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Amperometric tyrosinase based biosensor using an electropolymerized phosphate-doped polypyrrole film as an immobilization support. Application for detection of phenolic compounds

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ABSTRACT

An amperometric biosensor was constructed by immobilization of enzyme, tyrosinase, in an electrochemically synthesized phosphate ion-doped polypyrrole film on a Pt disk electrode. The tyrosinase maintains its bioactivity well within the polypyrrole thin film. A clearly defined reduction current proportional to the phenolic compounds concentration was observed in cyclic voltammetry, which attributed to the reduction of enzymatically produced quinone at the electrode surface.

Phenolic compounds were quantitatively estimated in aqueous medium by the direct electrochemical reduction of enzymatically liberated quinone species at −0.05 V by chronoamperometry. For the all six phenolic compounds analyzed, the kinetics of the enzymatic reaction fitted into a Michaelis–Menten type kinetics, as demonstrated by the h parameter close to 1 obtained from the Hill's plot.

The sensitivity followed the decreasing order catechol > phenol > 2-bromophenol > 2-chlorophenol > 2-iodophenol > 2-fluorophenol. The greater value of I_{max} and the lowest K_M^{app} was found for catechol.

The detection limits were in the range of 0.84–8.54 μM. The lowest detection limits were found for catechol and the highest for 2-fluorophenol.

1. Introduction

Phenolics constitute a large group of pollutants, which are widely distributed throughout the medical, food and environmental matrices [1–3]. They are used in variety of industrial processes such as manufacture of thermoset plastics, phenolic resins, polymer chemistry, wood industry, aerospace, building industry, automotive industry, abrasives, plasticizers, cleaning products, pesticide manufacturing, detergent applications, etc. [4,5]. They are major contaminants in ground and surface water [6,7]. Most of them are easily absorbed and have been shown to have detrimental effects on animal health [8].

In view of their high toxicity and persistence in the environment, the determination of phenolic compounds becomes an important subject.

Quantification of phenolics thus becomes significant and a number of methods have been developed such as spectrophotometric and chromatographic methods [9–11].

However, these analysis methods are relatively time-consuming, complex to perform, requiring samples pre-treatment, and may not be suitable for in situ monitoring. These disadvantages limit its practical applications.

Enzyme-based biosensors represent potential alternatives to these techniques. In particular, biosensors based on polyphenol oxidase (PPO) have been developed for the determination of phenolic compounds [12]. Tyrosinase (or polyphenol oxidase) is a binuclear copper metalloprotein that catalyzes two different reactions: hydroxylation of monophenols into diphenols and oxidation of diphenols to o-quinones [13].

A key factor in the construction of a biosensor is the need to achieve adequate and effective enzyme immobilization. Some of the common approaches that have been used for the immobilization of tyrosinase on to various substrates include carbon paste immobilization [14,15], sol–gel immobilization [16], physical adsorption [17], Langmuir–Blodgett thin films [18,19] and electrochemical entrapment of enzyme within polymer or composite matrix [20]. The use of conducting polymers for the fabrication of various biosensors have been studied extensively in last two decades due to their redox, optical, mechanical and electrical properties. Conducting polymer thin films have been investigated by our group as the sensing material for voltammetric electrodes in

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a variety of applications [21–23]. These polymers are unique in their capability to act both as transducers, as well as immobilization matrices for enzyme retention. In particular, polypyrrole has gained the most use for the fabrication of enzyme-based biosensors [20,24].

Electropolymerization of conducting copolymer is a simple process to construct biosensors. A sol–gel immobilization of tyrosinase followed by electropolymerization of pyrrole [25] or dip coating [24] has been reported for the fabrication of biosensors. Additionally, the direct electrochemical entrapment of tyrosinase during electropolymerization of pyrrole has been reported [20].

In this study, it has been investigated the use of cross-linking immobilization of tyrosinase into polypyrrole as a basis for fabrication of a stable amperometric biosensor for phenolic compounds. Cyclic voltammetry has been applied to study the detection of six phenolic compounds including phenol, catechol, 2-fluorophenol, 2-chlorophenol, 2-bromophenol, and 2-iodophenol. The response dependences and amperometric characteristics including sensitivity, kinetics, linear range, limits of detection and stability of the prepared enzyme electrode in the detection of phenolic compounds have been investigated.

2. Experimental

2.1. Materials

All the solutions were prepared using water purified in Millipore Milli-Q system. All chemicals were of analytical grade and used without further purification.

Mushroom tyrosinase (EC: 1.14.18.1 from mushroom, activity of 5370 U mg^{-1}, CAS 9002-10-2) was purchased from Sigma Chemical Co. (USA). A 4.5 mg mL^{-1} solution of tyrosinase in buffer phosphate 0.01 M (pH = 7) was used for the enzyme immobilization.

