Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 61 (2003) 501–507
Talanta
www.elsevier.com/locate/talanta

Biosensors for phenol derivatives using biochemical signal amplification

Sarmiza Elena Stanca [a,b,*], Ionel Catalin Popescu [c], Liviu Oniciu [c]

[a] Department of Physics, Chemistry and Informatics, Faculty of Environmental Protection, University of Oradea, 3700 Oradea, Romania
[b] Laboratory for Photonics and Interfaces, Instiute of Molecular and Biological Chemistry, Swiss Federal Institute of Technology, EPFL, ICMB LPI F1-496, Lausanne CH-1015, Switzerland
[c] Department of Physical Chemistry, University "Babes-Bolyai", 3400 Cluj-Napoca, Romania

Received 26 November 2002; received in revised form 11 April 2003; accepted 9 May 2003

Abstract

Two different approaches, both exploiting two enzymes cooperative functioning, to enhance the sensitivity of tyrosinase (PPO) based biosensor for amperometric detection of phenols have been compared. For this purpose, one monoenzyme electrode (PPO) and two bienzyme electrodes (PPO and D-glucose dehydrogenase, GDH; PPO and horseradish peroxidase, HRP) were constructed using agar–agar gel as enzyme immobilization matrix. The biosensors responses for L-tyrosine detection were recorded at −50 mV versus saturated calomel electrode (SCE). The highest sensitivity (74 mA M^{-1}) was observed for the PPO–GDH couple, while that recorded for PPO–HRP couple system was only 32 times higher than that measured for monoenzyme electrode (0.01 mA M^{-1}). The ability of the PPO-, PPO–GDH-, PPO–HRP-based biosensors to assay phenols was demonstrated by quantitative determination of phenol, 1,2-dihydroxybenzene, 1,3-dihydroxybenzene, 1,4-dihydroxybenzene, 2-amino-3 (4-hydroxyphenyl) propanoic acid, 2-hydroxytoluene, 3-hydroxytoluene, 4-hydroxytoluene, 4-clorophenol, 3-clorophenol, 2-clorophenol, 4-hydroxybenzoic acid.

Keywords: Biosensor signal amplification; Phenols determination; Tyrosinase; Glucose dehydrogenase; Horseradish peroxidase

1. Introduction

Among of numerous phenols, especially chloro-, nitro-, amino- phenols, present in environmental field via degradation of pesticides [1–5], chlorination and combustion of organic compounds [6] or from waste water of coal [4–10], oil processing [11–13], dyes [9,11,12], pharmaceutical [7,11,12], photographic [5] a virgule cellulose [5,7,13], petrochemical [7,12] industries, approximately 165 are known with a toxic effect on plants and animals [14,15]. Nevertheless, nitro- and chloro- phenols are inscribed on major pollutant list [3,4,15], and

* Corresponding author. Tel.: +41-21-693-3669/40-59-41-2550; fax: +41-21-693-4111.
E-mail addresses: sarmizaelena.stanca@epfl.ch, stanca@rdslink.ro (S.E. Stanca).

0039-9140/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0039-9140(03)00310-2

consequently, in order to minimize their hazardous effects it is important to use sensitive and fast techniques for detection and estimation levels.

Owing to their high selectivity electrochemical biosensors constitute powerful tools for analysis of biomolecular interactions, especially in the field of medical care and biochemistry [16–25]. More recently, this approach has been extended to environmental pollutants control [26–30], where their acceptance is growing steadily, as they demonstrate the advantage of high selectivity even in complex matrices. In addition, they have the potential for becoming portable and easy-to-use test devices. Limits of detection are not always as low as with chromatographic techniques, but for screening and field monitoring purpose electrochemical and, particularly, amperometric techniques are gaining more and more importance.

Several biosensors based on tyrosinase (PPO), were elaborated for phenols detection [29–31]. Since PPO catalyses the oxidation of phenol to *o*-quinone by dioxygen, various kinds of electrochemical detection were involved in these biosensors: (i) the detection of dioxygen consumption [32–34]; (ii) the direct reduction of the generated *o*-quinone [35–51]; (iii) the mediated reduction of *o*-quinone by hexacyanoferrate (II) [52,53], tetracyanoquinonedimethane [54], 1,2-naphtoquinone-4-sulphonate [55,56] and *N*-methylphenazonium [57].

