 μL and 2 μL of a 0.46 units/μL enzyme solution, respectively. Once deposited, the electrodic system was allowed to dry for 90 min at 4 °C.

2.4. *Characterization of TTF/SPCEs*

Once the electrodes were printed by the procedure described in previous sections, the presence of the mediator TTF was verified by cyclic voltammetry. Fig. 2 shows the cyclic voltammograms recorded between +800 and −400 mV in KCl 0.1 M using a TTF/SPCE. The voltammograms corresponded to the typical behavior of TTF with two oxidation peaks and their two corresponding reduction peaks ($TTF \rightarrow TTF^{+} \rightarrow TTF^{2+}$) [42].

2.5. *Electrochemical measurements*

All measurements were made at room temperature in a cell containing 5 mL of a supporting electrolyte solution of the desired pH, under constant stirring. The amperometric detection was performed by measuring the oxidation of TTF_{RED} at a potential of +250 mV vs. screen printed Ag/AgCl reference electrode (Scheme 1). The sample to be analyzed was added after reaching a stable baseline.

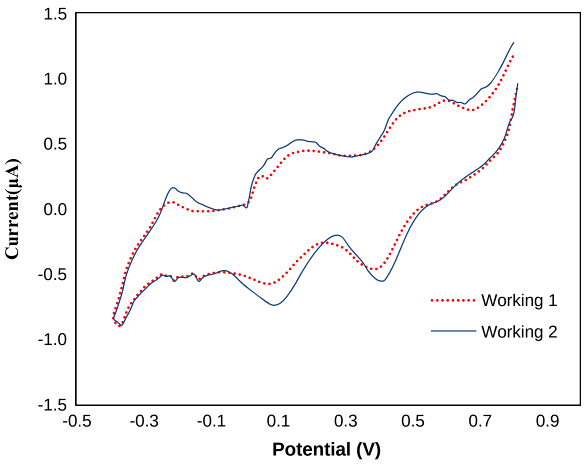
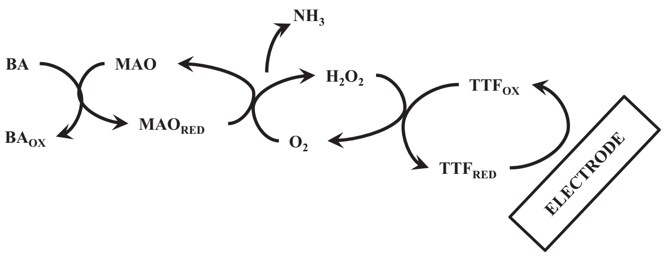


Fig. 2. Cyclic voltammograms recorded in a 0.1 M KCl solution using an array-TTF/SPCE.



Scheme 1. Principle of the electrochemical detection of Put and Cad at MAO-modified TTT/SPCEs .

2.6. Sample preparation

Fresh octopus samples were extracted with a simple preparation method, using an ethyl acetate–acetone mixture, according to the procedure described by Yigit et al. [43]. Thus, 5 g of the previously homogenized sample and 10 mL of a 5% $HClO_4$ solution were mixed in a vortex mixer for 1 min. The mixture was placed in an ultrasonic bath for 10 min and then, centrifuged for 10 min at 7000 rpm. The supernatant was recovered and neutralized with a 3% ammonia solution, saturated with NaCl. Next, it was extracted with 10 mL of an ethyl acetate–acetone (2:1) solvent system in a vortex mixer for 1 min. Subsequently, the mixture was briefly centrifuged. Finally, the organic phase was separated and dried with anhydrous Na_2SO_4, and the solvent was evaporated.

3. Results and discussion

It is well known that MAO catalyzes the oxidation process of both Put and Cad. However, previous experiments showed that the selectivity of MAO towards these molecules is different, being the K_m value for Put, 4.3×10^{-4} M and for Cad, 1.2×10^{-3} M [44]. The efficiency of an enzyme-catalyzed reaction is related to substrate selectivity. Differences in substrate selectivity for an enzyme toward different substrates may affect substrate sensitivity [45]. Thus, when both substrates, Put and Cad, are present in the same medium, they will compete for reaction with the enzyme. The amount of enzyme immobilized on the electrode could therefore affect the selectivity and sensitivity of a developed biosensor. In this way, a detailed study of the influence of the quantity of MAO immobilized in the working electrode surface has been carried out.

At the developed biosensor the BA was oxidized to aldehyde (BA_{OX}) through the action of the enzyme MAO. The hydrogen peroxide enzymatically generated is then oxidized by the mediator TTF_{OX}. The recorder signal corresponded to the electrooxidation of TTF_{RED}.

