Biosensors and Bioelectronics 25 (2010) 2454–2457

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A very thin overoxidized polypyrrole membrane as coating for fast time response and selective H_2O_2 amperometric sensor

Catherine Debiemme-Chouvy*

Laboratoire Interfaces et Systèmes Electrochimiques – UPR 15 du CNRS, Université Pierre et Marie Curie – Case courrier 133 - 4 place Jussieu, 75005 Paris, France

ARTICLE INFO

Article history:
Received 5 February 2010
Received in revised form 22 March 2010
Accepted 6 April 2010
Available online 13 April 2010

Keywords:
Overoxidized polypyrrole
H_2O_2 determination
Ascorbate rejection
Dopamine rejection
Permselective membrane
Amperometric biosensor

ABSTRACT

Pyrrole electrooxidation in the presence of weak-acidic anions allows one to locally, rapidly and in single step form on the electrode a pinhole-free very thin layer of overoxidized polypyrrole (OPPy). This membrane could be really useful for the design of amperometric biosensor based on oxidation of H_2O_2 generated by an oxidase. Indeed, using rotation disk electrode, the apparent diffusion coefficient of H_2O_2 within this OPPy film was found to be 10^{-8} cm^2 s^{-1}, which corresponds to a fast response time of 0.1 ms. Moreover, it is shown that with this system, platinum electrode coated with the very thin OPPy membrane, the H_2O_2 sensitivity is excellent (700 nA μM^{-1} cm^{-2}) and the H_2O_2 selectivity relative to potentially interfering species present in biological fluids, such as ascorbate and dopamine, is very good. Thus, this OPPy membrane which can be locally electrodeposited is ideal for the construction of oxidase-based microbiosensors.

1. Introduction

In the design of enzymatic amperometric biosensors, the enzyme could be either entrapped within an electrochemically deposited conducting polymer such as polypyrrole (PPy) or polyaniline or nonconducting polymer such as polyphenol (Bartlett et al., 1992), poly(2-naphthol) (Ciriello et al., 2000), poly(*o*-aminophenol) (Guerrieri et al., 2009), polyphenylendiamine (Malitesta et al., 1990; Dempsey et al., 1993) or overoxidized PPy (OPPy) (Freund et al., 1991; Witkowski et al., 1991; Descroix and Bedioui, 2001). Another possibility is the immobilization of the enzyme onto the surface of the polymer (Guerrieri et al., 1998; Carelli et al., 2006, 2007). In all cases, the polymeric membrane that covers a platinum or a gold electrode has to act as a protective layer which rejects interfering species, the membrane permselectivity arising from the charge or being based on size exclusion.

Recently, numerous oxidase-based biosensors have been developed for a wide range of analytes (Palmisano et al., 1993; Hamdi et al., 2006). Hydrogen peroxide is a side-product of the reactions catalyzed by the oxidase enzymes which means that the concentration of H_2O_2 should be directly related to the concentration of the substrate *i.e.* the analyte.

$$\text{substrate} + O_2 \xrightarrow{\text{oxidase}} \text{product} + H_2O_2 \quad (1)$$

$$\text{Transduction}: H_2O_2 \rightarrow O_2 + 2e^- + 2H^+ \quad (2)$$

The development of highly sensitive and interference-free H_2O_2 amperometric biosensor is very important in different fields such as medicine and food quality control. For biosensors used in medicine field, potentially interfering species like ascorbate and dopamine have to be prevented from reaching the surface of the electrode in order to make these biosensors very useful for the analysis of real samples.

For H_2O_2 specific amperometric detection, according to reaction (2), the literature shows that, among the possible polymers already cited, overoxidized polypyrrole is an excellent candidate. Indeed, for example, four different polymeric, permselective films have been compared for specific H_2O_2 detection (Nafion, polyaniline, OPPy and polyphenylenediamine) the best results were obtained with the OPPy membrane (Hamdi et al., 2005). It is now well known that OPPy film has ion-selective properties based on the exclusion of anionic species due to the presence of carbonyl groups in some pyrrole rings (Beck et al., 1987; Ge et al., 1994; Palmisano et al., 1995).

