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# Use of sinusoidal voltages with fixed frequency in the preparation of tyrosinase based electrochemical biosensors for dopamine electroanalysis

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ABSTRACT

Sinusoidal voltages (SV) of fixed frequency were used in the preparation of electrochemical biosensors based on gold (Au) disk microelectrode arrays (MEAs) modified with a bio-composite material consisting of poly(3,4-ethylenedioxythiophene) conducting polymer (PEDOT) and tyrosinase (Tyr). The SV was applied over a d.c. potential of 0.60 V vs. Ag/AgCl/KCl (3 M) in order to assess the contribution of the sinusoidal signal to the electrochemical polymerization of the monomer. The use of SV with fixed frequency ensured the preparation of bio-composite materials with given properties. A high porosity is expected, as the Tyr enzyme is entrapped within the polymeric layer by electrostatic interactions during the electrochemical polymerization process. The morphology and the chemical nature of the prepared coatings were studied by scanning electron microscopy, optical profilometry, and infrared reflection absorption spectroscopy. The MEA devices present two independent arrays separated by an insulating gap. One electrode from the device was modified by a PEDOT-Tyr layer, while the second electrode was modified with a PEDOT layer. The analytical determination of dopamine and hydroquinone was carried out via bipotentiostatic measurements by simultaneous polarization of both PEDOT-Tyr and PEDOT modified electrodes from one device using cyclic voltammetry. The analytical performance in terms of linear range, detection and quantification limits, sensitivity, repeatability, re-usability and operational stability, have been assessed. The PEDOT-Tyr based biosensor, prepared at 0.60 V d.c. potential value and SV signal with 50 mHz frequency and ±350 mV amplitude, exhibited a low detection limit of $2.4 \times 10^{-7}$ M dopamine, an excellent repeatability of 4.1%, and a recovery of 100.2% were achieved for dopamine determination. The proposed biosensor was also successfully applied in dopamine electroanalysis in pharmaceutical products.



## 1. Introduction

Organic conducting polymers (CPs) have attracted a great deal of interest in the last two decades thanks to peculiar features such as electrical conductivity, electrochromic properties, low ionization potentials and high stability in both organic and aqueous media. In recent years, CPs have been extensively used in the development of electrochromic devices [1], solar cells [2], batteries [3], sensors and biosensors [4,5]. CPs film-modified electrodes proved to be a feasible approach to entrap biological sensing elements such as enzymes, preserving their biocatalytic activity and enhancing the sensitivity of the analytical measurements [6,7].

Tyrosinase (Tyr, polyphenol oxidase, E.C. 1.14.18.1) is a copper-containing enzyme that, in the presence of molecular oxygen, catalyses two important reactions: (i) the *o*-hydroxylation of monophenols to catechols, and (ii) the oxidation of *o*-diphenols to *o*-quinones. Consequently, Tyr has been successfully employed in the fabrication of various electrochemical biosensors for dopamine [8–11], catechol [12–14], and phenols [15–18]. The immobilization of Tyr has been achieved by several protocols including carbon

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paste immobilization [8,16], adsorption [9], covalent attachment [10,11,17], chemical grafting on polymers [12], entrapment within conducting polymer films [13–15], and cross-linking [18]. Actually, these protocols are the most frequently used for enzymes and biomolecules immobilization in connection with various nano- and composite materials for biosensors' development [19–23]. Among these protocols, the entrapment of tyrosinase within conducting polymer films during the electropolymerization of the corresponding monomers was proved to enhance the stability and the sensitivity of the prepared biosensors, taking advantages of the capabilities of conducting polymers to act as transducers and to provide a favorable microenvironment for enzyme immobilization. Conducting polymers can also enhance the communication between enzyme active sites and electrode surface. Thus, they are considered as suitable for enzyme immobilization on microelectrodes and miniaturized devices, particularly because the thickness and the amount of immobilized enzyme can be easily controlled by the electropolymerization time. In this context, we have investigated the development of a novel electrochemical procedure for enzyme immobilization within conducting polymers by means of sinusoidal voltages (SV) superimposed on a d.c. potential [24–28]. Compared to other electrochemical methods like cyclic voltammetry, potentiostatic and galvanostatic methods, the new SV method ensured the preparation of conducting polymer matrices characterized by higher porosity and superior enzyme retention.

The principal aim of this work is to assess the advantages of a new biosensor consisting of a poly(3,4-ethylenedioxythiophene) (PEDOT) – Tyr biocomposite material electrodeposited onto gold microelectrode arrays (MEAs) and Au (disk of 3 mm diameter) electrodes by means of SV of fixed frequency onto a d.c. potential. Recently, we have applied a similar preparation method for biosensors development by using SV of fixed frequency range superimposed on a d.c. potential. The use of the SV procedure monitored with the electrochemical quartz crystal microbalance (EQCM) technique was recently addressed in our previous work [24]. EQCM measurements were performed on quartz crystals covered with a gold layer to understand the electrodeposition mechanism. The novelty and the improvement proposed here with respect to previous works [25–28] lie in the use of a SV of ±350 mV amplitude with a constant frequency at a d.c. potential value situated in a region where no faradic or electrochemical polymerization reaction takes place [24]. In the present work, the SV procedure based on fixed frequency has been applied to the development of electrochemical biosensors at microelectrode arrays. This new approach emphasizes the contribution of the SV signal in the development of the microelectrode arrays based electrochemical biosensors. The analytical performance of the obtained biosensors toward dopamine (DA), hydroquinone (HQ), catechol (CT), and their use in pharmaceutical products analysis have been studied.