Solutions of phenolic compounds including phenol, catechol, 2-fluorophenol, 2-chlorophenol, 2-bromophenol, and 2-iodophenol were prepared by dissolving the corresponding compound in phosphate buffer 0.01 M (pH 7).

2.2. Apparatus

Electropolymerizations and electrochemical measurements were performed using an EG&G Princeton Applied Research potentiostat/galvanostat (Model 263). A three-electrode electrochemical cell (25 mL) with a thermostatic jacket (Metrohm) and a temperature-controlled liquid system (Neslab) were used. All the electropolymerizations were carried out at a constant temperature (20 °C), and the solutions were deoxygenated by bubbling nitrogen for 10 min prior to use. An $Ag/AgCl/KCl_{sat}$ electrode was used as reference electrode. All potentials reported are relative to the corresponding reference. The counter electrode was a large surface area platinum (2 cm^2 surface area) gauze, which was flamed prior to use.

2.3. Preparation of phosphate-doped polypyrrole film (PO_4–Ppy/Pt)

The polypyrrole film (0.78 mm^2) was electrochemically synthesized on a Pt disk electrode from an aqueous solution containing 0.1 M pyrrole and 0.1 M PBS, by chronopotentiometry at a fixed current of 60×10^{-6} A for 15 s. Once prepared, the modified polymeric electrodes were extracted from the synthesis solution and washed thoroughly. The thickness of the film obtained was about 2.7 μm as calculated from the injected charge [23,26].

2.4. Fabrication of enzyme electrode (Tyr/PO_4–Ppy/Pt)

The enzyme, tyrosinase (Tyr), was immobilized on the above electrochemically polymerized Ppy film by a casting technique followed by cross-linking. 5 μL of 0.01 M phosphate buffer (pH 7.0) containing 4.5 mg mL^{-1} of enzyme, tyrosinase, was added onto 0.78 mm^2 area of polymer film. After drying, the Tyr/PO_4–Ppy films were exposed to a 2.5% (v/v) glutaraldehyde solution (in phosphate buffer 0.01 M of pH 7) for 20 min at room temperature [27]. The enzyme-immobilized film was dried at 10 °C and rinsed with phosphate buffer solution thrice to remove any unbound enzyme from the polymer film surface and was further dried at 10 °C and stored at 4 °C.

3. Results and discussions

3.1. Optimization of polypyrrole electrosynthesis

The appropriate immobilization of the enzyme in solid substrates is crucial for the development of the biosensors. The structure of the film used for immobilize Tyr should contribute to the maintenance of enzyme functionality. In this study polypyrrole (Ppy) films were electrosynthetized in the presence of 0.1 M PBS (pH 7.0). The idea was to use a polypyrrole matrix doped with phosphate ions in order to minimize the doping-dedoping effect in the polymer during the electrochemical measurements.

It is well known that after polymerization, the backbone of the polymeric film exists in the conducting oxidized state. The overall charge of the polymer is neutral due to the doping anions which are incorporated into the polymeric matrix during the electropolymerization process [23]. By using the phosphate anion as doping agent and controlling the pH during polymerization and immobilization of enzyme could be expected that biosensor to provide a fast and convenient detection of phenolic compounds in an aqueous medium.

In order to prepare high reproducible polypyrrole films chronopotentiometry technique was used. The RSD (relative standard deviation) of polypyrrole preparation is lower than 5%.

The effect of the thickness in the voltammetric response of the biosensors was evaluated. Since film thickness is controlled by the electropolymerization time, different electrodes were fabricated varying the deposition time between 5 and 150 s.

The effect of film thickness on the biosensor responses is illustrated in Fig. 1 for a Tyr/PO_4–Ppy/Pt electrode immersed in 0.01 M PBS (dashed line), and in 0.01 M PBS containing 4×10^{-4} M catechol (solid line). The polymerization time was 150 s and the thickness of the film was about 27 μm.

As observed in the figure, at large deposition times, a high background current is obtained and the resolution of cathodic peak is reduced. Therefore, a reduced thickness is necessary for high quality performance biosensors. On the other hand, polypyrrole thin films have a reduced mechanical stability. So, an equilibrium between the thickness and sensibility must be found.

The optimal current for polypyrrole deposition was 60×10^{-6} A and the polymerization time was 15 s. The polymeric film obtained under these conditions has an optimal thickness for an efficient immobilization of the enzyme. At the same time, this thickness produces low capacitive currents, enhancing the sensitivity of the biosensor (Fig. 2).