However, due to the thickness the OPPy membrane (about 0.1 μm) (Hsueh and Brajter-Toth, 1994), the main drawback of this film is a slow time response due to the diffusion of the analyte (H_2O_2) within the film. Using very thick OPPy films, the amperometric detection of H_2O_2 present in the electrolyte could be nearly impossible (Descroix and Bedioui, 2001). Therefore to overcome this problem, the rejection film has to be as thin as possible while retaining a good selectivity for H_2O_2. Usually, the OPPy films are

* Tel.: +33 1 44 27 41 49; fax: +33 1 44 27 40 74.
E-mail address: catherine.debiemme-chouvy@upmc.fr.

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.04.008

prepared in two steps, the first one being the pyrrole electropolymerization and the second one its overoxidation in electrolyte without pyrrole under potentiodynamic conditions (Gao et al., 1994) or under potentiostatic conditions, high anodic potential (0.7–0.85 V *vs.* Ag/AgCl) being applied for at least 5 h until a steady state background current was obtained.

Recently, we have shown that the presence of solely weak-acidic anions in the pyrrole solution allows one to rapidly and directly electrodeposit overoxidized polypyrrole film onto the electrode (Debiemme-Chouvy, 2007). It has already been shown that this film allows the rejection of large inorganic redox species such as $Ru(NH_3)_6^{2/3+}$ and anionic redox species such as $Fe(CN)_6^{3/4-}$ (Debiemme-Chouvy and Gallois, 2010). However, in order to use such membrane in the design of oxidase-based amperometric biosensor the response time and permselectivity against biological electrooxidizable species have to be determined. It is the aim of the present paper. First, rotating disk electrode (RDE) experiments were performed in order to evaluate the response time of the electrode coated with the OPPy film electrodeposited in our specific conditions. Then, the rejection of two biological compounds, ascorbic acid and dopamine was checked.

2. Experimental

Pyrrole, ascorbic acid, dopamine, H_2O_2 and phosphate buffered (PB) (pH = 7) solutions were purchased from Sigma. The polypyrrole film syntheses were carried out using pyrrole (Py) solution preliminarily distilled. All the solutions were prepared with bi-distilled water. The electrochemical experiments were performed in a classical three-electrode electrochemical cell. The working electrode was either Pt disk ($S = 0.07\ cm^2$) or very smooth and hard amorphous carbon nitride (a-CN_x) film elaborated in our lab using a radiofrequency magnetron sputtering technique (Lagrini et al., 2005; Cachet et al., 2006). Before electrodeposition of the OPPy film, the substrates were degreased with methanol, then rinsed with water and finally dried under an Ar stream. A Pt wire was used as counter electrode and a saturated calomel electrode (SCE) as the reference one. The potentiostat (Autolab PGSTAT30) and the rotating disk electrode system were from Eco Chemie. The OPPy films were deposited under potentiostatic conditions. The Pt electrode was polarized at 0.8 V/SCE for 15 min in an aqueous solution containing 0.2 M K_2HPO_4 plus 0.15 M Py. The SEM image was obtained from a SEM-FEG microscope, Ultra55 Zeiss, operating at 3 kV.

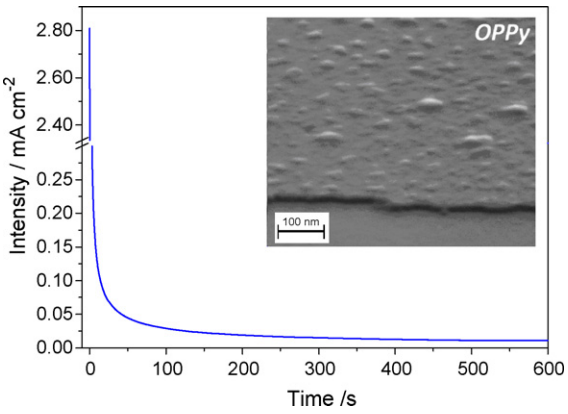

Fig. 1. OPPy film electrosynthesis. Current density *vs.* time at a polarized Pt electrode at 0.8 V/SCE for 15 min in 0.15 M Py + 0.2 M K_2HPO_4 aqueous solution. Inset: Tilted (60°) SEM view of the OPPy film deposited on a a-CN_x substrate.

