Available online at www.sciencedirect.com

SCIENCE DIRECT

Bioelectrochemistry 64 (2004) 47–52

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Phenols monitoring and Hill coefficient evaluation using tyrosinase-based amperometric biosensors

Sarmiza Elena Stanca[a,*,1], Ionel Catalin Popescu[b]

[a] *Department of Physics, Chemistry and Informatics, Faculty of Environmental Protection, University of Oradea, 3700 Oradea, Romania*
[b] *Department of Physical Chemistry, University "Babes-Bolyai", 400028 Cluj-Napoca, Romania*

Received 14 December 2002; received in revised form 11 November 2003; accepted 16 February 2004
Available online 26 April 2004

Abstract

Sensitive amperometric biosensors for phenols compounds, based on tyrosinase (polyphenoloxidase, PPO) immobilized on a Pt electrode in an electropolymerized poly-amphiphilic pyrrole matrix or cross-linked with glutaraldehyde, were constructed and compared. Steady-state amperometric measurements, performed at −50 mV vs. SCE in aqueous phosphate buffer containing $LiClO_4$ 0.1 M (pH 7) as well as in a chloroform solution containing 0.1 M $C_6H_5CH_2N(CH_3)_3Cl$, were used in order to compare the electroanalytical and kinetic parameters of the investigated amperometric biosensors in aqueous and nonaqueous media. It was established that the polypyrrole matrix has a higher efficiency for enzyme retention resulting in higher bioelectrode sensitivity, both in aqueous buffer (690 μA M^{-1}) and in chloroform (149 μA M^{-1}).

Keywords: Phenols; Tyrosinase; Amperometric biosensors

1. Introduction

Recently, a major progress in biomolecules immobilizing techniques, based on their integration in suitable polymers, resulting in robust and functional matrix has been registered [1–24]. The interest was strongly focused on the development of modified electrodes with a compact structure in order to preserve the enzyme activity and to promote the electronic and/or the mass transfer. For this purpose, three categories of polymers have been used to obtain the biomolecule matrix: electronic conducting polymers [8–17], ions exchanger polymers [22–25] and redox polymers [22,23,26]. Among them, the most studied and intensively reviewed are the conducting polymers [7,8] such as polyacetylene, polypyrrole, polyaniline; polytiophenole, poly(*p*-phenylenvinil), polytiophene, poly(*N*-methylpyrrole), which can be efficiently prepared "in situ" by electrochemical oxidizing of the monomer at the electrode surface. It is noteworthy to mention that if the electropolymerization potential is too high a polymer over-oxidation could occur, inducing a loss of the polymer conductivity. Nevertheless, in these conditions the obtained polymer has a porous structure allowing a better substrate diffusion toward the working electrode [23]. Consequently, if the applied potential is carefully chosen, the optimal matrix conductivity and porosity could be obtained.

On the other hand, according to Cosnier and Innocent [1] and Cosnier and Popescu [2], the usual technique of enzyme entrapping into conducting polymers matrix has as major drawback the necessity of using high enzyme concentrations in the electrolyte solution. Hence, the idea to compare the bioelectroanalytical and kinetic parameters of two biosensors, one obtained by electropolymerization of a small amount of pyrrole monomer–enzyme mixture deposited on the electrode surface [1], and the other obtained by enzyme cross-linking using glutaraldehyde as cross-linking agent, was examined in this work. For this purpose, tyrosinase (polyphenoloxidase, PPO) was selected as enzyme and the behavior of resulting biosensors was compared in respect of their bioanalytical parameters in aqueous and non-aqueous (chloroform) media.

* Corresponding author. Present address: Department of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland. Tel.: +353-1-716-2444; fax: +353-1-716-2127.
E-mail addresses: sarmiza.stanca@ucd.ie, stanca@rdslink.ro (S.E. Stanca).
[1] Fax: +40-259-43278.

1567-5394/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2004.02.004

The applicability of this kind of bioelectrode is extended toward detection of some important analytes, which act as enzyme inhibitors, and provided deeper information about the interaction mechanism between substrate and enzyme. In this work, an investigation of enzyme inhibition of the benzoic acid in aqueous and chloroform media has been attempted in order to estimate the hydrophobic solvent effect. Thus, measuring the current intensity depletion due to the solvent effect, and using Hill equation (Eq. (1)) [27], a deviation from the ideal case of Michaelis–Menten kinetic can be estimated by the difference between the experimental value of x and its theoretical value (equal to 1):

$$\log \frac{\Delta i}{i_{ss} - \Delta i} = x \log \Delta i \quad (1)$$

where Δi stands for the current intensity depletion under inhibitor influence (A), and i_{SS} is the steady-state intensity current corresponding to a given substrate concentration.

