STUDY OF A NOVEL [Cu^{II}(TPP)]/NAFION FILM ELECTRODE FOR THE DETECTION OF NITRIC OXIDE RADICAL (NO)

Ya-Zhen WANG[a1,b], Wan-Hua CHEN[a2] and Sheng-Shui HU[a,b1,*]
[a] *Department of Chemistry, Wuhan University, Wuhan 430072, P.R. China;*
e-mail: [1] yazhenwang@163.com, [2] wh_chen53@163.com
[b] *State Key Laboratory of Transducer Technology, Chinese Academy of Sciences,*
Shanghai 200050, P.R. China; e-mail: [1] sshu@whu.edu.cn

Received August 10, 2005
Accepted February 17, 2006

A novel modified platinum electrode coated with copper(II)-5,10,15,20-tetraphenylporphyrin, [Cu^{II}(TPP)], has been developed. The electrochemical behavior of NO at the modified electrode was studied by cyclic and differential pulse voltammetries. In a deoxygenated phosphate-buffered saline solution (pH 7.4), the [Cu^{II}(TPP)]/Nafion-modified Pt electrode exhibits a strong electrocatalytic effect and stability to oxidation with NO and can be employed as a NO sensor. In this system, the modification film is capable of removing some endogenous anionic electroactive substances in biological samples, such as nitrite and ascorbate. The oxidation currents increase linearly with the concentration of NO in the range 1.8×10^{-7}–0.9×10^{-4} M. The detection limit is 2.0×10^{-8} M. The nitric oxide radical, released from sodium nitroprusside (SNP), was also determined using chronoamperometry in the presence of light.

Keywords: Copper(II)-5,10,15,20-tetraphenylporphyrin; Nitric oxide radical; Chemically modified electrodes; Nafion; Sodium nitroprusside; Cyclic voltammetry; Electrochemistry.

Recent research in developing new electrode materials has been directed towards the use of biological macromolecules in the form of conductive polymers. Metalloporphyrins and metallophthalocyanines have attracted much attention due to their special electrochemical and electrocatalytic properties during the past decade[1]. The modification of the electrode surface by depositing macrocyclic metal complexes appears as an attractive way to design highly selective and sensitive sensors for direct electrochemical measurement of nitric oxide radical[2] (NO).

Nitric oxide is an extremely important messenger molecule, being responsible for the proper function of several physiological substances such as the endothelium-derived relaxing factor (EDRF). It has also been found that NO plays a physiological role in vascular relaxation, platelet activation, neurotransmission and immune response[3]. Due to its importance in

Collect. Czech. Chem. Commun. 2006, Vol. 71, No. 5, pp. 698–708

doi:10.1135/cccc20060698

physiological processes, there has been an increasing interest in the development of methods for detection of this compound. One of the main difficulties associated with its study and determination is the fact that free NO has a high spontaneous chemical reactivity, with a half-life of 6–50 s (ref.[4]) and can be easily oxidized with O_2 to nitrite (NO_2^-) and nitrate (NO_3^-). In recent reviews, Taha[5] has summarized some procedures for the measurement of NO, and Bedioui[6] has described various approaches involved in chemically modified microelectrodes.

In general, electrochemical methods are most advantageous because they are simple, rapid and sensitive, and can be performed in real time. The most widely used approach to NO detection is based on its electrochemical oxidation at a nickel- or cobalt-porphyrin and Nafion-coated carbon fiber electrode[2,7], but the way these porphyrin film electrodes work is not fully understood. It may be related to the well-known ability of the central metal ion of the porphyrin to axially complex small molecules such as O_2, CO and NO (ref.[2]). Thus, it is necessary to search for a sensing material with high selectivity and sensitivity in detecting of NO and to assess the exact role played by the porphyrin film.

In this study, a new Cu(II)-metalloporphyrin-modified platinum electrode is described. The experimental results show that the Cu(II)-5,10,15,20-tetraphenylporphyrin [Cu^{II}(TPP)]/Nafion-modified electrode, as an NO electrochemical sensor, displays good electrocatalytic activity in NO oxidation.