3. Results and discussion

3.1. Film electrosynthesis

The rapid and direct electrodeposition of an overoxidized polypyrrole (OPPy) film can be achieved if the anions present in the Py solution are all weak-acidic anions. Fig. 1 shows the current-time response obtained under potentiostatic conditions in the presence of monohydrogenophosphate ions. The anodic current is initially high, it rapidly decreases until a very low value which is in fact due to solvent oxidation *i.e.* to water oxidation. We have already explained why such response is observed (Debiemme-Chouvy, 2007). Briefly, it is due to the decrease of the pH at the electrode-film interface during Py oxidation. At the interface, the weak-acidic anions capture the protons released, therefore no more anions are available at the interface and the PPy film growth vanishes. Since a high potential is applied at the electrode the solvent oxidation occurs which leads to formation of hydroxyl radicals which overoxidize the PPy film already deposited (Debiemme-Chouvy and Tran, 2008). The structure of the OPPy film can be seen in the insert of Fig. 1. Its thickness is very low, about 10 nm. The reproducibility of the electrosynthesis of the OPPy film is excellent. Indeed, the shape of the $I(t)$ response is always the same, the final current density which is due to water oxidation at the Pt–OPPy interface is always very low (see Fig. 1). However, it is important to notice that very good reproducibility is obtained only if the Py solution is not polluted with non weak-acidic anions coming from the reference electrode, for example.

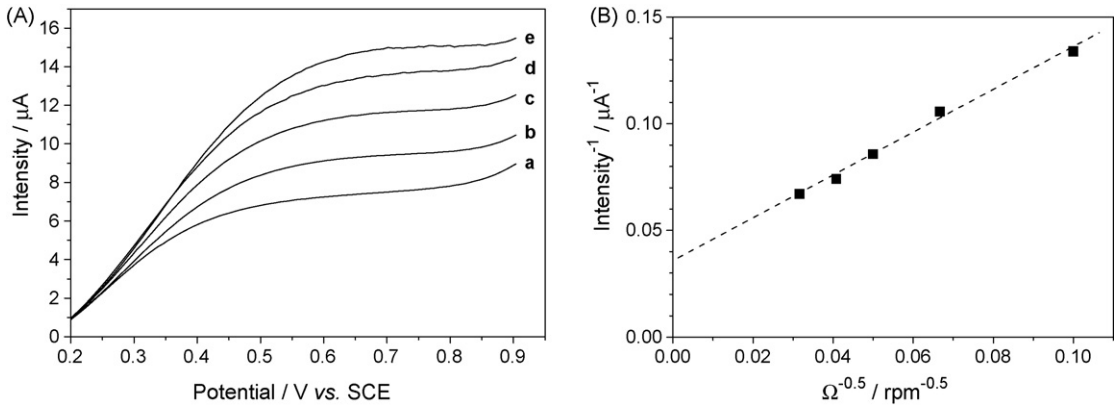

Fig. 2. H_2O_2 oxidation at OPPy-covered Pt electrode. (A) Voltammograms obtained at the OPPy-covered Pt RDE in 2×10^{-4} M H_2O_2 PB solution. Electrode rotation rate: 100 (a), 225 (b), 400 (c), 600 (d), 1000 (e) rpm. Potential scan rate: 20 mV s^{-1}. (B) Koutecky–Levich plot, data are from graph A (intensity at 0.65 V/SCE).

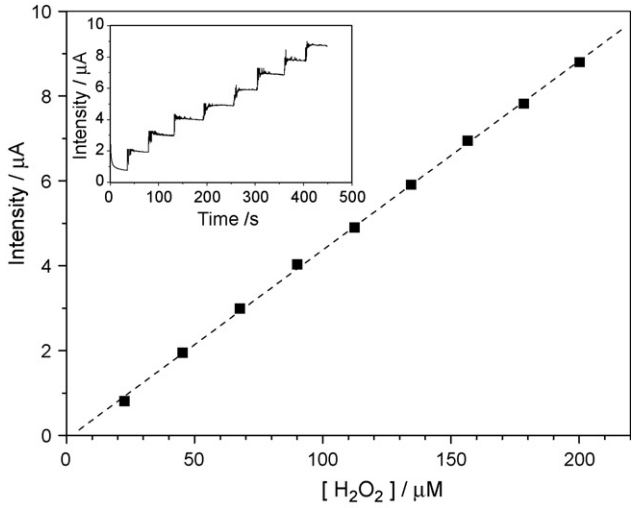

Fig. 3. H_2O_2 determination. Intensity *vs.* H_2O_2 concentration recorded at an OPPy-covered Pt electrode polarized at 0.65 V/SCE. The experimental curve (Intensity *vs.* time after successive additions of H_2O_2) is shown in the inset. Electrolyte: 20 mL PB solution + 0.023 mM H_2O_2 + 8 successive additions of 50 µL of 9.1 mM H_2O_2 solution. Electrode rotation rate: 600 rpm.