2. Experimental

2.1. Reagents

Tyrosinase (EC 1.14.18.1, from mushroom, 4200 Sigma units/mg) was purchased from Sigma. Phenol, glutaraldehyde, chloroform, benzoic acid, KH_2PO_4, K_2HPO_4, $LiClO_4$, 1,2-dihydroxybenzene, 1,3-dihydroxybenzene, 1,4-dihydroxybenzene, 2-amino-3(4-hydroxyphenyl)propionic acid, 2-hydroxytoluene, 3-hydroxytoluene, 4-hydroxytoluene, 2-clorophenole, 3-clorophenole, 4-clorophenole, 4-hydroxybenzoic acid were obtained from Merck and used as received. The amphiphilic pyrrole derivative, [12-(pyrrole-1-yl)dodecyl]triethylammonium tetrafluoroborate], was generously supplied as a gift by Dr. Serge Cosnier, LEOPR, University J. Fourier, Grenoble (France).

$LiClO_4$ (0.1 M) and phosphate buffer (0.1 M) (pH 7, obtained by mixing of the corresponding volumes of 0.1 M KH_2PO_4 and 0.1 M K_2HPO_4) were used as supporting electrolytes in aqueous solutions employed for pyrrole electropolymerisation and for amperometric measurements, respectively. $C_6H_5CH_2N(CH_3)_3Cl$ (0.1 M), supplied by Aldrich, was employed as supporting electrolyte for voltammetric and amperometric measurements in chloroform solutions.

2.2. Electrochemical measurements

All measurements were performed using a computer-assisted potentiostat (Autolab-PGSTAT-10, Eco Chemie, Utrecht, The Netherlands), connected to a conventional electrochemical cell equipped with three electrodes. The bioelectrode was the working electrode. In both aqueous and non-aqueous experiments, a saturated calomel electrode (SCE) was used as reference electrode and a Pt foil as counter electrode.

Steady-state amperometric measurements were done as follows: the bioelectrode was immersed in 10 ml of testing solution (aqueous or non-aqueous) at room temperature, and poised at the desired value of the applied potential. When the recorded signal attained a stable value, a known volume of standard solution of substrate (phenol) was added under a vigorous stirring. Subsequently, the signal variation corresponding to the reduction of enzymatically produced o-quinone was recorded for 1–2 min. Thus, the calibration curve was constructed by means of successive additions of small volumes of standard aqueous solution of the substrate.