Since immobilization material is an important aspect for the stability of the enzyme, conducting phosphate-doped polypyrrole porous film was used as a suitable matrix for enzyme immobilization.

The method used for tyrosinase immobilization consists in two steps: adsorption of enzyme within polypyrole film and cross-

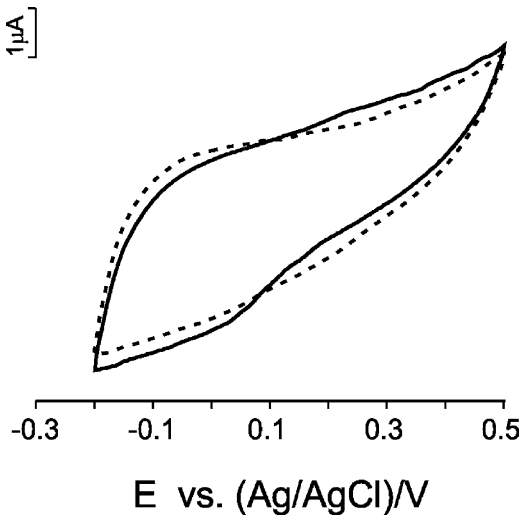


Fig. 1. CVs of Tyr/PO_4–Ppy/Pt electrode immersed in 0.01 M PBS (dashed line), and in 0.01 M PBS containing 4×10^{-4} M catechol (solid line). The thickness of the film was about 27 μm.

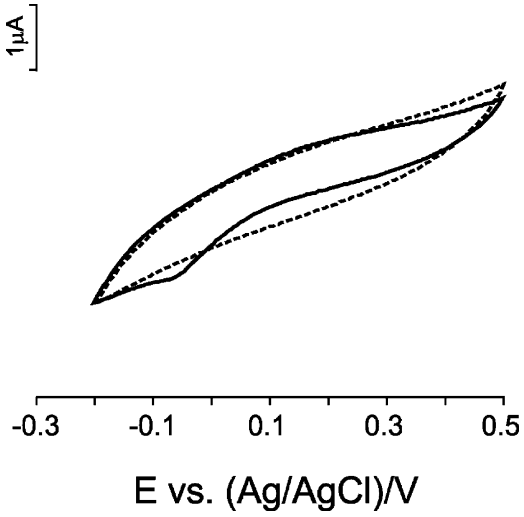


Fig. 2. Cyclic voltammograms of Tyr/PO_4–Ppy/Pt electrode in the absence (dashed line) and in the presence of 4×10^{-4} M catechol (solid line). The thickness of the film was about 2.7 μm.

linking with glutaraldehyde. The optimal conditions presented in experimental part were obtained considering a high signal-noise ratio, and stability of biosensor response. The idealized structure of biological active layer of biosensor is presented in Scheme 1.

3.2. Cyclic voltammograms for phenolic compounds at the Tyr/PO_4–Ppy/Pt electrode

Cyclic voltammograms of the PO_4–Ppy/Pt electrode immersed in catechol solution did not present any peak in the potential range studied (Fig. 1). Similarly, no redox activity was observed for all phenolic compounds under study. Thus, for detection of such compounds immobilization of Tyr within polypyrrole film is necessary.

Tyrosinase catalyzes the oxidation of phenol and various phenol derivatives (Phenol-R) to o-quinone derivatives via catechol derivatives (Catechol-R) [28], as shown in Eqs. (R1) and (R2).

$$\text{Phenol-R} + \text{Tyrosinase}(O_2) \rightarrow \text{Catechol-R} \quad \text{(R1)}$$

$$\text{Catechol-R} + \text{Tyrosinase}(O_2) \rightarrow \text{o-Quinone-R} + H_2O \quad \text{(R2)}$$

$$\text{o-Quinone-R} + 2H^+ + 2e^- \rightarrow \text{Catechol-R} \quad \text{(R3)}$$

Therefore, the generated o-quinones can be reduced electrochemically at low potential without any mediator (Eq. (R3)). Phenolic compounds can be detected by electrochemical reduction of the o-quinones.

Fig. 2 shows the CV of the Tyr/PO_4–Ppy/Pt electrode before and after addition of catechol in 0.01 M PBS (pH 7). In the absence of catechol, only a relative low background current was observed; after addition of catechol to the PBS, the CV gave a well defined peak locating at −0.05 V, which was due to the reduction of o-quinone species formed from the enzymatic reaction on the electrode surface.

This result is in good agreement with results published using tyrosinase-polypyrrole based sensors where the cathodic peak corresponding to the reduction o-quinone appears at ca. +0.05 V vs. Ag/AgCl [24].