3.2. H_2O_2 response at the OPPy-covered Pt electrode

Fig. 2 shows the responses obtained at various electrode rotation rates at an OPPy-covered Pt RDE in the presence of H_2O_2. The anodic current increases as the electrode rotation rate is increased. The plot of the inverse of the current (at 0.65 V/SCE, curves Fig. 2A) versus the inverse of the square root of the electrode rotation rate (Koutecky–Levich plot) is a linear relationship (Fig. 2B). The apparent diffusion coefficient within the OPPy membrane (D_{app}) can be determined from this plot (Leddy et al., 1985). Indeed, for a membrane-covered RDE, the membrane diffusion limited current, i_k, obtained from the inverse of the intercept of the Koutecky–Levich plot is given by:

$$\frac{1}{i_K} = \frac{1}{nFSC_{ox}P_m} \tag{3}$$

where n is the number of exchanged electrons, F (in C mol^{-1}) is the Faraday's constant, C_{ox} (in mol cm^{-3}) is the bulk concentration of the electroactive species, P_m (in cm s^{-1}) is the permeability of the membrane, $P_m = D_{app}/\delta$ (Gough and Leypoldt, 1979; Spurlock et al., 1996), δ (in cm) being the thickness of the membrane. From Fig. 2B one can notice that $1/i_K = 0.0355$ µA^{-1}, therefore Eq. (3) allows one to calculate the apparent diffusion coefficient of H_2O_2 within the OPPy film. It is found to be 10^{-8} cm^2 s^{-1}. The response time of the system (t) could be estimated using the following expression (Witkowski and Brajter-Toth, 1992):

$$t = \frac{\delta^2}{D_{app}} \tag{4}$$

In our conditions, the response time is equal to 0.1 ms which is a very low value, very useful for an amperometric oxidase-based biosensor.

The OPPy membrane was also checked for the H_2O_2 determination under potentiostatic conditions. The rotating disk Pt electrode coated with the OPPy film was used and aliquots of H_2O_2 were successively added to the PB solution. The chronoamperometric response is shown in the inset of Fig. 3. The relationship between

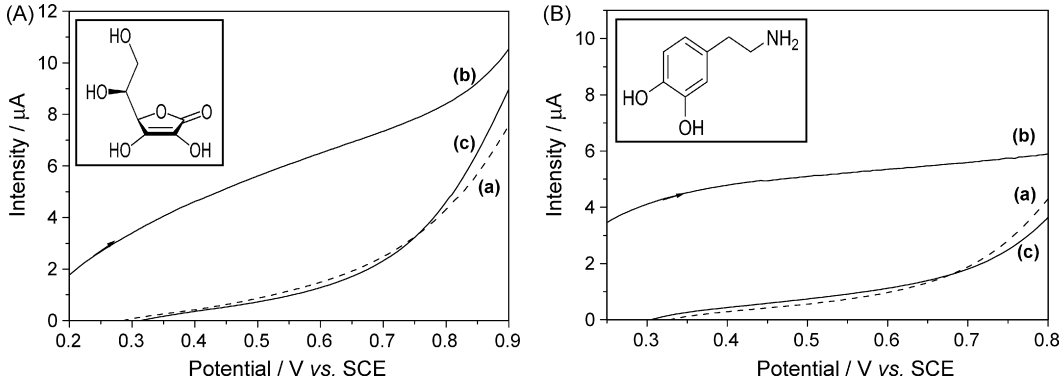

Fig. 4. AA and DA rejection. Voltammograms obtained at a Pt RDE (a, b) bare electrode, (c) OPPy-covered electrode, in (a) PB solution (b, c) + 10^{-4} M AA (A) or DA (B). Potential scan rate: 20 mV s^{-1}. Electrode rotation rate: 600 rpm. Inset: structures of ascorbic acidic (A) and dopamine (B).