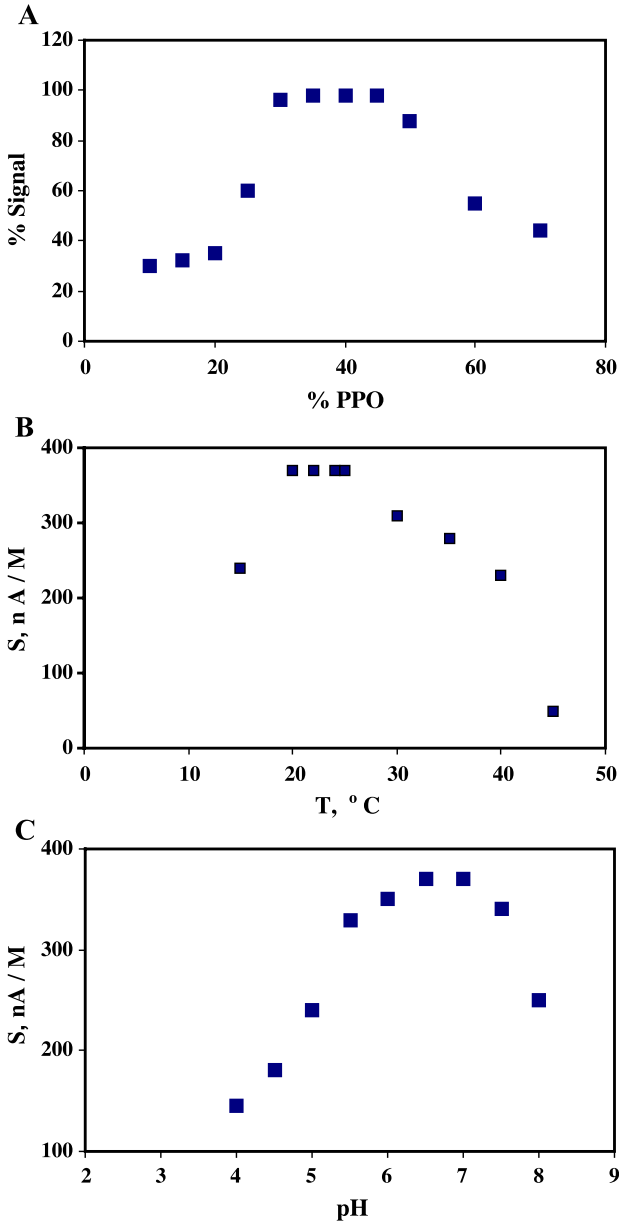

Fig. 1. The dependence of the Pt/pPy–PPO bioelectrode response to phenol on enzyme loading (A), temperature (B) and pH (C). Experimental conditions: applied potential, −0.05 V vs. SCE; phenol concentration, 0.1 mM; supporting electrolyte, 0.1 M phosphate buffer containing 0.1 M $LiClO_4$ (pH 7).

Before using the bioelectrode was kept at 5 °C in a humid atmosphere. The procedure presented above was repeated unchanged in all tests carried out using the amperometric bioelectrode in both aqueous and chloroform solutions.

2.3. The bioelectrode preparation

2.3.1. Pt/pPy–PPO bioelectrode

The technique of enzyme entrapment into a polypyrrole matrix consisted in the electro-polymerization of the amphiphilic pyrrole monomer, after the adsorption on electrode surface of a mixture of enzyme and monomer [1]. Thus, 2.5 mg of monomer were ultrasonically dispersed in 1 ml of distillated water and 3 mg of PPO was added per ml of dispersion. A volume of 10 μl from the above-described mixture was deposited on a Pt disk electrode (3-mm diameter) and the water was removed by keeping the coated electrode during 2 h under reduced pressure. Finally, the monomer–enzyme film was potentiostatically electropolymerized in a 0.1 M $LiClO_4$ aqueous solution at +0.7 V/SCE.

2.4. Pt/pPy–(PPO–GA) and Pt/(PPO–GA) bioelectrodes

On a polypyrrole derivative modified Pt electrode, prepared following the above described technique excepting the presence of the enzyme, 5 μl of PPO (2.5 mg/ml) with 10 μl glutaraldehyde (0.1 M) was successively deposited two times [24]. Similarly, 10 μl of PPO (2.5 mg/ml) mixed with 10 μl glutaraldehyde (0.1 M aqueous) were dropped on a naked Pt electrode.

3. Results and discussion

In order to optimize the behavior of the PPO entrapped in amphiphilic polypyrrole based bioelectrode, its steady-state amperometric response to phenol was recorded at different enzyme loading. As it can be observed from Fig. 1A, an enzyme loading higher than 50% (w/w) was not productive. Consequently, all further experiments were carried out with bioelectrodes having 35% enzyme loading.

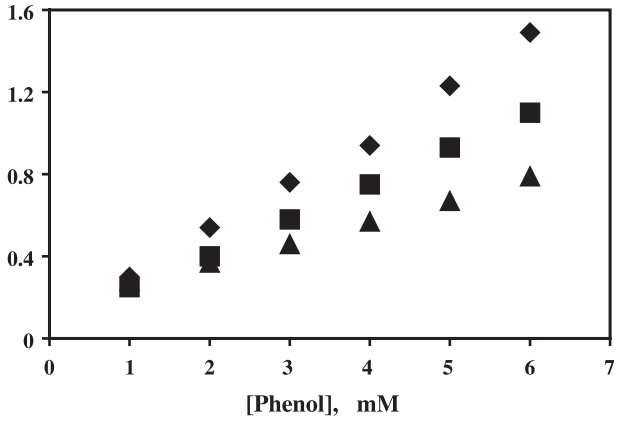

Fig. 2. The calibration curves for phenol of Pt/pPy–(PPO–GA) (▲) (R=0.9944), Pt/(PPO–GA) (■) (R=0.9994) and Pt/pPy–PPO (◆) (R=0.9916) bioelectrodes in aqueous buffer. Experimental conditions: working potential, −50 mV/ESC; supporting electrolyte, 0.1 M phosphate buffer containing 0.1 M $LiClO_4$ (pH 7); stirred solutions; t=21 °C.