In the presence of phenol (10^{-2} M) in PBS, the CV curves gave a well-defined peak located at −0.05 V. This peak was due to the reduction of o-quinone species liberated from the enzymatic reaction on the electrode surface (Fig. 3).

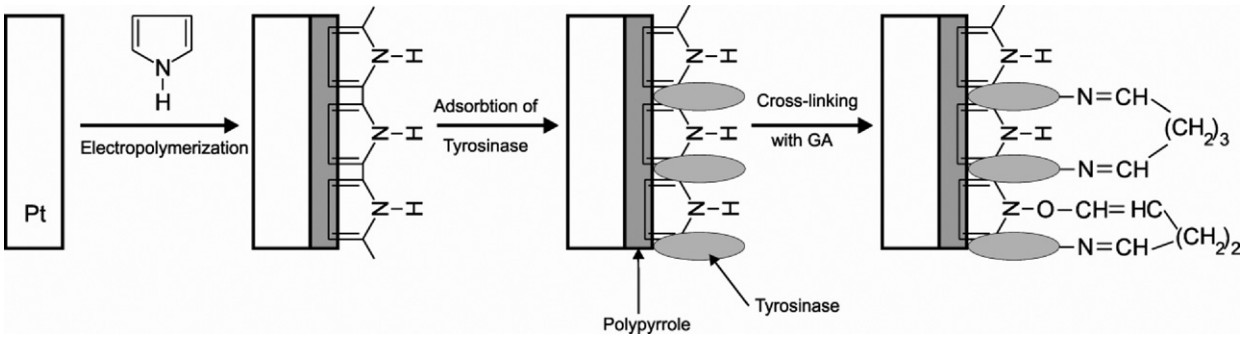
As observed in Fig. 4, the cyclic voltammogram of Pt/Ty electrode immersed in 10^{-4} M catechol presents only a small activity in the potential range studied.

These results demonstrate that the tyrosinase enzyme retains its bioactivity to a large extent when immobilized on phosphate-doped polypyrrole film.

3.3. Optimization of the experimental variables

The modification of biosensor response on the applied potential was investigated over a potential range of −0.2 to +0.5 V, using 4×10^{-4} M catechol in 0.01 M phosphate buffer (pH 7.0).

The effect of applied potential for the Tyr/PO_4–Ppy/Pt electrode on the amperometric signal and background current is shown in Fig. 5.



Scheme 1. The scheme of fabricating biological active layer.

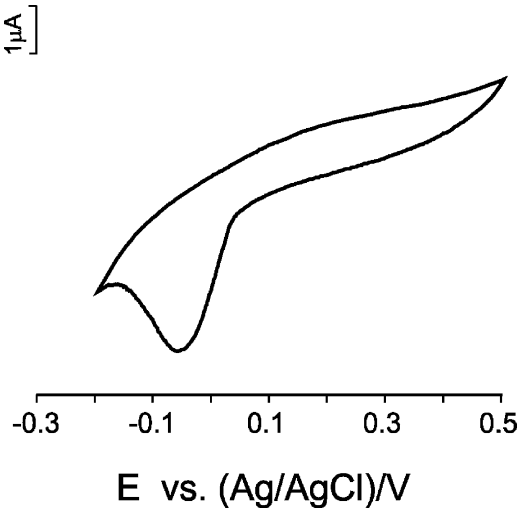


Fig. 3. Cyclic voltammogram of biosensor in 10^{-2} M phenol (pH 7.0). Scan rate 0.02 V s^{-1}.

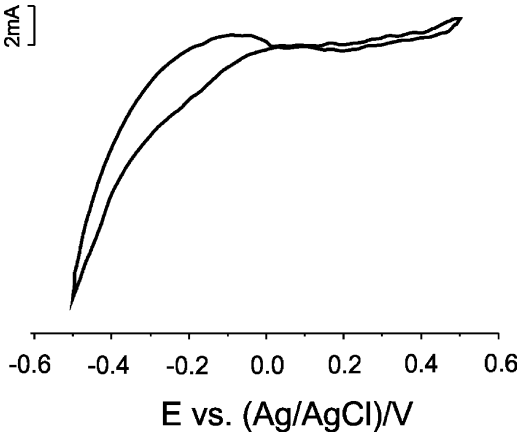


Fig. 4. Cyclic voltammogram of Pt/Ty electrode immersed in 10^{-4} M catechol. Scan rate 0.05 V s^{-1}.

The maximum of the signal vs. background current is obtained at −0.05 V. When applied potential more negative than −0.05 V, a higher current is achieved, but background current increases more rapidly because of possible reduction of dissolved oxygen. Therefore, −0.05 V was used as the applied potential. This potential is favorable, since only some chemical species expected to be present in samples are reduced at such a low potential [29,30].

