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# Amperometric biosensors precision improvement. Application to phenolic pollutants determination

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ABSTRACT

Electrodes fouling associated with the electroenzymatic phenols determination was characterized in this work, applying various techniques such as cyclic voltammetry, amperometry, EQCM, and optical microscopy. An approach to overcome the fouling effects and hence to improve the precision of the phenolic compounds determination was suggested and tested. This approach consists of pulsed potential waveform application with a cleaning potential step of + 1.4 V vs. Ag, $AgCl/KCl_{sat}$ with a duration of 166.66 ms, while the determination was carried out at 0.0 V vs. Ag, $AgCl/KCl_{sat}$ applied for 66.64 ms. As a result a RSD of 2.97% for 0.6 mmol $L^{-1}$ o-catechol determination was achieved, compared with 6.53% without the cleaning step application. The method was successfully used for the precise phenolic and triazine pollutants determination.



## 1. Introduction

Phenols or phenolics are a large group of compounds of natural and anthropogenic origin [1]. The indiscriminate release of wastes generated during dyes, chemicals, textiles, resins, and plastics production, as well as during the processes of wood preservation, and petroleum refining causes phenolic compounds pollution [1]. Since phenolics are persistent in the environment and highly toxic [1], they are included in the EPA list of priority pollutants [2]. EPA sets the maximum concentration limit of phenol in water for the protection of public health at 3.5 μg $L^{-1}$ [3], while in the European Community the maximum admissible concentration of phenolic compounds in drinking water is 0.5 μg $L^{-1}$ for the total content and 0.1 μg $L^{-1}$ for the individual content [4]. The maximum levels of phenolics in surface water for drinking purposes should be in the 1-10 μg $L^{-1}$ range, depending on how it is treated [5]. Hence, a number of high sensitive chromatographic methods were suggested for phenolic compounds determination [6,7]. Nevertheless, they are not well suited for *in situ* analysis. Therefore, recent trends in analytical chemistry rely on the use of biosensors as an alternative to the above mentioned traditional techniques. Several biosensors were developed for phenolic compound determination [8]. Most of them are amperometric and are based on tyrosinase. This enzyme catalyzes the o-hydroxylation of the monophenols to o-diphenols (monophenolase or cresolase activity), and the oxidation of the o-diphenols to the corresponding o-quinones (diphenolase or cathecolase activity) in the presence of $O_2$. The analytical signal is the current of o-quinone reduction, proportional to the phenolic compound concentration. Since tyrosinase is an instable enzyme, current efforts are devoted to the development of appropriate immobilization protocols using various approaches and immobilization matrices to guarantee the operational and storage stability of the biosensors. These protocols typically involve enzyme entrapment in polymers [9–11] and hydrogels [12–14], incorporation within carbon paste [15], or immobilization onto nanomaterials modified electrodes [16–24]. Nevertheless, little attention is paid to another important drawback of the tyrosinase-based amperometric sensors for phenolics determination, namely sensitivity and precision alterations resulting from electrode fouling induced by the polymerization of the radicals formed during the enzymatic and electrochemical reactions sequence [25]. Hence, tyrosinase sensors based on the mediated reduction of o-quinone [26–33], and tyrosinase sensors which monitor the consumption of the oxygen cofactor applying Clark type electrode [34–36] were developed to bypass this problem. In contrast to these strategies, a simple and effective way to overcome electrode fouling problems is suggested in this work. It is based on the registration of the quinone reduction current in the absence of any mediator, along with the continuous

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cleaning and reactivation of the electrode surface achieved by applying an appropriate pulse potential waveform. The technique, known as pulsed amperometry, was successfully applied to address surface-related challenges associated with alcohols, carbohydrates, amino acids, and organo-sulfur compounds detection [37–40]. By this way the precision of the determinations is greatly improved. The efficiency of the suggested approach was recently demonstrated performing organophosphorus pesticides (OPs) quantification by applying an organophosphorus hydrolase-based electrochemical sensor [41]. The oxidation current of p-nitrophenol released upon organophosphorus hydrolase-catalyzed hydrolysis of paraoxon, parathion and methyl parathion, which is directly proportional to the OPs concentration, was recorded as analytical signal. RSD of 0.41% at 40 mmol $L^{-1}$ p-nitrophenol determination was achieved after 10 determinations, instead of 43.93% without the pulsed cleaning application.

## 2. Experimental

### 2.1. Reagents

Phenol, catechol, dopamine and atrazine, analytical reagent grade, were purchased from Sigma and were used without further purification. Fresh solutions of phenol, catechol, and dopamine were prepared in deionized water. Atrazine was dissolved in methanol. 0.2% w/v solution of chitosan (medium molecular weight, Sigma) was obtained by dissolving the substance in $CH_3COOH$ 0.1 mol $L^{-1}$. All the experiments were performed in phosphate buffer solution (PBS) 0.1 mol $L^{-1}$, pH 6.5, consisting of a mixture of appropriate amounts of $K_2HPO_4$ and $KH_2PO_4$.