EXPERIMENTAL

Reagents and Chemicals

Copper(II)-5,10,15,20-tetraphenylporphyrin, [Cu^{II}(TPP)], was synthesized as described in the literature[8]. Nafion solution (5%, w/v) (Sigma), sodium nitroprusside (SNP) (Guangzhou Reagent Factory, Guangzhou, P.R. China) and other chemicals used were of reagent grade. High-purity nitrogen gas was used for deaeration. Phosphate-buffered saline (PBS) containing 1.37×10^{-2} M NaCl, 2.7×10^{-3} M KCl, 8.0×10^{-3} M Na_2HPO_4 and 1.5×10^{-3} M KH_2PO_4 was prepared in water and adjusted to pH 7.4. NO gas was generated by dropping slowly 2 M H_2SO_4 into a glass flask containing a saturated $NaNO_2$ solution. Before the addition of H_2SO_4, the apparatus was flushed with nitrogen for 30 min to exclude O_2. The gas generated was bubbled twice through a 30% NaOH solution and once through water in order to trap any NO_2 formed as a result of oxidation of NO with traces of oxygen. To produce a saturated NO solution (at 25 °C, ca. 1.8×10^{-3} M NO in ref.[9]), PBS (2 ml) was also bubbled with nitrogen for 30 min to remove oxygen. The solution was then bubbled with pure NO gas for 30 min and kept under an NO atmosphere before use. Standard NO solutions were freshly prepared by serial dilutions of the saturated NO solution as previously reported[4], and kept in a glass flask with a rubber septum. The saturated solution was diluted using deoxygenated PBS.

Apparatus

Electrochemical experiments were performed with a CHI830 electrochemical analyzer (CHI, USA) in conjunction with an IBM-compatible PC. The three-electrode system consisted of a platinum disc working electrode modified with [Cu^{II}(TPP)]/Nafion, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode. On NO determination, a hermetic electrochemical cell was employed to avoid NO leakage and O_2 permeation. All electrochemical experiments were performed at room temperature.

Preparation of Modified Electrode

[Cu^{II}(TPP)] (2.0 mg) was dissolved in $CHCl_3$ (1 ml). Prior to coating with the [Cu^{II}(TPP)] complex, the platinum disc electrode was polished with 0.3 and 0.05 μm Al_2O_3 powder on a Buehler felt pad, followed by continuous sonication in 1:1 nitric acid, ethanol and water. Then, 20 cyclic scans were carried out in 0.5 M H_2SO_4 in the potential range from –0.8 to 1.2 V. The [Cu^{II}(TPP)] solution (10 μl) was pipetted onto the surface of the clean platinum electrode. The solvent was then evaporated in air. The [Cu^{II}(TPP)]-modified electrode was covered with Nafion by depositing 1% Nafion solution in ethanol (5 μl) on the surface of the electrode and evaporating the solution under an infrared lamp.

Procedure

Prior to NO measurements, the [Cu^{II}(TPP)]/Nafion-modified electrode was placed in a deoxygenated PBS solution and the potential was cycled between 0.0 and 1.0 V at a scan rate of 100 mV s^{-1} until a stable cyclic voltammogram was obtained (after continuous scanning for ca. 10 cycles). Selectivity tests of the electrode were evaluated by employing differential pulse voltammetry (DPV). DPV was performed with a potential sweep rate of 40 mV s^{-1} and 5 pulses per second (pulse height 50 mV, pulse width 50 ms). Calibrations of NO were made by chronoamperometry (pulsed from 0.5 to 0.8 V). NO solutions were added continuously with a gas-tight syringe to a stirred PBS solution under nitrogen, and the oxidation currents of NO were recorded after each addition. Chronocoulometry (pulsed from 0.5 to 0.8 V) was used to confirm the one-electron transfer character of NO oxidation.