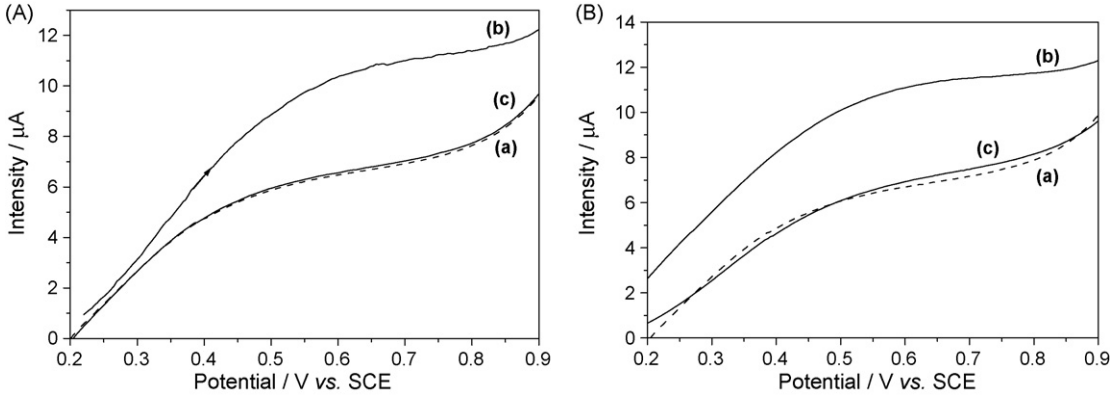

Fig. 5. Permselective OPPy membrane. Voltammograms obtained at a Pt RDE (b) bare electrode, (a, c) OPPy-covered electrode, in (a) 10^{-4} M H_2O_2 PB solution (b, c) + 10^{-4} M AA (A) or DA (B). Potential scan rate: 20 mV s^{-1}. Electrode rotation rate: 600 rpm.

the H_2O_2 concentration and the anodic current is depicted in Fig. 3, it is linear as expected for a sensor. Moreover, the slope of the curve allows us to determine the H_2O_2 sensitivity of the OPPy-covered Pt electrode, it is found to be 700 nA μ M^{-1} cm^{-2}. This value is excellent, about 2-fold higher than the value reported by Hamdi et al. (2005) and obtained using a thicker OPPy membrane prepared in two steps, the electrode rotation rate being 500 rpm.

3.3. Interferent rejection

Finally, in order to detect specifically analytes *i.e.* quantitatively to H_2O_2 released (Eqs. (1) and (2)), no other electrooxidizable species should reach the electrode surface. In extracellular fluid the interference species are mainly ascorbic acid (AA) and dopamine (DA). The structure of these compounds is given in the inset of Fig. 4A and B, respectively. Both compounds have low molecular weight. At physiological pH, AA is an anion ($pK_a = 4.1$) whereas DA is a cation ($pK_a = 8.9$). For rejection based on size exclusion, the membrane has to be compact and pinhole-free.

The voltammograms obtained in the presence of AA or DA at a bare Pt electrode are shown in Fig. 4 (curves (b)), for comparison the curves recorded without redox species in the medium are also depicted (curves (a)). Curves (c) were recorded in the presence of AA or DA at the electrode covered with the OPPy membrane. In both cases, it is obvious that curves (a) and curves (c) are very similar indicating that AA and DA are not oxidized at the Pt electrode when it is coated with the OPPy film. Thus, we can assert that the rejection of AA and DA by the OPPy film synthesized in our conditions is very efficient. To confirm this finding, same experiments were conducted in the presence of H_2O_2 plus AA or DA. The results are shown in Fig. 5. When the Pt electrode is coated with the OPPy membrane, the presence of AA (Fig. 5A) or DA (Fig. 5B) in the medium does not modify the electrochemical response (see curves a and c). On the contrary, at a bare Pt electrode the anodic current is really higher, it is due to H_2O_2 and AA or DA oxidation (curves b). The AA and DA rejections were tested for concentrations up to 1 mM and 0.1 mM, respectively. In all cases the rejection was excellent.

The compactness of the OPPy film obtained in our conditions can be explained taking into account the reactional mechanism which occurs during the film electrosynthesis. Indeed, in the presence of small weak-acidic anions in the Py solution, under potentiostatic conditions, after a few minutes the anodic current is stable and very low, it corresponds to solvent oxidation (Debiemme-Chouvy, 2007). Py oxidation does not occur anymore because the already form polymer has been overoxidized and forms a barrier against Py. Thus, this OPPy film can act on the one hand as a steric barrier layer due to its compactness and on the other hand, as already mentioned, as an electrostatic repulsion barrier for anions. Several OPPy membranes have been synthesized and tested, in all cases the rejection of AA and DA was very good.