For phenol amperometric biosensors working via the electrochemical reduction of the quinone product a partial substrate recycling was suggested, inducing amplification on the biosensor response [37,41,47–50]. This "intrinsic" amplification effect was supposed to be responsible for the very low detection limits reported for phenol and *o*-diphenols.

Recently, significant response amplification of PPO-based biosensors has been reported involving (i) a cyclic chemical reaction between the enzyme-generated *o*-quinone and a deliberately added reducing agent as ascorbate [58] or NADH [59], and (ii) a cooperative functioning of PPO and HRP [60].

In this context, we describe here a study aiming to compare two approaches for response amplification of the PPO based biosensor, applied for amperometric detection of phenols. For this purpose mono- and bienzyme electrodes were constructed using agar–agar gel as enzyme immobilization matrix. The biochemical approaches, consisting of two enzymes cooperative functioning, have been investigated for PPO and D-glucose dehydrogenase (GDH) couple (Fig. 1), as well as for PPO and horseradish peroxidase (HRP) couple (Fig. 2). Both bienzyme electrodes were tested for L-tyrosine detection.

2. Experimental

2.1. Reagents

Tyrosinase from mushroom (EC 1.14.18.1; 385 Sigma, units mg^{-1}), glucose dehydrogenase (E.C.1.1.1.47; 250 Sigma, units mg^{-1}) and horseradish peroxidase (EC.1.11.1.7; 250 Sigma, units mg^{-1}) were purchased from Sigma.

Phenol, L-tyrosine, KH_2PO_4, K_2HPO_4 and $LiClO_4$ were obtained from Merck and used as received. The agar–agar powder, 1,2-dihydroxybenzene, 1,3-dihydroxybenzene, 1,4-dihydroxybenzene, 2-amino-3 (4-hydroxyphenyl) propanoic, 2-hydroxytoluene, 3-hydroxytoluene, 4-hydroxytoluene, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 4-hydroxybenzoic and acid polyoxyethylenesorbitan monolaurate were obtained from "Reactivul" Bucharest and were used without any further purification.

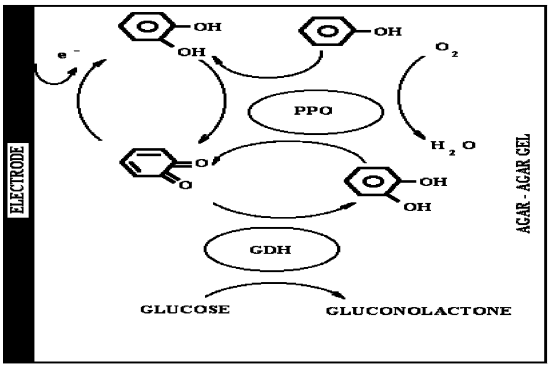

Fig. 1. Schematic recycling of *o*-quinone between PPO and GDH within a PPO–GDH containing matrix used for phenol amperometric detection.

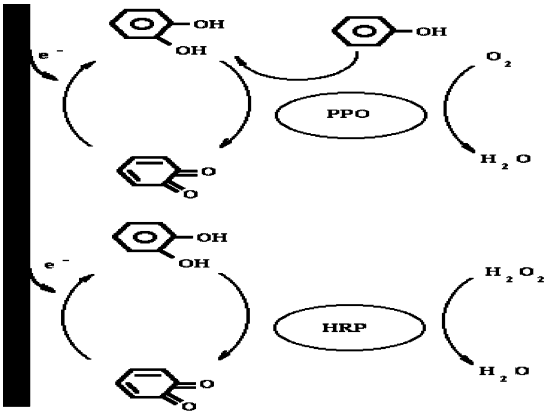

Fig. 2. Schematic amplification of the phenol response at PPO–HRP bienzyme bioelectrode.