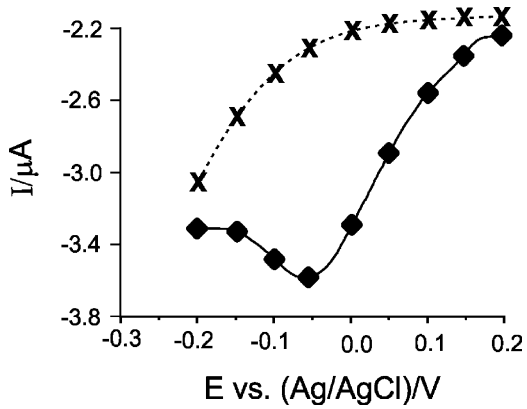


Fig. 5. Current–potential curves in 0.01 M PBS (pH 7.0) without (dashed line) and with (solid line) 4×10^{-4} M catechol under stirring.

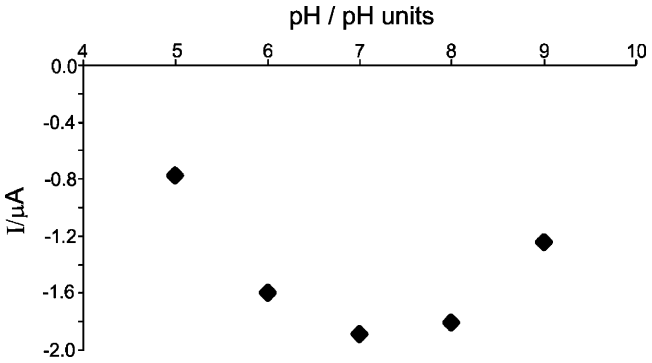


Fig. 6. The effect of pH value in 0.01 M PBS containing 4×10^{-4} M catechol. Applied potential: −0.05 V.

The performance of the Tyr/PO_4–Ppy/Pt electrode is affected by the pH value of solution due to the participation of protons in the enzymatic reaction. The effect of pH for the Tyr/PO_4–Ppy/Pt electrode in 0.01 M PBS containing 4×10^{-4} M catechol is shown in Fig. 6.

The reduction current increases slightly as the pH changing from 5.0 to 7.0, and then decreases gradually from 7.0 to 9.0. The current achieves the maximum value at a pH of 7. This pH value is in accor-

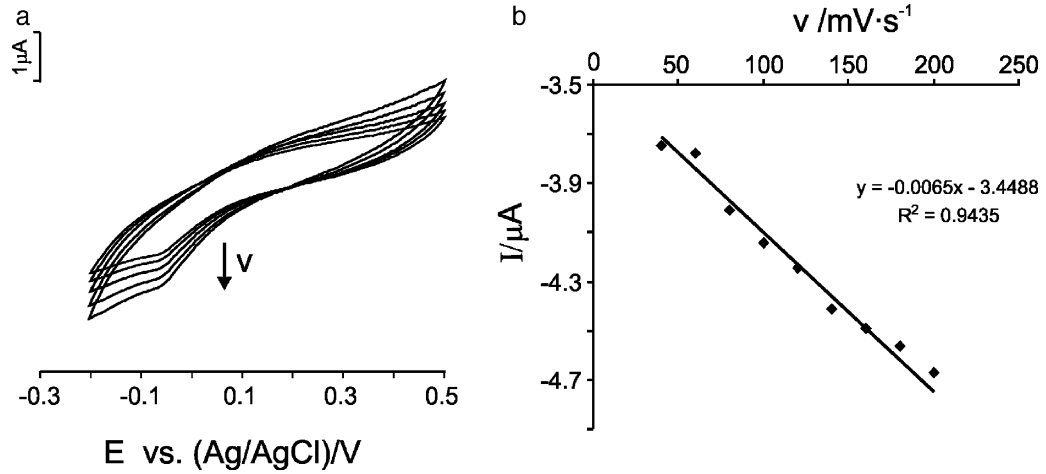


Fig. 7. (a) Cyclic voltammograms of biosensor in 4×10^{-4} M catechol (pH 7.0) registered with different scan rates (0.03–0.21 V s^{-1}). (b) Plot of I_{pc} vs. V.

dance with the pH at which the enzymatic activity is maximum in solution [31].

To obtain the maximum response, a pH of 7.0 for the PBS was selected for the following studies.