RESULTS AND DISCUSSION

Cyclic Voltammetric Behavior of the [Cu^{II}(TPP)] and [Cu^{II}(TPP)]/Nafion-Modified Pt Electrode

Figure 1 shows the cyclic voltammograms of the Pt electrode modified with [Cu^{II}(TPP)] and [Cu^{II}(TPP)]/Nafion in a deoxygenated PBS solution. A pair of redox peaks was observed; the anodic and cathodic peak potentials are –0.20 and –0.32 V (curve a), respectively. The peaks have been assigned to the redox activity of Cu^{II}/Cu^{I} in the porphyrin complex[10]. However, the Cu^{II}/Cu^{I} voltammetric peaks do not appear after depositing 1% Nafion solution on the surface of the [Cu^{II}(TPP)] film electrode (curve b). The Cu^{II}/Cu^{I} redox reaction requires diffusion of Cl^{-} to neutralize the charge generated in the [Cu^{II}(TPP)] film, which is impossible through Nafion. After repeated potential scans, the CV waves remain almost unaffected. This result indicates the stability of [Cu^{II}(TPP)]/Nafion-modified electrode in PBS.

Electrocatalytic Oxidation of NO at the [Cu^{II}(TPP)]/Nafion-Modified Electrode

The CV responses of NO at the [Cu^{II}(TPP)]/Nafion-modified electrode are shown in Fig. 2. A broad anodic peak is observed at ca. 0.76 V, the peak current increasing with addition of NO solution. Meanwhile, no cathodic

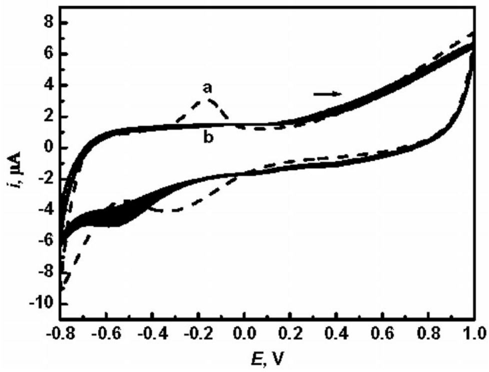

FIG. 1

Cyclic voltammograms at platinum electrodes modified with [Cu^{II}(TPP)] (a) and [Cu^{II}(TPP)]/Nafion (b) in a deoxygenated PBS solution (pH 7.4) at a scan rate of 100 mV s^{-1}

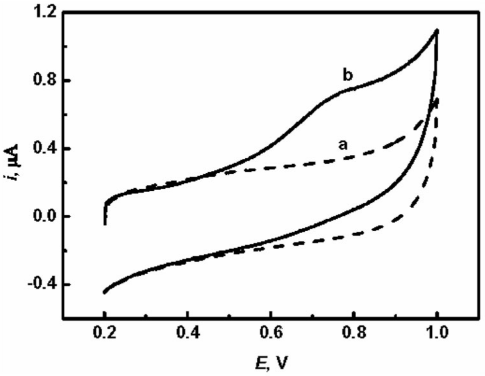

FIG. 2

Cyclic voltammograms of the [Cu^{II}(TPP)]/Nafion-modified electrode at the following NO concentrations (in M): 0.0 (a) and 7.2 × 10^{-5} (b). The potential scan rate was 100 mV s^{-1}

peak appears, which indicates that NO oxidation is an irreversible process. Figure 3 shows the differential pulse voltammograms (DPV) of NO at different electrodes. At the bare platinum electrode, no DPV response is observed (curve a). The DPV response to NO at the Nafion-modified platinum electrode exhibits only a broad small peak (curve b), which indicates that a moderate volume of Nafion could accumulate NO. However, at the [Cu^{II}(TPP)]/Nafion-modified electrode, an anodic peak is observed at 0.69 V, with the peak current increasing significantly (curves c, d). Compared with the Nafion-modified platinum electrode, the oxidation potential of NO at the [Cu^{II}(TPP)]/Nafion-modified platinum electrode is more negative. These experimental results suggest that [Cu^{II}(TPP)] can act as a catalyst for electro-oxidation of NO.