Electrochemical measurements were made using as supporting electrolyte 0.1 M $LiClO_4$ in 0.1 M phosphate buffer (pH 6.5 and 7), obtained by mixing the corresponding volumes of 0.1 M KH_2PO_4 and 0.1 M K_2HPO_4.

2.2. Enzyme electrode preparation

The technique of enzyme entrapment in agar–agar gel [61,62] consisted in two steps:

i) 20 mg of agar–agar powder was homogenized with 0.9 ml of 0.1 M $LiClO_4$ in 0.1 M phosphate buffer (pH 7). The obtained mixture was heated at 100 °C and, subsequently, it was cooled at 50 °C. Then, 1 ml of enzyme/enzymes solution was added. The concentration of each enzyme solution was 2.5 mg ml^{-1}, and for bienzyme bioelectrodes the enzyme ratio was 1:1 (w/w). All enzyme solutions were prepared by dissolving pure enzyme in distilled water.

ii) The above-described mixture was deposited on a dialysis membrane of 0.3 mm thickness. The so obtained enzyme-modified membrane was stored at 5 °C into phosphate buffer (pH 6. The enzyme-modified membrane was attached to a Pt disc electrode (5 mm diameter) and fixed with O ring.

2.3. Electrochemical measurements

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

Amperometric measurements were done as follows: the bioelectrode was immersed in 10 ml of testing solution (0.1 M phosphate buffer containing 0.1 M $LiClO_4$) at room temperature and poised at the desired value of applied potential. When the recorded signal attained a stable value, a known volume of standard solution of substrate (phenols or L-tyrosine) was added, under a vigorous stirring. Subsequently, the bioelectrode amperometric response was recorded for 1–2 min. Thus, the calibration curve was constructed by mean of successive additions of small volumes of standard aqueous solution of substrate.

Before using, the bioelectrode was kept at 5 °C in a humid atmosphere.

3. Results and discussions

3.1. Biosensors characterization

The effects of temperature (Fig. 3A), pH (Fig. 3B) and enzyme loading (Fig. 3C) on the biosensors sensitivity have been investigated in order to characterize the biosensors behavior and to define the optimal experimental conditions. Generally, the obtained results were found in good agreement with similar reported data [61–63]. Thus, the temperature dependence of the biosensor response (Fig. 3A) revealed that the optimum temperature range was between 20 and 25 °C. For decreasing, as much as possible, the enzyme thermal denaturation, the working temperature of 21 °C was chosen for all further investigations. Focusing on the pH influence, the optimal pH range for PPO activity was found placed between 5.5 and 7.5 (Fig. 3B). As can be seen from Fig. 3C an enzyme loading