3.4. Kinetic of the biosensor

Kinetic studies were performed by registering the cyclic voltammograms of the Tyr/PO_4–Ppy/Pt biosensor at different scan rates, from 0.02 to 0.20 V s^{-1} (Fig. 7). The peak currents were proportional to sweep rates (linear equation of the plot was $y = -0.0065x - 3.4488$; $R^2 = 0.9435$), pointing to a charge transfer limited process (due to the electrochemical activity of the enzyme deposited in the surface of the electrode). From the slope of this line and using the Laviron equation:

$$I_p = \frac{n^2F^2\nu A\Gamma}{4RT}$$

where Γ is the surface coverage of the redox species (o-quinone) (mol cm^{-2}), A is the electrode area (cm^2), ν is the potential sweep rate and n, I_p, F, R and T have their usual meanings [32], the total surface coverage calculated was 9.83×10^{-9} mol cm^{-2}.

3.5. Amperometric response of the Tyr/PO_4–Ppy/Pt electrode

Fig. 8a illustrates a typical amperometric response for the Tyr/PO_4–Ppy/Pt electrode at −0.05 V after the addition of successive aliquots of phenolic compounds to the 0.01 M PBS (pH 7.0) under constant stirring. A well-defined reduction current proportional to the concentration of phenolic compounds is observed, which results from the electrochemical reduction of o-quinone species enzymatically formed.

The Tyr/PO_4–Ppy/Pt electrode achieves 95% of steady-state current in less than 5 s. The response rate is much faster than that of 10 s reported in a conducting polymer film [33] and 50 s reported in the pure silica sol–gel matrix [29]. Such fast response is attributed to a rapid electron transfer between the enzymatically produced quinone and the PO_4–Ppy/Pt electrode.

3.6. Effect of catechol concentration

Fig. 8b shows the relationship between the response current of the biosensor and the catechol concentration in PBS (pH 7.0) at −0.05 V (calibration curve).

It can be seen from Fig. 8c that the response current is linear with catechol concentration in the range from 10 to 120 μM, indicating that the enzyme catalytic reaction of Tyr is the first-order reaction. Then, with further increasing catechol concentration, the current increases slowly, and the enzyme reaction shows a transition from first to zero-order. The sensitivity of the biosensors is 0.047 μA μM^{-1}. The corresponding detection limits were calculated according to the $3s_b/m$ criterion, where m is the slope of the calibration graph, and s_b was estimated as the standard deviation ($n = 7$) of the voltammetric signals from different solutions of the substrate at the concentration level corresponding to the lowest concentration of the calibration plot. The detection limits calculated were 0.84 μM. The value obtained is lower than that reported by Rajesh et al. (1.5 μM) [24] and slight lower than that obtained by Ameer and Adeloju (1 μM) [20]. So the biosensor can satisfy the requirements of practical applications.

From the calibration data, the Hill coefficient (h) can be calculated by representing the $\log[I/(I_{max} - I)]$ vs. $\log [S]$ (the logarithm of substrate concentration). A Hill coefficient of 1.09 was calculated for the reduction process of o-quinone formed from the enzymatic reaction on the electrode surface ($R^2 = 0.952$). The value obtained for the h parameter, calculated from the corresponding Hill's plot,