Some investigations indicate that the transition metal macrocyclic complexes such as metalloporphyrin and metallophthalocyanine can be used as biomimetic oxidation catalysts and oxygen carriers, called inorganic enzymes[11]. It has also been reported that these metal complexes were used as electrocatalysts to improve the redox response of NO (ref.[2]), although the catalytic mechanism for the NO oxidation and the role of the metal site remain unclear. This may be related to the well-known axial complexation ability of the central metal ion in a porphyrin complex towards small molecules such as O_2, CO and NO.

Here, we employed Pt electrode modified with a copper(II) porphyrin complex for determination of NO in a deoxygenated PBS solution. The

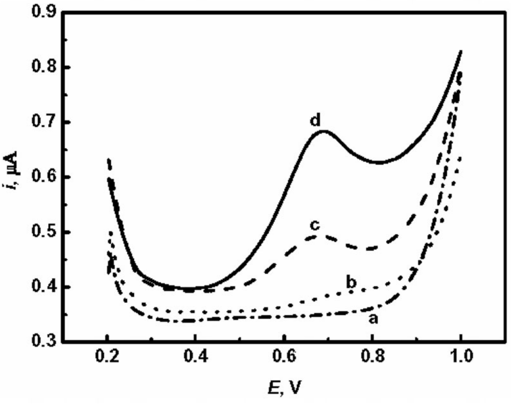

FIG. 3

Differential pulse voltammograms for different electrode surfaces: Bare platinum electrode (a), Nafion- (b) and [Cu^{II}(TPP)]/Nafion-modified platinum electrode (c, d) in a deoxygenated PBS solution at the following NO concentrations (in M): 3.6×10^{-5} (a, b, c) and 7.2×10^{-5} (d) enhancement of the current response was probably due to catalytic effects of the modified electrode. To our knowledge, the axial coordination ability of the d^9 Cu(II) center is very limited; nevertheless, the metal ion shows a strong electron affinity. NO is an electron-rich free radical and, consequently, can interact to form an adduct like [Cu^{II}(NO)(TPP)]. Thus, the mechanism of NO oxidation at the [Cu^{II}(TPP)]/Nafion-modified electrode could be described as given by Eqs (*1*) and (*2*).

$$[\mathrm{Cu^{II}(TPP)}] + \mathrm{NO} \rightarrow [\mathrm{Cu^{II}(NO)(TPP)}] \qquad (1)$$

$$[\mathrm{Cu^{II}(NO)(TPP)}] - \mathrm{e} \rightarrow [\mathrm{Cu^{II}(TPP)}] + \mathrm{NO^+} \qquad (2)$$

Chronocoulometry

The one-electron oxidation of NO at the [Cu^{II}(TPP)]/Nafion-modified electrode could be confirmed by chronocoulometry. Figure 4 depicts the linear plot of charge Q vs $t^{1/2}$ after the subtraction of the background charge while adding 3.6×10^{-7} M NO to a deoxygenated PBS solution. According to the Cottrell equation[12], the number of electrons involved in the process of NO oxidation could be estimated from the Cottrell slope of this plot (Eq. (*3*))

$$\text{slope} = 2nFAD^{1/2}C^{*}\pi^{-1/2} \qquad (3)$$

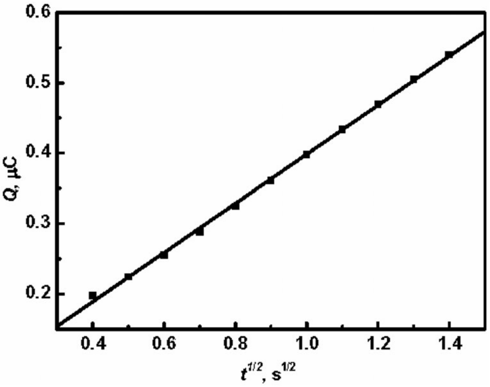

FIG. 4
Linear plot of a chronocoulometric response at the [Cu^{II}(TPP)]/Nafion-modified electrode. Initial potential 0.5 V, step potential 0.9 V