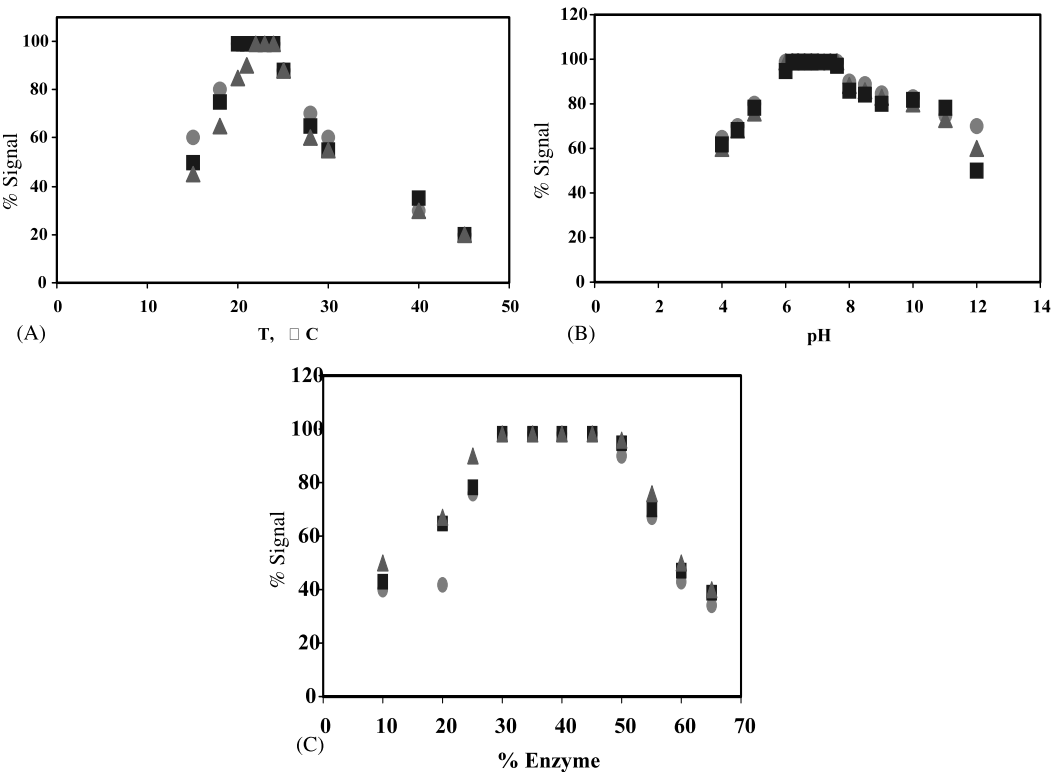

Fig. 3. The dependence of the bioelectrodes (▲, PPO–GDH; ●, HRP–PPO; ■, PPO) response to phenol on temperature (A), pH (B), and enzyme loading (C). Experimental conditions: applied potential, −50 mV vs. SCE; supporting electrolyte, 0.1 M phosphate buffer containing $LiClO_4$ 0.1 M (pH 6.5); substrate, phenol 0.1 mM. In the supporting electrolyte 1 mM H_2O_2 (for PPO–HRP bioelectrode) or 2 mM glucose (for PPO–GDH bioelectrode) was added.

higher than 50% (w/w), was not productive. Consequently, all further experiments were carried out with bioelectrodes having this enzyme loading.

The ability of the PPO-, PPO–GDH- and PPO–HRP-based biosensors to assay phenols was demonstrated by quantitative determination of various phenolic derivatives mentioned in Table 1.

In the temperature range from 15 to 50 °C, the enzyme thermostability was evaluated at three different polyoxyethylenesorbitan monolaurate concentrations. The significant effect of the investigated stabilizer consists in strengthening of the substrate binding at the enzyme active center at higher temperatures. Consequently, the enzyme activity remains unchanged till 50 °C (Fig. 4). A correlation between stabilizer concentration and the biosensor sensitivity, at a constant catechol concentration, was noticed: increased biosensor stability is related with higher stabilizer concentrations. Thus, 0.1 M stabilizer kept the enzyme activity unchanged till 50 °C, while 0.01 mM stabilizer exerted no significant effect on the enzyme thermal stability.

3.2. Bienzyme cooperative functioning

A comparison of the amperometric response to L-tyrosine was done for monoenzyme PPO-based bioelectrode and bienzyme bioelectrodes, based on PPO–GDH and PPO–HRP enzyme couples. The obtained results are presented in Fig. 5.

In all cases, in order to facilitate the comparison, the enzyme matrix contained the same amount of PPO, and the amperometric detection was performed measuring the current intensity corresponding to *o*-quinone reduction. As can be

Table 1
The relative sensitivity of PPO-, PPO–GDH-, PPO–HRP-based biosensors toward various phenolic derivatives

Phenolic compounds	Relative signal (%)		
	PPO	PPO–GDH	PPO–HRP
Phenol	100	100	100
1,2-Dihydroxybenzene	200	150	180
1,3-Dihydroxybenzene	0	10	2
1,4-Dihydroxybenzene	1	50	100
2-Amino-3 (4-hydroxyphenyl) propanoic	50	25	47
2-Hydroxytoluene	1	41	28
3-Hydroxytoluene	125	190	176
4-Hydroxytoluene	160	100	200
4-Chlorophenol	20	30	90
3-Chlorophenol	12	60	120
2-Chlorophenol	1	40	20
4-Hydroxybenzoic acid	2.2	30	10