The temperature dependence of the biosensor response, showed in Fig. 1B, revealed that the optimum temperature range for enzyme activity was between 20 and 25 °C. In order to decrease as much as possible the enzyme thermal denaturation, the temperature of 21 °C was chosen as working temperature for all further investigations. The pH influence on the phenol biosensor response was investigated in the pH range 5.0–8.0. As can be seen from Fig. 1C, the optimal pH range is 6.5–7.5; outside this interval a significant decrease of the biosensor signal was observed.

The biosensor selectivity for different phenolic compounds detection was tested measuring its response in aqueous solutions of phenole, 1,2-dihydroxybenzene, 1,3-dihydroxybenzene, 1,4-dihydroxybenzene, 2-amino-3 (4-hydroxyphenyl) propionic acid, 2-hydroxytoluene, 3-hydroxytoluene, 4-hydroxytoluene, 4-clorophenol, 3-clorophenol, 2-clorophenol, 4-hydroxybenzoic acid (Table 1).

The calibration curves, recorded by successive additions of standard phenol solution in aqueous buffer, corres-

Table 1
The selectivity of Pt/pPy–PPO biosensor

Phenolic compounds	Relative signal (%)
Phenol	100
1,2-Dihydroxybenzene	200 ± 2.50
1,3-Dihydroxybenzene	0
1,4-Dihydroxybenzene	1 ± 0.01
2-Amino-3(4-hydroxyphenyl)propionic acid	50 ± 0.4
2-Hydroxytoluene	1 ± 0.02
3-Hydroxytoluene	125 ± 2.1
4-Hydroxytoluene	160 ± 2.40
4-Clorophenol	20 ± 0.25
3-Clorophenol	12 ± 0.14
2-Clorophenol	1 ± 0.01
4-Hydroxybenzoic acid	2.2 ± 0.04

Experimental conditions: applied potential, −0.05 V vs. SCE; phenolic derivatives concentration 0.1 mM; 0.1 M phosphate buffer containing 0.1 M $LiClO_4$ (pH 7); t=21 °C. The signal corresponding to 100% was a current intensity of 43 ± 0.02 μA.

Table 2
Sensitivity to phenol registered with three different bioelectrodes in aqueous buffer (0.1 M phosphate buffer containing 0.1 M $LiClO_4$, pH 7) and in chloroform (0.1 M $C_6H_5CH_2N(CH_2)_3Cl$)

Solvent	Sensitivity (μA/M)			Linear range (mM)	No. of exp. points
	Pt/pPy–PPO	Pt/pPy–(PPO–GA)	Pt/(PPO–GA)		
H_2O	690 ± 15	331 ± 12	69 ± 2	up to 7	10
$CHCl_3$	149 ± 8	69 ± 3	95 ± 5	up to 2.5	10

Experimental conditions: applied potential, −50 mV vs. SCE; temperature 21 °C; stirred solutions.

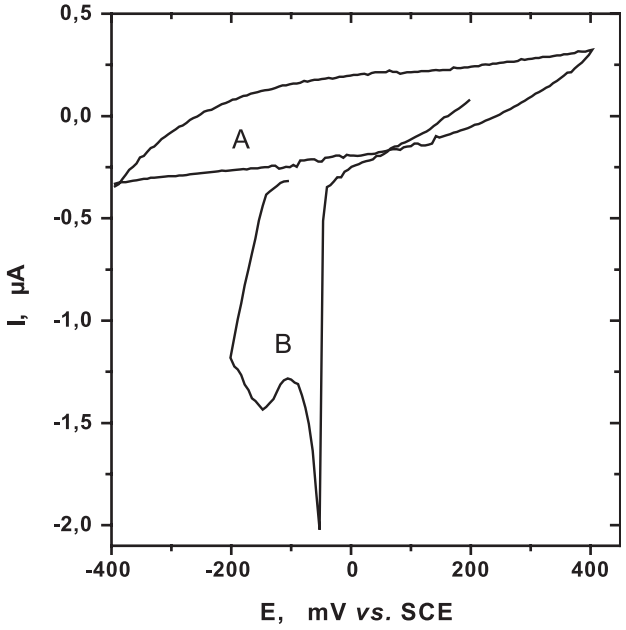

Fig. 3. Cyclic voltammograms recorded at Pt/pPy–PPO bioelectrode in chloroform in the absence of phenol (A) and in the presence of 0.1 mM phenol (B). Experimental conditions: potential scan rate, 50 mV/s; starting potential, −400 mV vs. SCE (A) and +200 mV vs. SCE (B).