Several biosensors have been developed by immobilization of different amounts of MAO ranging from 0.1 to 2 units onto the TTF/SPCEs surface and their amperometric response was then measured. This study has revealed that the developed biosensor responded to the presence of Put for amounts of MAO higher than 0.46 units. In contrast, Cad detection was only possible for quantities of MAO higher than 0.92 units. The amperometric response obtained for the developed biosensors, however, was relatively poor for Cad. The problem was solved by depositing AuNPs onto the working electrode. In this way, an electrode modified with AuNPs and 0.92 units of MAO, responded quantitatively to the presence of both Cad and Put.

Bearing in mind the above results, one of the working electrodes (working 1) of the developed array-TTF/SPCE system has been modified immobilizing 0.46 units of MAO, resulting in a sensitive response for Put only. The second working electrode (working 2) has been modified with AuNPs and 0.92 units of MAO being, therefore sensitive to both molecules. With this device, the simultaneous determination of both molecules in the same sample could be carried out using a single calibration set.

Previous to the simultaneous determination of Cad and Put, an optimization process of the experimental parameters has been carried out separately.

3.1. Determination of Put and Cad with MAO/AuNPs/TTF/SPCEs

As described above, the enzymatic oxidation of Cad or Put, in the presence of TTF, allowed the quantitative determination of these amines following the amperometric oxidation of the mediator TTF. An applied potential of +250 mV vs. Ag/AgCl was selected as the optimum value of this parameter for the amperometric analysis of Put and Cad at the MAO/AuNPs/TTF/SPCEs (Fig. 2). The best amperometric signals were obtained at pH 11. Thus this value was chosen as the optimum supporting electrolyte pH. With the aim of carrying out the characterization of these processes, several calibrations sets were performed for each amine.

Calibration parameters were calculated using the program PROGRESS [46], which evaluated the presence of anomalous points using a least median square regression (LMS). Once those points were removed from the calibration set, a calibration curve was built with the remaining points by ordinary least square regression (OLS). This OLS regression provided a proper assessment of the slope (sensitivity) and the independent term of calibration. Both terms were important for judging the quality of calibration and, indeed, the analytical method. In this way, a linear response in the range from 19.6 till 107.1 μM was obtained for Cad and from 9.9 till 74.1 μM for Put.

Reproducibility was then evaluated in terms of sensitivity using the slope of regression built using different MAO/AuNPs/TTF/SPCEs. The sensors showed an acceptable relative standard deviation (RSD) value of 4.9% and 10.3% ($n=4$) for Put and Cad, respectively. The capability of detection was calculated for a probability of false positive (α) and negative (β) equal to 0.05, according to the literature [47,48]. The average capability of

Table 2
Characterization of biosensor for Cad and Put determination.

Biosensor	Analyte	Sensitivity (nA/μM)	Reproducibility RSD (%)	Capability of detection (μM)	Linear range (μM)
MAO/AuNPs/TTF/SPCEs	Put	1.16 1.27 1.15 1.16	4.9	9.9*	9.9–74.1
	Cad	1.05 1.22 1.26 1.03	10.3	19.9	19.6–107.1
MAO/TTF/SPCEs	Put	1.65 1.41 1.47 1.65	8.0	12.6	11.9–77.0

*The minimum detectable net concentration found for $\alpha=\beta=0.05$, was 2.1 ± 0.5 μM. Since this concentration value was lower than the first value of the standards, 9.9 μM was taken as average analytical capability of detection.

detection found was 19.9 ± 0.9 μM for Cad and 9.9 μM for Put ($\alpha=\beta=0.05$) ($n=4$). Table 2 summarizes these results.

3.2. *Determination of Put with MAO//TTF/SPCEs*

As it has been explained above, when the working electrode surface was modified with 0.46 units of MAO, only Put gave an analytical amperometric response. Thus, this electrode was characterized for Put. The MAO/TTF/SPCE biosensor presented a linear response in the Put concentration range from 11.9 till 77.0 μM at pH 11. This biosensor showed a capability of detection of 13 ± 2 μM ($\alpha=\beta=0.05$) and a precision of 8% ($n=4$) in terms of RSD (Table 2).