where n is the number of electron, A is the surface area of the electrode, C^* is the bulk concentration of NO and D is the diffusion coefficient of NO in aqueous solution, t is time of electrolysis and other symbols have their usual meaning. The following values were used: $A = 0.018\ \mathrm{cm}^2$, $C^* = 3.6 \times 10^{-8}\ \mathrm{mol\ cm}^{-3}$, $D = 2.5 \times 10^{-5}\ \mathrm{cm}^2\ \mathrm{s}^{-1}$ (based on the value reported by Colucci et al.[13]) and slope = $3.491 \times 10^{-7}\ \mathrm{C\ s}^{-1/2}$. Thus, the calculated value of n is equal to 0.99, which is in agreement with the value reported by Manlinski and Taha[2].

Effects of Nafion on the Response of the Modified Electrode

Nafion, a cation exchanger, can not only exclude some anionic interfering analytes such as nitrite, nitrate and ascorbate, diminish the electrode fouling due to non-specific adsorption of macromolecules in biological sample, but can also enhance the mechanical strength of the film[14,15]. Therefore, it is widely used to modify the electrode surface in electrochemistry. For the determination of NO, it is common to coat the electrode surface with Nafion, as NO_2^- and NO have similar oxidation potentials. The Nafion film can prevent the diffusion of NO_2^- to the modified electrode surface, while the neutral NO can diffuse easily through the coatings. In addition, the Nafion film should also stabilize NO^+ formed by the oxidation of NO and prevent a complicated pattern of reactions that could possibly lead to the formation of NO_2^- and NO_3^- (ref.[2]). However, it is equally important to optimize the coverage of Nafion. A series of experiments were carried out with the same concentration of NO at the [Cu^{II}(TPP)]-modified platinum electrode further coated with 0, 1, 3, 5 and 8 µl of 1% (w/v) Nafion solution (data not shown). The results indicate that the NO anodic peak current slightly decreases with the increasing amount of Nafion. At the same time, the results of the study of selectivity for NO_2^- show that thick films can provide the highest barrier against diffusion of NO_2^-. Thus, 5 µl of 1% (w/v) Nafion solution was chosen as the modification concentration. From previous studies[16], the thickness of Nafion-film is in the range of 2–3 µm.

Selectivity of the [Cu^{II}(TPP)]/Nafion-Modified Electrode

Figure 5 shows differential pulse voltammograms of solutions of 2.0×10^{-4} M NO_2^- (curve a), 3.6×10^{-5} M NO (curve b) and 3.6×10^{-5} M NO + 2.0×10^{-4} M NO_2^- (curve c) using the modified electrode. No anodic peak in curve a appears between 0.2 and 1.0 V, which indicates that NO_2^- is not oxidized at the [Cu^{II}(TPP)]/Nafion-modified electrode. No current and potential change for the oxidation of NO in curves b and c is observed, indicating that the [Cu^{II}(TPP)]/Nafion-modified electrode could provide high selectivity for NO_2^-. From the other viewpoint, it also indirectly demonstrates the one-electron transfer reaction of NO at the modified electrode, which leads to the formation of NO^+ but no NO_2^- and NO_3^-. Otherwise, we would observe increased current due to the NO_2^- oxidation. Under the chosen conditions, 3 × 10^{-4} M ascorbic acid and 1.0 × 10^{-5} M dopamine did not interfere with the determination of 1.8 × 10^{-5} M NO.