ponding to the three different bioelectrodes (Fig. 2) showed a linear response up to 7 mM and a sensitivity strongly depending on the bioelectrode type (Table 2). The Pt/pPy–PPO bioelectrode was one order of magnitude more sensitive than Pt/(PPO–GA) bioelectrode, and two times more sensitive than the Pt/pPy–(PPO–GA) bioelectrode.

As previously reported [2], the phenol amperometric detection can be accomplished by applying a potential of −0.2 V vs. SCE to detect amperometrically the biocatalytically generated *o*-quinone. Additionally, it was shown that using $[Fe(CN)_6]^{4-}$ as mediator it is possible to obtain a significant increase of the bioelectrode selectivity by using a

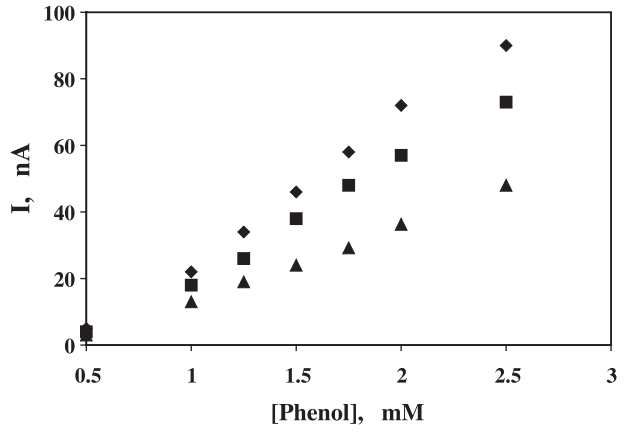

Fig. 4. Calibration curves for phenol of Pt/pPy–(PPO–GA) (■) ($R=0.9995$), Pt/(PPO–GA) (▲) ($R=0.9993$) and (♦) Pt/pPy–PPO bioelectrodes ($R=0.9968$) in chloroform containing 0.1 M $C_6H_5CH_2N(CH_2)_3Cl$. Experimental conditions: applied potential, −50 mV vs. SCE; stirred solutions; temperature 21 °C.

Table 3
The parameters of Lineweaver–Burk plots for the inhibited and uninhibited response to phenol of the Pt/pPy–PPO biosensor

Response type	Slope (M/μA)	$1/i_{max}$ (nA^{-1})	Regression coefficient/number of experimental points
Inhibited (benzoic acid)	2.678×10^{-6}	0.0175	0.9965/12
Uninhibited	1.440×10^{-6}	0.0178	0.9908/12

lower value of the applied potential (−0.05 V vs. SCE), well placed in the optimal domain of the amperometric detection [24]. Operated in this manner, the optimized bioelectrode developed a well-shaped Michaelis–Menten response, proven by the excellent agreement between the experimental data and the calculated curve (results not shown).

As expected [28,29], cyclic voltammetry measurements performed in chloroform proved that the Pt/pPy–PPO bioelectrode maintains its bioelectrocatalytic response for phenol (Fig. 3). However, the chloroform being, to some extent, a hydrophilic compound can gradually remove water from the enzyme environment [28]. Consequently, after each measurement the bioelectrodes should be kept in phosphate aqueous buffer. Additionally, it was noticed that even an applied potential lower than −50 mV vs. SCE could be used to perform amperometric measurements in chloroform.

For all investigated bioelectrodes the linear range of the calibration curves to phenol in chloroform was extended towards higher phenol concentration, in comparison with that observed in aqueous buffer (Fig. 4), but their sensitivities in chloroform were much lower than those observed in aqueous buffer (Table 2). For the Pt/pPy–PPO bioelectrode the detection limit was 50 nM in chloroform and 0.8 nM in aqueous media, being comparable with the lowest detection limit reported for PPO containing bioelectrodes [30,31]. The shortest time of response (less than 1 min) was noticed for