## 2. Material and methods

### 2.1. Reagents

Tyrosinase (E.C. 1.14.18.1, from mushroom, 3610 units/mg solid, Sigma, St. Louis, MO, USA), dopamine hydrochloride (Fluka, Steinheim, Germany), hydroquinone (Sigma-Aldrich, Steinheim, Germany), catechol (Sigma-Aldrich, Steinheim, Germany), $Na_4[Fe(CN)_6]$ x $10H_2O$ (Riedel-de-Haën, Seelze, Germany), $K_3[Fe(CN)_6]$ (Sigma-Aldrich, Steinheim, Germany), $KNO_3$ (Sigma-Aldrich), KCl (Riedel-de-Haën), $KH_2PO_4$ (Riedel-de-Haën), $K_2HPO_4 \times 3H_2O$ (Merck, Darmstadt, Germany), and 3,4-ethylenedioxythiophene (EDOT, Aldrich) were of analytical reagent grade. Dopamine hydrochloride 5 mg/mL concentrate solution for intravenous infusion was purchased from Zentiva Romania. The poly(3,4-ethylenedioxythiophene) (PEDOT) polymer was electrodeposited from an aqueous buffered solution containing 0.05 M EDOT, 0.1 M $KNO_3$, and 0.05 M phosphate buffer of pH 7. Double distilled water was used for the preparation of all aqueous solutions.

### 2.2. Electrochemical measurements

All the electrochemical measurements were carried out using an Autolab potentiostat/galvanostat 302N (Ecochemie, The Netherlands) with bipotentiostat module coupled to a PC running the GPES software, in a three-electrode configuration. Gold disk microelectrode arrays (MEAs, where microelectrodes have a diameter of 20 μm and a center-to-center separation of 100 μm) were used as working electrode. Au disk electrode with diameter of 3 mm was also used as working electrode. Ag/AgCl/KCl (3 M) electrode (Metrohm) and a platinum wire (Metrohm) were used as reference and auxiliary electrode, respectively. Before use, the working MEAs devices have been activated by using an electrochemical procedure previously described [25,26]. Cyclic voltammetry was performed in bipotentiostatic mode by simultaneous polarization of both microelectrode arrays from the MEA devices. All the electrochemical measurements were performed under a high purity argon (5.0) atmosphere. The analytical determinations of the target analytes using the developed biosensors were carried out in air-saturated buffered aqueous solutions, since the presence of oxygen is required for proper functioning of the Tyr enzyme.

### 2.3. Fabrication of microelectrode arrays

The microelectrode arrays devices, MEAs, used as working electrodes, contain 135 microelectrodes, 20 μm in diameter and 100 μm center-to-center distance (see Fig. 1A).

### 2.4. Deposition procedures of PEDOT-enzyme films

The microbiosensors were prepared by the electrodeposition of the PEDOT-Tyr bio-composite material onto one of the microelectrode arrays from a MEA device in a solution containing the optimum concentrations of 0.05 M EDOT, 2 mg/mL Tyr, 0.1 M $KNO_3$, and 0.05 M phosphate buffer solution (PBS) of pH 7 using the following procedure: sinusoidal voltages (SVs) of fixed frequency values of 100 kHz, 1 kHz, and 50 mHz (see Fig. 1B), with excitation amplitude ($\Delta E_{ac}$) of ±350 mV, were applied at fixed d.c. potential ($E_{dc}$) values of 0.60 V and/or 0.95 V to one of the arrays of the MEA device. The investigated deposition times were of 60, 120, 240, and 600 s, respectively. Deposition time controls the final thickness of the electrodeposited bio-composite material, and the resulting modified electrodes were referred to as Au-MEA/PEDOT-Tyr-SV.

The electrodeposition of PEDOT coatings was also achieved by using the SV procedure described above in a solution containing 0.05 M EDOT, 0.1 M $KNO_3$, and 0.05 M PBS of pH 7, and the resulting electrode was denoted as Au-MEA/PEDOT-SV. The SV procedure was also applied to Au disk electrodes with a diameter of 3 mm. The SV preparation procedure was implemented using the FRA manual control feature of the potentiostat. The maximum value of the amplitude allowed by the FRA manual control is ±350 mV. The applied SV signal was measured with a Keysight Infinii Vision MSO-X 4154A oscilloscope.

### 2.5. Surface characterization

#### 2.5.1. Infrared spectroscopy

InfraRed Reflection Absorption Spectroscopy (IRRAS) was used to study the electrodeposited coatings. IRRAS spectra were done in reflection geometry at a grazing-incidence angle of 65° using a