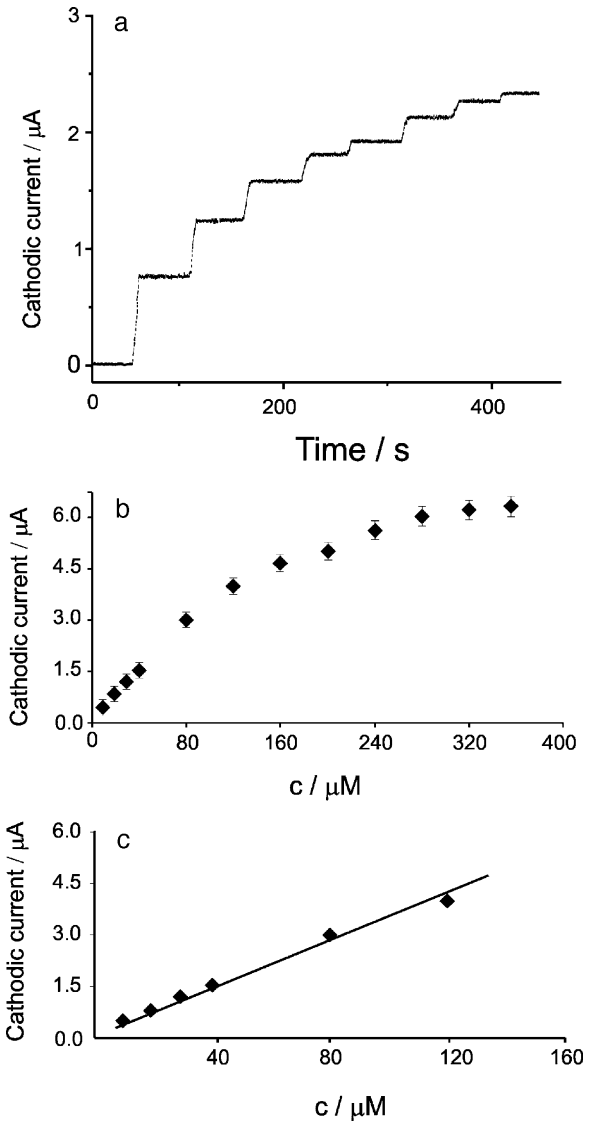


Fig. 8. (a) Amperometric response of tyrosinase biosensor to catechol in 0.1 M PBS solution (pH = 7). Applied potential −0.05 V. (b) The calibration curve between the reduction current and the concentration of catechol in PBS (pH 7.0) at −0.05 V. (c) Plot of the intensity of reduction current vs. the catechol concentration in the linear range (10–120 μM).

was close to unity demonstrated that the kinetics of the enzymatic reaction fitted into a Michaelis–Menten type kinetics [34]. The value, slightly higher than 1 demonstrate a positive cooperative effect between the occupied active sites.

The apparent Michaelis–Menten constant (K_M^{app}) is calculated for the immobilized Tyr by the amperometic method as suggested by Shu and Wilson [35].

The kinetic parameters may be related through Eq. (1) and were obtained using the linearization of Lineweaver–Burk expressed by Eq. (2):

$$I = \frac{I_{max}[S]}{[S] + K_M^{app}} \quad (1)$$

$$\frac{1}{I} = \frac{1}{I_{max}} + \frac{K_M^{app}}{I_{max}[S]} \quad (2)$$

where $[S]$ is the concentration of the oxidized substrate, I is the cathodic current at −0.05 V, K_M^{app} is the apparent Michaelis–Menten constant for the enzymatic reaction and I_{max} is the steady-state current.

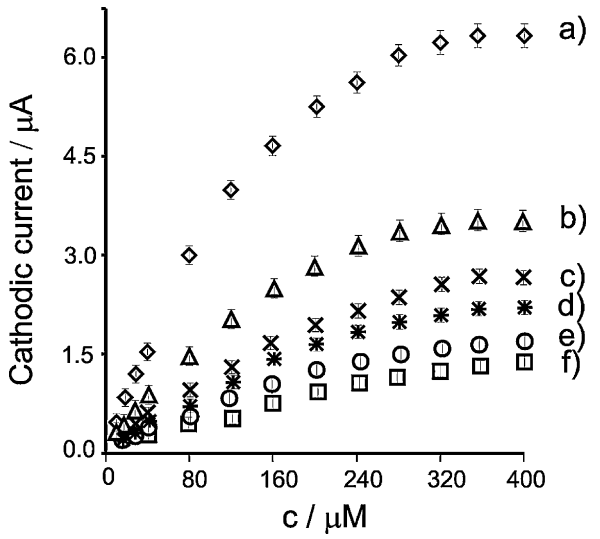


Fig. 9. The calibration curves between the reduction current and the concentration of different phenolic compounds: (a) catechol; (b) phenol; (c) 2-bromophenol; (d) 2-chlorophenol; (e) 2-iodophenol; (f) 2-fluorophenol.

Table 1
Response characteristics of the Tyr/PO_4–Ppy/Pt biosensor to phenolic compounds.

Compound	h	Sensitivity/μA μM^{-1}	LD/μM	I_{max}/μA	K_M/μM
Phenol	1.00	0.023 ± 0.0011	1.71	3.62	116.7
2-Fluorophenol	0.99	0.0046 ± 0.0002	8.54	1.51	240.2
2-Chlorophenol	0.99	0.007 ± 0.0003	5.61	2.43	173.5
2-Bromophenol	0.94	0.0075 ± 0.0003	5.24	2.92	160.2
2-Iodophenol	0.94	0.005 ± 0.0002	7.86	1.75	198.9
Catechol	1.09	0.047 ± 0.0022	0.84	6.25	80.2

The maximum current response (I_{max}) and apparent Michaelis–Menten constant K_M^{app} can be calculated from the intercept and slope. The I_{max} is 6.25 μA, with K_M^{app} = 80.2 μM. The K_M^{app} is lower but comparable to that obtained by Arslan et al. [36] or Li et al. [29] using catechol as the substrate. According to the inherent characteristic of Michaelis–Menten constant (K_M^{app}), the less the value of K_M^{app}, the stronger will be the affinity between Tyr and substrate. The biosensor with lower K_M^{app}, can detect both low and high concentrations [37]. This indicates that the Tyr/PO_4–Ppy/Pt biosensor makes it possible to obtain a wide linearity range for the measurements of catechol concentration.