Linearity, Sensitivity, Reproducibility and Stability of the Modified Electrode

Figure 6 displays the chronoamperometric response of the modified electrode to successive additions of NO. The chronoamperogram clearly shows an increase in anodic current with the number of NO injections. The response curve gradually decreased after injections of the standard NO solution since the radical could react with some species at the electrode surface. Previously reported papers dealing with polymer-modified electrode showed a similar declining pattern while the solution was deaerated using a nitrogen gas[17]. A linear relationship with a correlation coefficient of 0.9994 was obtained over the range from 1.8 × 10^{-7} to 9.0 × 10^{-4} M NO (see insert

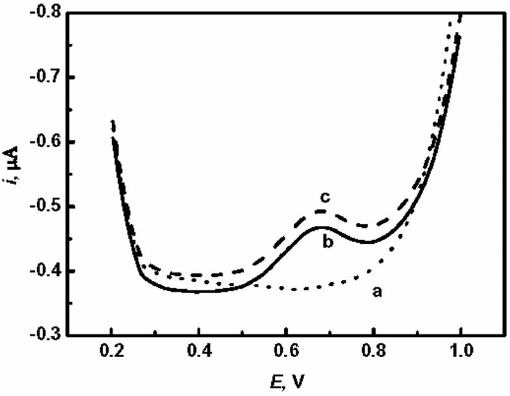

FIG. 5
Differential pulse voltammograms of [Cu^{II}(TPP)]/Nafion-modified electrode in a deoxygenated PBS solution containing 2.0 × 10^{-4} M NO_2^- (a), 3.6 × 10^{-5} M NO (b), 3.6 × 10^{-5} M NO + 2.0 × 10^{-4} M NO_2^- (c)

in Fig. 6). The minimum detectable concentration of NO is estimated to be 2×10^{-8} M (based on a signal-to-noise ratio of 3). The DPV responses of the modified electrode in ten repeated measurements of 1.8×10^{-5} M NO solutions remained almost the same, with a relative standard deviation of 6.3%. Even with the modified electrode kept in phosphate buffer solution for two weeks, the peak current for the same NO concentration decreased only to ca. 7.4%. This suggests that the modified electrode shows a good reproducibility and stability in the determination of NO.

Direct Measurement of NO Release from Sodium Nitroprusside

Nitric oxide (NO) has been confided as a mediator of vasodilatation in the cardiovascular system and as a neurotransmitter in the central nervous system[3,4]. NO imbalance in endothelium may be used as an early sign of vascular diseases such as atherosclerosis, hypertension and myocardial ischemia. Recently, much attention has been focused on the development of drugs that can release NO either spontaneously or enzymatically. Sodium nitroprusside (SNP), an NO donor, is a quick-acting vasodilator which is used in clinical medicine to cure hypertension. The mechanism of cardioprotection of SNP is still debatable. Nevertheless, it has been reported that the mechanism appears to be related to the ability to release NO. In our experiment, the determination of NO released from SNP has been

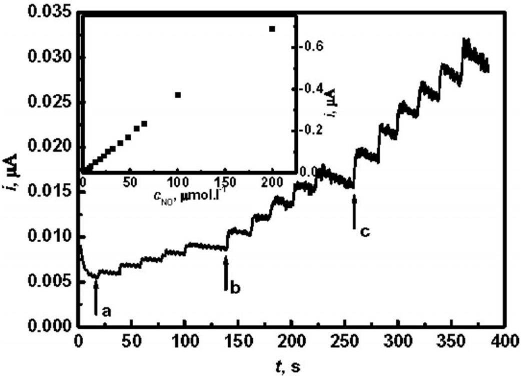

FIG. 6

Chronoamperometry of [Cu^{II}(TPP)]/Nafion-modified electrode in a deoxygenated PBS solution with successive additions of NO (in M): 0.9×10^{-6} (5 times) (*a*), 2.2×10^{-6} (5 times) (*b*), 4.5×10^{-6} (6 times) (*c*). Inset: Linear variations of the measured currents with the NO concentration achieved by the chronoamperometric method, as shown in Fig. 7. Significant current responses are observed. They increase almost linearly with time in the presence of light. After the illumination, the current gradually decreases. This result also confirmed a report in the literature[18], claiming that light exposure releases NO from SNP in a rapid photodegradation process, and confirming the usefulness of the [Cu^{II}(TPP)]/Nafion-modified electrode for the determination of NO.