Experimental conditions: applied potential, −0.05 V vs. SCE; phenols concentration 0.1 mM; phosphate buffer (pH 7) containing 0.1 M $LiClO_4$ (t 21 °C). The current intensity recorded for 0.1 M phenol was 23 μA.

seen (Fig. 5), the highest response was recorded for the biosensor using the PPO–GDH couple and the lowest for the PPO-based bioelectrode. This sequence of bioelectrochemical responses is in good agreement with recently published results on the

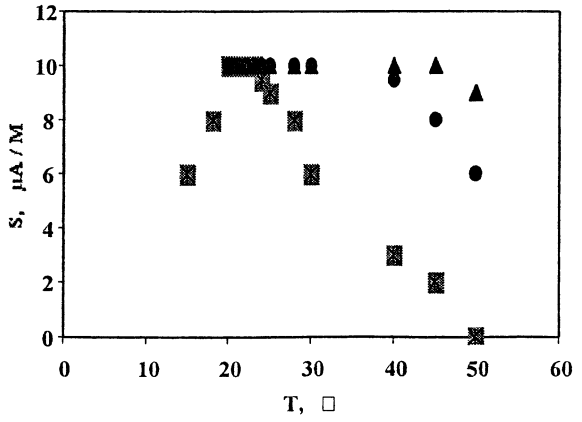

Fig. 4. The temperature influence on the HRP-based biosensor sensitivity to catechol, measured in the presence of different stabilizer concentrations (■, 0.01 mM; ●, 0.05 M; ▲, 0.1 M). Experimental conditions: applied potential, −50 mV vs. SCE; supporting electrolyte, 0.1 M phosphate buffer containing $LiClO_4$ 0.1 M (pH 6.5), 0.1 M catechol and 1 M H_2O_2.

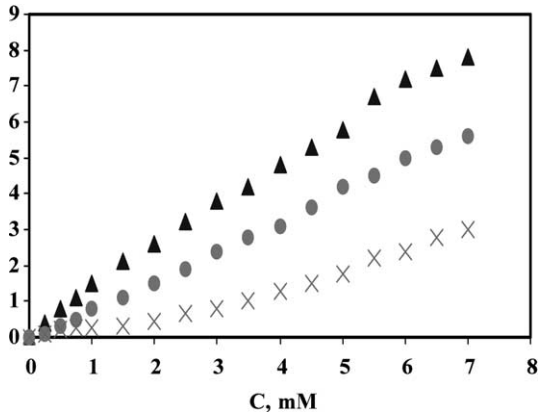

Fig. 5. Calibration curves to phenol for bienzyme biosensors (▲, PPO–GDH and ●, PPO–HRP) and monoenzyme biosensor (×, PPO). Experimental conditions: applied potential, −50 mV vs. SCE; supporting electrolyte, 0.1 M phosphate buffer containing $LiClO_4$ 0.1 M (pH 6.5); working temperature, 21 °C. In the supporting electrolyte 1 mM H_2O_2 (for PPO–HRP bioelectrode) or 2 mM glucose (for PPO–GDH bioelectrode) was added.

beneficial effect of H_2O_2–HRP couple on the phenol–PPO reaction [60], as well as on the efficient substrate recycling evidenced in the case of PPO–GDH couple [61].

For all three investigated bioelectrodes, the bioelectrochemical parameters (I_{max} and K_m, estimated using the Lineweaver–Burk linearization of the bioelectrodes response to L-tyrosine) together with the biosensors sensitivities (estimated from calibration curves) are presented in Table 2.