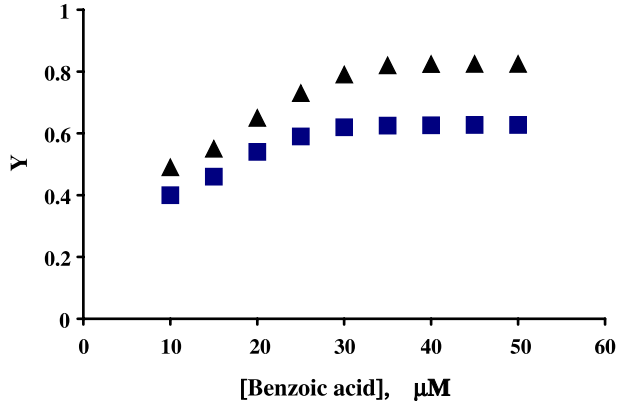

Fig. 5. The relative current intensity depletion ($Y=\Delta i/i_{SS}$) induced by the benzoic acid presence on the phenol response, recorded at Pt/pPy–PPO bioelectrode: in chloroform (■) and in aqueous buffer (▲). Applied potential: −50 mV vs. SCE; $t=22$ °C; phenol concentration, 0.4 mM; stirred solution; $i_{SS}=5.75$ nA (chloroform, 0.1 M $C_6H_5CH_2N(CH_2)_3Cl$), $i_{SS}=4.33$ μA (0.1 M phosphate buffer containing 0.1 M $LiClO_4$; pH = 7).

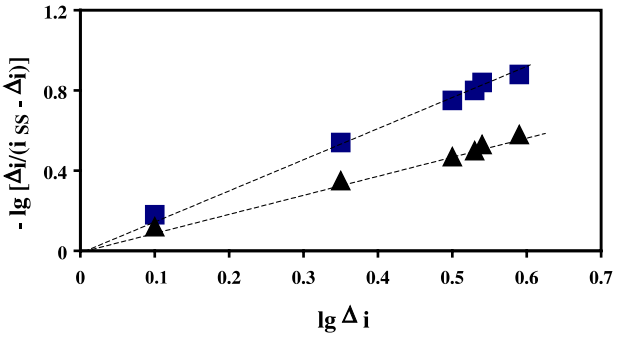

Fig. 6. Hill plot for benzoic acid inhibition recorded at Pt/pPy–PPO bioelectrode in chloroform (■) and in aqueous buffer (▲). Experimental conditions: see Fig. 5.

Pt/pPy–PPO bioelectrode, too. It is interesting to mention that, irrespective of enzyme immobilization variant, the bioelectrodes signal decrease, induced by the solvent effect, was roughly comparable (Table 2).

In order to check the chloroform effect on the short time operational stability of Pt/pPy–PPO bioelectrode, its amperometric response for 0.1 mM phenol was recorded after 2 h of continuous immersion in chloroform. If this experiment was done without any previous bioelectrode hydration, the absence of any response proved the complete removal of the enzyme hydration layer by chloroform. Contrarily, if the biosensor was preconditioned 1 day in a humid atmosphere, the signal to 0.1 mM phenol was ~ 95% recovered.

The presence of benzoic acid induces a strong inhibitory effect on the response to phenol of tyrosinase-based bioelectrodes [32]. The characteristic parameters for the inhibited response of the Pt/pPy–PPO bioelectrode were estimated from the calibration curves to phenol, recorded in the absence and presence of benzoic acid, using the Lineweaver–Burk plots (Table 3). Taking into account that in the both cases, inhibited and uninhibited response, the maximum current intensity has practically the same value (I_{max} ~ 57 nA), it was concluded that the benzoic acid inhibition is competitive with the phenol response at the cresolase active site of the enzyme [33]. Based on this supposition, the values of the inhibition constant, $(1.16 \pm 0.01) \times 10^{-6}$ M, and the inhibition coefficient, $(1.24 \pm 0.15) \times 10^{-3}$ M, were calculated and were found in good concordance with that recently published for a PPO-based bioelectrode [27,34], based on oxygen consumption measurement.

The inhibitor influence on the amperometric response of the Pt/pPy–PPO, for a constant substrate concentration (0.4 mM phenol), was estimated by the current intensity depletion (Δi) due to the benzoic acid presence. The effect of increasing inhibitor concentration on the amperometric signal was measured both in chloroform and aqueous media (Fig. 5). Based on Eq. (1) and using the data from Fig. 5, the plots presented in Fig. 6 were obtained. From these plots the Hill coefficients were estimated as the slopes of the linear regression lines (Fig. 6). In aqueous media the Hill coefficient was found equal to 1.31, while in choroform it was 2.23, both being in a good concordance with previous published data [27]. These results prove the suitability of the investigated biosensor for kinetic studies, recommending it as an efficient, fast, non-expensive, and accurate alternative of spectrophotometric measurements.