3.7. Sensing properties of the Tyr/PO_4–Ppy/Pt biosensor towards different phenolic compounds

Similar studies were carried out in different phenolic compounds, with the results for catechol just presented used for comparison.

Fig. 9 shows the calibration curve of Tyr/PO_4–Ppy/Pt biosensor to phenol, catechol, 2-fluorophenol, 2-chlorophenol, 2-bromophenol, and 2-iodophenol, respectively. A clearly defined reduction current proportional to the phenolic compounds concentration was observed, which attributed to the reduction of enzymatically produced quinone at the electrode surface.

The kinetic parameters and the analytical characteristics listed in Table 1. As can be observed, for all six phenolic compounds analyzed, the kinetics of the enzymatic reaction fitted into a Michaelis–Menten type kinetics, as demonstrated by the *h* parameter close to 1 obtained from the Hill's plot.

Studies of the dependence of substrate reduction showed that K_M^{app} varied with the nature of the substrate. In general, lower K_M^{app} values were obtained for the phenolic compounds exhibiting a higher sensitivity.

The sensitivity followed the decreasing order catechol > phenol > 2-bromophenol > 2-chlorophenol > 2-iodophenol > 2-fluorophenol. The greater value of I_{max} and the lowest K_M^{app} was found for catechol. These results are in accordance with results reported in the literature for other tyrosinase biosensor designs [38,39], thus revealing a good affinity of the enzyme immobilized on polypyrrole film for these substrates. The difference in sensitivity between each phenolic compound might depend on the hydrophobic characteristics of the immobilized matrix [38,40] and molecular hindrance.

The detection limits were in the range of 0.84–8.54 μM. The lowest detection limits were found for catechol and the highest for 2-fluorophenol.

3.8. Sensor stability and storage conditions

The Tyr/PO_4–Ppy/Pt biosensor was also studied for the enzyme stability both at room temperature as well as in refrigerated conditions. The stability of the enzyme was monitored with time to a sample of 50 μM catechol. A very slow decreasing in response current was obtained up to a period of 1 month. It was observed that the Tyr/PO_4–Ppy/Pt biosensor retained 80% of initial enzyme activity for 1 month, when stored at 4 °C in a refrigerator. This long-term stability of the Tyr/PO_4–Ppy/Pt biosensor is more than the recently reported literatures (<15 days) [41]. However, at room temperature (ca. 25 °C) the electrode loosed about 70% of its initial enzyme activity within 10 days and thereafter showed a rapid enzyme inactivation.

3.9. Application to real samples

Real environmental water samples were analyzed with Tyr/PO_4–Ppy/Pt biosensor to evaluate its real feasibility. A simple sample pretreatment was carried out. The water samples were mixed with 0.1 M phosphate buffer solution pH = 7.0 to its final concentration of 0.01 M.

The phenolic content of two environmental water samples (collected along the course of Danube River near Galaţi city) was estimated by standard addition analysis.

The uncertainty of phenol concentration (c) was calculated using the Eq. (3) [42]:

$$u(c, Q) = \frac{S_{xy}}{a} \cdot \sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(c - \bar{c})^2}{\sum_{i=1}^{n}(c_i - \bar{c})^2}} \quad (3)$$

where S_{xy} is residual standard deviation, p is number of repetitions (2 in this case), n is number of calibration points (5 in this case), $\bar{c}$ is the mean of all concentration values of the calibration experiment and a is a slope of calibration curve.

The mean content of phenolic compounds, expressed as phenol concentration, was found to be 176.3 ± 3.6 μg L^{-1}.

To perform the recovery study environmental water sample was spiked with 100 μg L^{-1} of phenol and the sample was analyzed. For this sample phenol concentration was found to be 282.1 ± 4.4 μg L^{-1}. Based on these concentration values, the recovery factor was found to be 105.8%.

The results suggest that developed biosensor can be successfully used for the determination of natural waters contaminants at low concentration levels.

4. Conclusion

This study has demonstrated the feasibility of developing a conducting polypyrrole based biosensor for monitoring phenolic

compounds in aqueous medium. Additionally, it has confirmed that the conducting polypyrrole having phosphate dopant anion inserted over it, can be utilized as a suitable matrix for the immobilization of enzyme, tyrosinase. The biosensor exhibits fast response, high sensitivity and stability for the amperometric detection of phenolic compounds because of the high loading of tyrosinase and the rapid electron transfer between the enzymatically produced quinones and the prepared electrode. The biosensor exhibited high sensitivity and stability for the amperometric detection of phenolic compounds. Possible applications of this type of biosensor include environmental and industrial monitoring.

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