Tyrosinase (EC 1.14.18.1) was supplied by Spectrum Chemical, USA. Enzyme activity unit was defined as the amount of enzyme that liberates 1 μmol of o-quinone per minute from catechol under the assay's conditions (PBS 0.1 M, pH 7.0, 25°C). The rate of quinone formation was evaluated by spectrophotometric measurements using a PC controlled Evolution 60S UV-VIS spectrophotometer (λ=400 nm; $\varepsilon_{quinone}$=1450 $mol^{-1}$ L $cm^{-1}$ [42]). The specific enzyme activity was found to be 224 units $mg^{-1}$.

### 2.2. Instrumentation and procedures

Electrochemical studies were performed by means of a CH Instruments model 440 A electrochemical analyzer (CH Instruments Inc., USA), and an electrolysis cell of conventional type (10 mL of volume). Pt wire was used as auxiliary electrode and Ag, $AgCl/KCl_{sat}$ as a reference. A bare electrode fabricated from spectrally pure graphite (Ringsdorf Werke, Germany, 3 mm diameter) served as working electrode for electrode fouling and passivation tests. The electrode surface was preliminary treated by polishing, degreasing with alcohol, and ultrasonic cleaning. Further experiments for phenolic compounds determination applying pulsed amperometric detection were performed using the electrode from spectrally pure graphite, modified by spin-coating on its surface of 5 μL of a mixture prepared from chitosan and enzyme solutions in 1:1 ratio. The hydrogel film of chitosan with entrapped tyrosinase was formed after 60 min at ambient temperature. Chitosan cross-linking was achieved by dropping onto the film surface of 5 μL of glutaraldehyde 2.5% and allowing it to react for 10 min. The excess of glutaraldehyde was removed by rinsing the modified electrode with PBS pH 6.5. Then, a second enzyme-entrapped chitosan layer was formed following the same procedure.

Electrochemical measurements in combination with QCM measurements were accomplished using the model CHI400A series electrochemical quartz crystal microbalance (EQCM). The model includes a potentiostat/galvanostat CHI440A, an external box with oscillator circuitry, and a QCM cell. The density of the gold coated quartz crystals (13.7 mm crystal diameter, and 5.11 mm diameter of the gold disk) was 2.648 g $cm^{-3}$, and a shear modulus was $2.947\times10^{11}$ g $cm^{-1}$ $s^{-2}$. For the 8 MHz crystal, the mass change is 0.14 ng for 0.1 Hz frequency change. QCM cleaning procedure involved immersing of the crystal for 5 min into a 1:1:5 solution of $H_2O_2$ (30%), $NH_3$ (25%), and deionized water heated to a temperature of 75 °C, rinsing with deionized water, and drying. The gold electrode of the EQCM served as a working electrode. Pt wire was used as auxiliary electrode and Ag, $AgCl/KCl_{sat}$ as a reference.

Optical microscopy images were obtained using a XJP-H100 microscope (magnificacion 1000x), equipped with a

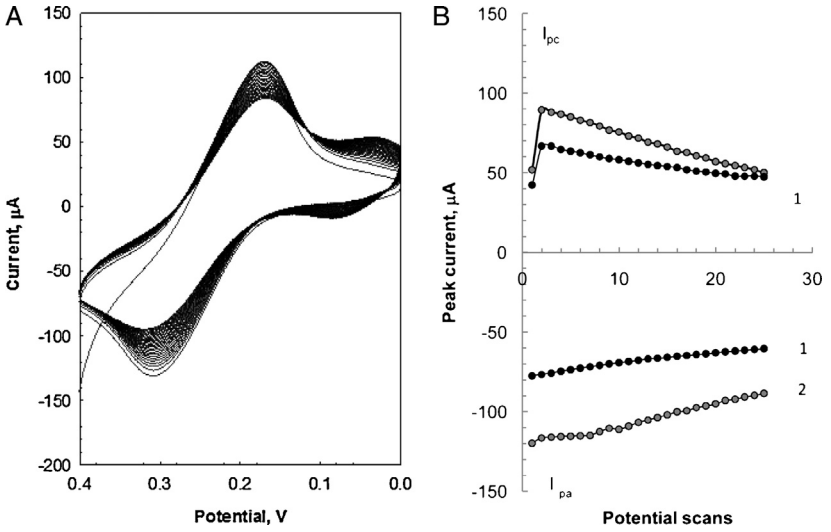


**Fig. 1.** (A) Cyclic voltammogram resulting of the reversible reduction and oxidation of quinone at a bare electrode from spectrally pure graphite. pH 6.5, 25°C, 25 potential scans, scan rate 0.1 V $s^{-1}$. (B) Cathodic peak current $I_{pc}$ and anodic peak current $I_{pa}$ dependence on the number of the successive potential scans and catechol concentration: 1) 2 mmol $L^{-1}$; 2) 3 mmol $L^{-1}$; PBS 0.1 mol $L^{-1}$.