4. Conclusions

The present study has proven the suitability of conducting polymers, especially of the amphiphilic polypyrrole, to obtain polymer matrix for biosensors construction. Thus, when PPO was entrapped in an amphiphilic polypyrrole matrix a rapid stabilization of the background current, a high sensitivity to phenol and a good operational stability (higher than 3 months) both in aqueous and chloroform media was observed. Contrarily, the bioelectrode based on PPO cross-linked with glutaraldehyde showed a low reproducibility and a poor operational stability.

A kinetic interpretation of the amperometric response to phenol recorded in the absence and in the presence of benzoic acid allowed identifying the inhibition process as a competitive one, with the inhibition constant of $(1.16 \pm 0.01) \times 10^{-6}$ M, and the inhibition coefficient of $(1.24 \pm 0.15) \times 10^{-3}$ M. The inhibitory effect of chloroform on the biosensor response was characterized by the Hill coefficient both in aqueous (1.31) and choroform (2.23) media.

Acknowledgements

The authors thank Dr. Serge Cosnier for generously supplying the amphiphilic pyrrole derivative, [12-(pyrrole-1-yl)dodecyl]triethylammonium tetrafluoroborate].

References

[1] S. Cosnier, C. Innocent, A novel biosensor elaboration by electropolymerization of an adsorbed amphiphilic pyrrole–tyrosinase enzyme layer, J. Electroanal. Chem. 328 (1992) 361–366.
[2] S. Cosnier, I.C. Popescu, Poly(amphiphilic pyrrole)–tyrosinase–peroxidase electrode for amplified flow injection-amperometric detection of phenol, Anal. Chim. Acta 319 (1996) 145–151.
[3] H. Kotte, B. Grundig, K.D. Vorlop, B. Strehlitz, U. Stottmeister, Methylphenazonium-modified enzyme sensor based on polymer thick films for subnanomolar detection of phenols, Anal. Chem. 67 (1995) 65–70.
[4] J. Kulys, R.D. Schmid, A sensitive enzyme electrode for phenol monitoring, Anal. Lett. 23 (1990) 589–597.
[5] C. Kranz, H. Wolhlschlager, H.L. Schmidt, W. Schuhmann, Controlled electrochemical preparation of amperometric biosensors based on conducting polymer multilayers, Electroanalysis 10 (1998) 546–552.
[6] B. Eggins, Biosensors, An Introduction, Wiley-Teubner, New York, Teubner, Stuttgart, Leipzig, Wiley, Chichester, New York, 1996, pp. 99–151.