It is interesting to notice that comparing the bioelectrochemical parameters for PPO- and PPO–GDH-based biosensors in spite of the dramatic sensitivity increase (74 times) the K_m value remained practically unchanged. This behavior confirms the amplification scheme presented in Fig. 2. Contrarily, the PPO–HRP-based biosensor, besides an improved sensitivity (32 times) showed a significant higher value for K_m than that observed for PPO-based biosensor. This K_m increase suggests a decrease of the substrate–enzyme affinity or a supplementary diffusion constraint existing in the enzyme matrix, both associated with the increase of enzyme activity, induced by the presence of H_2O_2 [61]. A possible explanation could be formulated if the HRP effect

Table 2
The bioelectrochemical parameters corresponding to L-tyrosine response for PPO-, PPO–GDH- and PPO–HRP-based bioelectrodes

Enzyme matrix	Sensitivity (mA M^{-1})	I_{max} (μA)	K_m (mM)
PPO	0.010	0.196	10
PPO–HRP	0.326	19.6	55
PPO–GDH	0.741	16.4	14

on the agar–agar gel permeability is taken into consideration. Thus, HRP being an enzyme with a lower molecular weight than GDH will induce higher transport constraints for substrate diffusion than GDH. Obviously, this circumstance will determine a K_m value increase.

4. Conclusions

Two different approaches, both exploiting two enzymes cooperative functioning, to enhance the sensitivity of tyrosinase (PPO) based biosensor for amperometric detection of phenols have been compared. For this purpose, one monoenzyme electrode (PPO) and two bienzyme electrodes (PPO and D-glucose dehydrogenase, GDH; PPO and horseradish peroxidase, HRP) were constructed using agar–agar gel as enzyme immobilization matrix. The biosensors responses for L-tyrosine detection were recorded at -50 mV versus SCE. An optimization study was performed in order to establish the optimal experimental conditions (pH, temperature and enzymes loading).

Taking into account the bioelectrochemical parameters of the investigated biosensors, the signal amplification for L-tyrosine detection was found the highest for PPO–GDH couple. Moreover, the PPO–GDH biosensor showed, besides the highest sensitivity, the shorter response time.

When entrapped in the agar–agar gel all enzymes kept their specific activity, pointing out this matrix as a very convenient one for enzyme immobilization.

The ability of the PPO-, PPO–HRP-, PPO–GDH-based biosensors to assay phenols was demonstrated by quantitative determination of several phenol derivatives. The HRP thermostability, tested for cathecol determination, was extended toward high temperature domain ($\sim$ 50 °C) using polyoxyethylenesorbitan monolaurate as enzyme thermo-stabilizer.

Acknowledgements

The Agronomy Faculty from Cluj Napoca (Romania) and the Medicine Faculty from Oradea (Romania) are gratefully acknowledged for their generous supply of enzymes, L-tyrosine and dialysis membrane. Also, the authors thank the Swiss Federal Commission for the funding fellowship of the corresponding author and to Dr Raman Suri for his valuable discussions regarding enzyme stabilization.

References

[1] A. Hamilton, H.L. Hardy (Eds.), Industrial Toxicology, third ed, Publishing Sciences Group, Acton, MA, 1974, pp. 3–369.
[2] G.W. Ware (Ed.), Reviews of Environmental Contamination and Toxicology, vol. 109, Springer, New York, 1987, pp. 15–45.
[3] V.C. Dall'Orto, C. Dominguez, J. Hurst, A.L. Balbo, I. Rezzano, Electroanalysis 10 (1998) 127.
[4] F.A. Patty, Industrial Hygiene and Toxicology, in: D.W. Fassett, D.D. Irosh, K. Kumler (Eds.), Toxicology, vol. II, Interscience Publishers, New York, 1963, pp. 1363–2148.
[5] P.I. Ortiz, P.R.A. Nader, H.A. Mottola, Electroanalysis 5 (1993) 165.
[6] C. Saby, J.H.T. Luong, Electroanalysis 10 (1998) 7.
[7] U. Wollenberger, B. Neumann, Electroanalysis 9 (1997) 366.
[8] A. Kaisheva, I. Iliev, R. Kazareva, S. Christov, U. Wollenberger, F. Scheller, Sens. Actuat. B 33 (1996) 39.
[9] G.W. Ware (Ed.), Reviews of Environmental Contamination and Toxicology, vol. 126, Springer, New York, 1992, pp. 100–115.
[10] A. Lindgren, J. Emnéus, T. Ruzgas, L. Gorton, G.M. Varga, Anal. Chim. Acta 347 (1997) 51.
[11] L.H. Keith (Ed.), Identification and Analysis of Organic Polluants in Water, Ann Arbor Science Publishing, Michigan, 1979, p. 227.
[12] J. Paasivirta, K. Heinola, T. Humppi, A. Karjalainen, J. Knuutinen, K. Mäntykoski, R. Paukku, T. Piilola, K. Surma-Aho, J. Tarhanen, L. Welling, H. Vihonen, J. Särkkä, Chemosphere 14 (1985) 469.