Finally, the stability of the OPPy film has been verified. After 1 month of storage in ambient atmosphere, the OPPy-covered Pt electrode always exhibits rejection against AA and DA and its sensitivity to H_2O_2 remains unchanged.

4. Conclusion

The OPPy film obtained by electropolymerization of pyrrole in the presence of solely weak-acidic anions is a very good candidate as a permselective membrane in the construction of oxidase-based biosensor. Indeed, the apparent diffusion coefficient of H_2O_2 through this film is high, thus the response time of the system is very short. Moreover, the OPPy-covered Pt electrode displays a very good sensitivity to H_2O_2 and selectivity toward small organic molecules such as ascorbate (electrostatic repulsion) and dopamine (steric congestion).

References

Bartlett, P.N., Tebbutt, P., Tyrrell, C.H., 1992. Anal. Chem. 64, 138–142.
Beck, F., Braun, P., Oberst, M., 1987. Ber. Bunsenges. Phys. Chem. 91, 967–974.
Cachet, H., Debiemme-Chouvy, C., Deslouis, C., Lagrini, A., Vivier, V., 2006. Surf. Interf. Anal. 38, 719–722.
Carelli, D., Centonze, D., De Giglio, A., Quinto, M., Zambonin, P.G., 2006. Anal. Chim. Acta 565, 27–35.
Carelli, D., Centonze, D., Palermo, C., Quinto, M., Rotunno, T., 2007. Biosens. Bioelectron. 23, 640–647.
Ciriello, R., Cataldi, T.R.I., Centonze, D., Guerrieri, A., 2000. Electroanalysis 12, 825–830.
Debiemme-Chouvy, C., 2007. Electrochem. Sol. State Lett. 10, E24–E26.
Debiemme-Chouvy, C., Tran, T.T.M., 2008. Electrochem. Commun. 10, 947–950.
Debiemme-Chouvy, C., Gallois, M., 2010. Surf. Inter. Anal., doi 10.1002/sia.3171.
Dempsey, E., Wang, J., Smyth, M.R., 1993. Talanta 40, 445–451.
Descroix, S., Bedioui, F., 2001. Electroanalysis 13, 524–528.
Freund, M., Bodalbhai, L., Brajter-Toth, A., 1991. Talanta 38, 95–99.
Gao, Z., Zi, M., Chen, B., 1994. J. Electroanal. Chem. 373, 141–148.
Ge, H., Qi, G., Kang, E., 1994. Polymer 35, 504–508.
Gough, D.A., Leypoldt, J.K., 1979. Anal. Chem. 51, 439–444.
Guerrieri, A., De Benedetto, G.E., Palmisano, F., Zambonin, P.G., 1998. Biosens. Bioelectron. 13, 103–112.
Guerrieri, A., Ciriello, R., Centonze, D., 2009. Biosens. Bioelectron. 24, 1550–1556.
Hamdi, N., Wang, J., Monbouquette, H.G., 2005. J. Electroanal. Chem. 581, 258–264.
Hamdi, N., Wang, J., Walker, E., Maidment, N.T., Monbouquette, H.G., 2006. J. Electroanal. Chem. 591, 33–40.
Hsueh, C.C., Brajter-Toth, A., 1994. Anal. Chem. 66, 2458–2464.
Lagrini, A., Charvet, S., Benlahsen, M., Debiemme-Chouvy, C., Deslouis, C., Cachet, H., 2005. Thin Solid Films 482, 41–44.
Leddy, J., Bard, A., Maloy, J., Savéant, J.-M., 1985. J. Electroanal. Chem. 187, 205–227.
Malitesta, C., Palmisano, F., Torsi, L., Zambonin, P.G., 1990. Anal. Chem. 62, 2735–2740.
Palmisano, F., Centonze, D., Guerrieri, A., Zambonin, P.G., 1993. Biosens. Bioelectron. 8, 393–399.
Palmisano, F., Malitesta, C., Centonze, D., Zambonin, P.G., 1995. Anal. Chem. 67, 2207–2211.
Spurlock, L.D., Jaramillo, A., Praserthdam, A., Lewis, J., Brajter-Toth, A., 1996. Anal. Chim. Acta 336, 37–46.
Witkowski, A., Freund, M.S., Brajter-Toth, A., 1991. Anal. Chem. 63, 622–626.
Witkowski, A., Brajter-Toth, A., 1992. Anal. Chem. 64, 635–641.