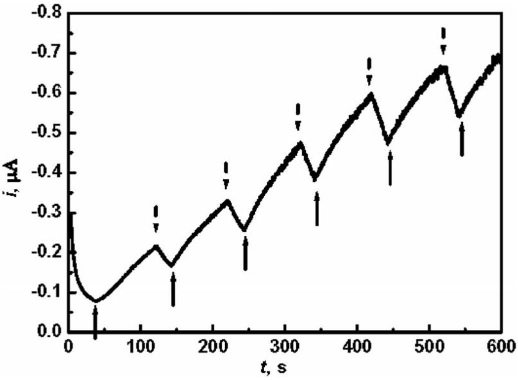

FIG. 7
Chronoamperometry of [Cu^{II}(TPP)]/Nafion-modified electrode in a deoxygenated PBS solution containing 6.4×10^{-3} M sodium nitroprusside (SNP) in the presence of light. The arrows (↑) and (↓) indicate the start and the end of illumination, respectively

CONCLUSION

This study reveals that [Cu^{II}(TPP)] is an excellent material for the electro-oxidation of NO. The [Cu^{II}(TPP)]/Nafion-modified platinum electrode is suitable for the electrochemical detection of NO in the concentration range of 1.8×10^{-7}–9.0×10^{-4} M and, therefore, can be developed as a new electrochemical sensor for the determination of NO in aqueous solution. The results show that NO release from SNP is a rapid process, which is helpful in studying the mechanism of drug action. In addition, a probable one-electron character of NO oxidation is presented, which will provide a new insight into the mechanism of NO sensors based on chemically modified electrodes.

This research was supported by the National Natural Science Foundation of P.R. China (grants No. 30370397 and No. 60571042) and by the provincial Natural Science Foundation of Hubei (grant No. 2004ABA116).

REFERENCES

1. Trevin S., Bedioui F., Villegas M. G. G., Bied-Charreton C.: *J. Mater. Chem.* **1997**, *7*, 923.
2. Malinski T., Taha Z.: *Nature* **1992**, *358*, 676.
3. Shibuki K.: *Neurosci. Res.* **1990**, *9*, 69.
4. Moncada S., Palmer R. M. J., Higgs E. A.: *Pharmacol Rev.* **1991**, *43*, 109.
5. Taha T. H.: *Talanta* **2003**, *61*, 3.
6. Bedioui F., Villeneuve N. L.: *Electroanalysis* **2003**, *15*, 5.
7. Gryglewski R. J., Palmer R. M., Moncada S.: *Nature* **1986**, *320*, 454.
8. Thomas D. W., Martell A. E.: *J. Am. Chem. Soc.* **1959**, *81*, 5111.
9. Butler A. R., Williams D. L. H.: *Chem. Soc. Rev.* **1993**, 233.
10. Zhuang Q. K.: *Microchem. J.* **2000**, *65*, 333.
11. Bedioui F.: *Coord. Chem. Rev.* **1995**, *144*, 39.
12. Bard A. J., Faulkner L. R.: *Electrochemical Methods, Fundamentals and Applications*, p. 210. Wiley, New York 2001.
13. Colucci J. A., Foral M. J., Langer S. H.: *Electrochim. Acta* **1985**, *30*, 521.
14. Moussy F., Harrison D. J.: *Anal. Chem.* **1994**, *66*, 674.
15. Moussy F., Jakeway S., Harrison D. J., Rajottet R. V.: *Anal. Chem.* **1994**, *66*, 3882.
16. Guadalupe A. R., Liu K. E., Abruna H. D.: *Electrochim. Acta* **1991**, *36*, 881.
17. Park J.-K., Tran P. H., Chao J. K. T., Qhodadra R., Rangarajan R., Thakor N. V.: *Biosens. Bioelectron.* **1998**, *13*, 1187.
18. Joseph J., Kalyanaraman B., Hyde J. S.: *Biochem. Biophys. Res. Commun.* **1993**, *192*, 926.