[13] A.G. Arroyo, Sens. Actuat. B 1 (1993) 15.
[14] A. Xyz, in: S. Marshall (Ed.), Priority Toxic Polluants, Health Impacts and Allowable Limits, Noyes Data Corporation, Park Ridge, NJ, USA, 1980, pp. 143–370.
[15] US Environmental Protection Agency, Pesticide Fact Handbook, Noyes Data Corporation, Park Ridge, NJ, USA, pp. 5–802.
[16] W. Göpel, K.D. Schierbaum, in: W. Göpel, T.A. Jones, M. Kleitz, J. Lundström, T. Seiyama (Eds.), Definition and Typical Examples in Sensors. A Comprehensive Survey, Chemical and Biochemical Sensors Part I, vol. 2, VCH, Weinheim, 1991, pp. 2–26.
[17] T.H. Scheper, K.F. Reardon, in: W. Göpel, T.A. Jones, M. Kleitz, J. Lundström, T. Seiyama (Eds.), Sensors in Biotechnology, in Sensors. A Comprehensive Survey, Chemical and Biochemical Sensors, Part I, vol. 2, VCH, Weinheim, 1991, pp. 1024–1042.
[18] W. Göpel, Sens. Actuat. A 37-38 (1993) 1.
[19] D.A. Bradley, in: K.T.V. Graattan, A.T. Augousti (Eds.), Sensors in Manufacturing Systems in Sensors. Technology, Systems and Applications, IOP Publishing Ltd, 1993, pp. 411–421.
[20] K.R. Rogers, Biosens. Bioelectron. 10 (1995) 533.
[21] A.L. Harmer, in: K.T.V. Grattan, A.T. Augousti (Eds.), European Research on Advanced Sensors, in Senors. Technology, Systems and Applications, IOP Publishing Ltd, Bristol, 1991, pp. 475–503.
[22] P. Gueneau, Les biocapteurs environmentaux en France, CNRS Press, Grenoble, 1994, pp. 1–35.
[23] K. Colbow, K.L. Colbow, in: W. Göpel (Ed.), Sensors and Monitoring Systems in Environmental Control, in Sensors. A Comprehensive Survey, Chemical and Biochemical Sensors, Part II, vol. 3, VCH, Weinheim, 1991, pp. 970–978.
[24] C. Tran Menh, Biosensors, Chapman & Hall, London, 1993, pp. 25–50.
[25] F. Scheller, F. Schubert, Biosensoren, Akademie Verlag, Berlin, 1989, pp. 23–47.
[26] U.E. Spichiger-Keller, Chemical Sensors and Biosensors for Medical and Biological Aplications, Wiley-VCH, Weinheim, 1998, pp. 203–250.
[27] B. Hock, D. Barcelo, K. Cammann, P.D. Hansen, A.P.F. Turner (Eds.), Biosensors for Environmental Diagnostic, Teubner Verlag, Stuttgart-Leibzig, 1998, pp. 23–45.
[28] F. Schubert, U. Wollenberger, F. Scheller, Biotechnol. Lett. 5 (1983) 239.
[29] J.M. Kauffmann, G.G. Guibault, in: C.H. Suelter (Ed.), Enzyme Electrode Biosensors: Theory and Application; Bioanalytical Applications of Enzymes, vol. 36, Wiley, 1992, pp. 63–113.
[30] D. Griffits, G. Hall, TIBTECH 11 (1993) 122.