3.3. *Substrate selective determination*

The possible interference from other BAs was evaluated. The BAs selected in this work were those most commonly found in food samples, namely Tyr, His, Tryp, Spd, and Spm. Under the optimal operational conditions, only Tyr interfered at a concentration of 60 μM in determination of Cad and Put when using the MAO/AuNPs/TTF/SPCE biosensor by giving a well-defined amperometric response. Tyr interfered also in the analysis of Put when using MAO/TTF/SPCE biosensors.

A deep study of the possible interference caused by the presence of other BAs was performed, using His as the selected interference. In this way, different calibration sets for Put and Cad were carried out using the MAO(0.92 units)/AuNPs/TTF/SPCE in the presence of two different levels of His (9 and 77 μM). The sensitivity (slope) of these calibrations was not altered by the presence of His. Similarly, the sensitivity of MAO(0.46 units)/TTF/SPCEs biosensor towards Put was not altered by the presence of His even at a high level of concentration.

3.4. *Application in octopus samples analysis*

The simultaneous determination of Put and Cad in octopus samples was performed using the standard addition method with array-TTF/SPCEs. The extraction of amines was performed according to the procedure described in Section 2.6. The extract obtained was dissolved in 2 mL of water. A volume of 500 μL of this extract was placed into the electrochemical cell, containing 5 mL of buffer solution pH 11, and following by successive additions of 100 μL of Put 5 mM solution (Fig. 3). The concentration of Put and Cad found were 22.1 ± 1.6 and 15.6 ± 1.1 mg/Kg ($n=3$, $\alpha=0.05$), respectively. These results were in agreement with previously described works. [49].

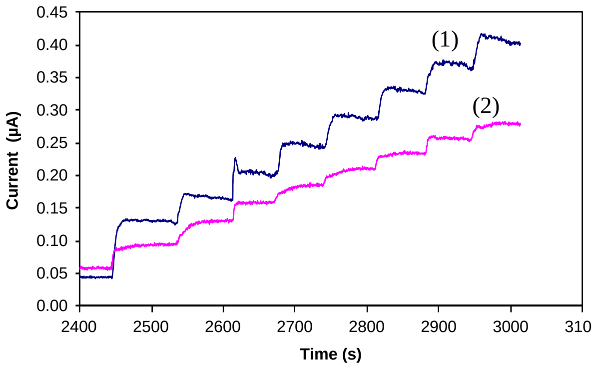


Fig. 3. Chronoamperogram recorded for the simultaneous determination of Put and Cad in octopus samples in the array-TTF/SPCEs biosensor. (1) Working 1: MAO/TTF/SPCE and (2) working 2: MAO/AuNPs/TTF/SPCE. The first addition (2450 s) corresponds to 500 μL of sample extract and then successive additions of 100 μL of a 5 mM Put solution were made. pH 11, $E_{ap}=+250$ mV vs. Ag/AgCl screen-printed reference electrode.

Table 3
Recovery (%), confidence interval for mean and RSD values for extracted Put and Cad from spiked octopus samples, using MAO/TTF/SPCEs and MAO/AuNPs/TTF/SPCEs based biosensors ($N=4$).

Analyte	Spiked concentration (mg/Kg)	Concentration found (mg/Kg)	Recovery (%)
Put	–	22 ± 2	–
Cad	–	16 ± 1	–
Put	21.93	44 ± 3	100 ± 6
Cad	15.4	29 ± 1	95 ± 3

In order to test the reliability of this procedure for the simultaneous determination of Put and Cad in octopus samples, a recovery study was also performed. The total amount of Put and Cad in a spiked octopus sample was determined by the standard addition method in quadruplicate. The concentration of Put and Cad found was 44 ± 3 and 29 ± 1 mg/Kg ($n=4$ $\alpha=0.05$), respectively. The average recovery found was 100 ± 6% and 95 ± 3% for Put and Cad, respectively (Table 3). On the basis of these data the method can be considered appropriate to be applied to simultaneous determination of Put and Cad in these samples.

4. Conclusions

A new and disposable, screen-printed array electrodic system including two working electrodes, an auxiliary and a reference electrode has been developed, in order to perform the simultaneous determination of Cad and Put.

The screen-printed TTF mediator reagent allows measuring at a low operational potential of+250 mV, thus, reducing the possible interfering by substances present in the complex sample. The biosensors construction is reproducible, allowing sensors with very similar sensitivities to be obtain The biosensors present a linear response range from 9.9 to 74.1 μM for Put and 19.6 to 107.1 μM for Cad with a capability of detection of 19.9 μM and 9.9 μM for Cad and Put, respectively.

The biosensor was successfully used as an efficient screening tool to simultaneously quantify Put and Cad in cephalopod mollusc samples.

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