[7] M. Trojanowicz, T.K. Krawczyk, Electropolymerized film based electrode. A review, Mikrochim. Acta 121 (1995) 167–177.
[8] W. Schuhmann, Electron transfer pathways in amperometric biosensors ferrocene-modified enzymes entrapped in conducting-polymer layer, Biosens. Bioelectron. 10 (1995) 181–193.
[9] H. Ju, D. Zhou, Y. Xiao, Hz. Chen, Amperometric biosensor for glucose based on a nanometer-sized microband gold electrode coimmobilized with glucose oxidase and poly(*o*-phenylendiamine), Electroanalysis 10 (1998) 541–545.
[10] S. Sasso, R. Pierce, R. Walla, A. Yacynych, Electropolymerized 1,2-diaminobenzene as a means to prevent interferences and fouling and to stabilize immobilized enzyme in electrochemical biosensor, Anal. Chem. 62 (1990) 1111–1119.
[11] T. Nagaoka, H. Nakao, T. Suyama, K. Ogura, Electrochemical characterization of soluble conducting polymers as ion exchangers, Anal. Chem. 69 (1997) 1030–1037.
[12] E. Pringsheim, E. Terpetschnig, O.S. Wolfbeis, Optical sensing of pH using thin films of substituted polyanilines, Anal. Chim. Acta 357 (1997) 247–252.
[13] W. Trettnak, I. Lionti, M. Mascini, Cholesterol biosensors prepared by electropolymerization of pyrrole, Electroanalysis 5 (1993) 753–763.
[14] J. Wang, E. Gonzales-Romero, Amperometric biosensing at electrochemically pretreated glassy carbon enzyme electrodes, Electroanalysis 5 (1993) 427–432.
[15] T.A. Sergeyeva, N.V. Lavrik, S.A. Piletsky, A.E. Rachkov, A.V. El'Skaya, Polyaniline label-based conductometric sensor for Ig G detection, Sens. Actuators, B, Chem. 34 (1996) 283–288.
[16] S.K. Dhawan, D. Kumar, M.K. Ram, S. Chandra, D.C. Trivedi, Application of conducting polyaniline as sensor material for ammonia, Sens. Actuators, B, Chem. 40 (1997) 99.
[17] A.A. Karyakin, O.A. Bobrova, L.V. Lukachova, E.E. Karyakina, Potentiometric biosensors based on polyaniline semiconductor films, Sens. Actuators, B, Chem. 33 (1996) 34–38.
[18] F.F. Bier, E. Ehrentreich-Fórster, R. Dólling, A.V. Eremenko, F.W. Scheller, A redox-label immunosensor on basis of a bioenzyme electrode, Anal. Chim. Acta 344 (1997) 119–124.
[19] G. Bidan, Electroconducting polymers: new sensitive matrices to build up chemical or electrochemical sensors, A review, Sens. Actuators, B, Chem. 6 (1992) 45–53.
[20] D. Puig, D. Barcelo, Determination of phenolic compounds in water and waste water, Trends Anal. Chem. 15 (1996) 362–375.
[21] J. Wang, S. Chen, M. Lin, Use of different electropolymerization condition for controlling the size-exclusion selectivity at polyaniline, polypyrrole and polyphenol films, J. Electroanal. Chem. 273 (1989) 231–242.
[22] Y. Sakai, Design of polymer electrolytes-based humidity sensors, in: T. Seiyama (Ed.), Chemical Sensors Technology, vol. 2, Kodansha, Elsevier, Amsterdam, 1989, pp. 117–123.
[23] K. Colbow, K.L. Colbow, Sensors and monitoring systems, in: W. Göpel (Ed.), Environmental Control, Sensors, Chemical and Biochemical Sensors, Part II, vol. 3, VCH, Wenheim, 1991, pp. 970–978.
[24] A.E. Cass, Biosensors. A practical Approach, IRL Press, Oxford, University Press, 1990, pp. 120–193.
[25] A.H. Hall, Biosensors, Open Univ. Press, Buckingham, 1990, pp. 115–129.
[26] K. Streffer, H. Kaatz, Ch. Bauer, A. Makower, T. Schulmeister, F.W. Scheller, M.G. Peter, U. Wollenberger, Application of a sensitive cathecol detector for determination of tyrosinase inhibitors, Anal. Chim. Acta 362 (1998) 81–90.
[27] Q. Deng, S. Shaojun, Amperometric biosensor for tyrosinase inhibitors in a pure organic phase, Analyst 121 (1996) 1979–1982.
[28] J. Wang, Y. Lin, On-line organic phase enzyme detector, Anal. Chim. Acta 271 (1993) 53–58.
[29] L. Campanella, A. Fortuney, M.P. Sammartino, M. Tomasseti, Tyrosinase biosensor response as a function of physical properties of organic solvents, Talanta 41 (1994) 1397–1404.
[30] G. Marko-Varga, J. Emneus, L. Gorton, Development of enzyme-based amperometric sensors for the determination of phenolic compounds, Trends Anal. Chem. 14 (1995) 319–328.
[31] H. Kotte, B. Grundig, K.-D. Vorlop, B. Strehlitz, U. Stottmeister, Methylphenazonium-modified enzyme sensor based on polymer thick films for subnanomolar detection of phenols, Anal. Chem. 67 (1995) 65–70.
[32] S.E. Stanca, I.C. Popescu, Amperometric study of the inhibitory effect of carboxylic acids on tyrosinase, J. Mol. Catal., B Enzym. 27 (2004) 221–225.
[33] D.V. Roberts (Ed.), Enzyme Kinetics, Cambridge Univ. Press, Cambridge, 1977, pp. 56–87.
[34] G.A. Evtugyn, H.C. Budnikov, N. Kolskaya, Sensitivity and selectivity of electrochemical enzyme sensors for inhibitor determination, Talanta 46 (1998) 465–484.