[31] P. Vadgama, P.W. Crump, Analyst 117 (1992) 1657.
[32] L. Macholan, L. Schanel, Collect. Czech. Chem. Commun. 42 (1977) 3667.
[33] L. Campanella, M.P. Sammartino, M. Tomassetti, Sens. Actuat. B 7 (1992) 383.
[34] S. Canofeni, S. Di Sario, J. Mela, R. Pilloton, Anal. Lett. 27 (1994) 1659.
[35] G.F. Hall, D.J. Best, A.P.F. Turner, Anal. Chim. Acta 213 (1988) 113.
[36] P. Skladal, Collect. Czech. Chem. Commun. 56 (1991) 1427.
[37] S. Cosnier, C. Innocent, J. Electroanal. Chem. 328 (1992) 361.
[38] J. Wang, N. Naser, H.S. Kwon, M.Y. Cho, Anal. Chim. Acta 7 (1992) 264.
[39] J. Wang, A.J. Reviejo, S. Mannino, Anal. Lett. 25 (1992) 1399.
[40] F. Ortega, E. Dominguez, G. Jönsson-Pettersson, L. Gorton, J. Biotechnol. 31 (1993) 289.
[41] S. Cosnier, C. Innocent, Bioelectrochem. Bioenerg. 31 (1993) 147.
[42] J. Wang, Y. Lin, Q. Chen, Electroanalysis 5 (1993) 23.
[43] J. Wang, Y. Lin, L. Chen, Analyst 118 (1993) 277.
[44] J. Wang, L. Fang, D. Lopez, Analyst 119 (1994) 455.
[45] J.L. Besombes, S. Cosnier, P. Labbé, G. Reverdy, Anal. Lett. 28 (1995) 405.
[46] S. Cosnier, Electroanalysis 9 (1997) 894.
[47] J. Wang, F. Lu, D. Lopez, Biosens. Bioelectron. 9 (1994) 9.
[48] F. Liu, A.J. Reviejo, J.M. Pingarrón, Talanta 41 (1994) 455–459.
[49] M. Lutz, E. Burestedt, J. Emnéus, H. Lidén, S. Gobhadi, L. Gorton, G. Marko-Varga, Anal. Chim. Acta 305 (1995) 8.
[50] J.G. Schiller, A.K. Chen, C.C. Liu, Anal. Biochem. 25-33 (1978) 85.
[51] C. Nistor, J. Emneus, L. Gorton, A. Ciucu, Anal. Chim. Acta 387 (1999) 309.
[52] M. Bonakdar, J.L. Vilchez, H.A. Mottola, J. Electroanal. Chem. 226 (1989) 47.
[53] P.I. Ortiz, P.R.A. Nader, H.A. Mottola, Electroanalysis 5 (1993) 165.
[54] J. Kulys, R.D. Schmid, Anal. Lett. 23 (1990) 589.
[55] M.H. Smit, G.A. Rechnitz, Electroanalysis 5 (1993) 747.
[56] W.R. Everett, G.A. Rechnitz, Anal. Chem. 70 (1998) 807.
[57] H. Kotte, B. Grundig, K.D. Vorlop, B. Stuhlitz, U. Stottmeister, Anal. Chem. 67 (1995) 3922.
[58] S. Uchiyama, Y. Hasebe, H. Shimizu, H. Ishihara, Anal. Chim. Acta 276 (1993) 341.
[59] R.S. Brown, K.B. Male, J.H.T. Luong, Anal. Biochem. 222 (1994) 131.
[60] S. Cosnier, I.C. Popescu, Anal. Chim. Acta 319 (1996) 145.
[61] A.V. Eremenko, A. Makower, C.G. Bauer, I.N. Kurochkin, F.W. Scheller, Electroanalysis 9 (1997) 288.
[62] A.E. Cass, Biosensors. A practical Approach, IRL Oxford University Press, 1990, pp. 2–62.
[63] G. Robinson, D. Leech, M.R. Smyth, Electroanalysis 7 (1995) 952.