Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 33 (2003) 735–744
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Electrochemical oxidation of biological molecules at carbon paste electrodes pre-treated in guanine solutions

Robson Pinho da Silva, Silvia H.P. Serrano *

Departamento de Química Fundamental-Instituto de Química, Universidade de São Paulo, Av. Prof. Dr. Lineu Prestes, 748-B 8sup, CEP: 05508-900, São Paulo (SP), Brazil

Received 22 December 2002; received in revised form 30 April 2003; accepted 14 May 2003

Abstract

Carbon paste electrodes were modified in guanine solutions under an applied potential of 1.1 V and used for electrochemical detection of NADH, NADPH, uric acid and 8-oxoguanine. Detection limits were 3.3, 3.7, 6.6 and 2.0×10^{-6} M, respectively, with sensitivity of 0.13, 0.10, 0.26 and 0.40 A mol^{-1} l cm^{-2}, respectively. The electrodes showed high reproducibility and absence of surface poisoning effects. Good analytical performance was attributed to the formation of superficial dimer or trimers species of guanine during the modification process.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Carbon paste modified electrodes; Differential pulse voltammetry; NAD(P)H; Guanine; Uric acid; 8-Oxoguanine; Guanine dimer

1. Introduction

Chemically modified electrodes have been used in biomedical [1], environmental [2,3] and food analysis [3,4], where high sensitivity and selectivity are required, and electrode poisoning effects are to be avoided. Several materials can be used to modify [5–8] and/or to design new electrodes [9–11], that are used in various electroanalytical applications.

High sensitivity and selectivity are obtained using differential pulse and square wave voltammetry associated with pre-concentration steps [3,5,6,9–11]. Superficial modifier agents can promote selective recognition due to charge expulsion [5], size [6,11], adsorption and ion exchange [9], or specific biological interactions.

A number of DNA-modified electrodes have been described [12–17] in the last few years. Applications in the field of electroanalysis have included drug determinations [13,18–22], protein fast electron transfer [23], DNA–drug interactions [20,24–30], detection of DNA hybridisation [31–34], and detection of single-base mismatches or mutations [35,36]. DNA has also been used to immobilize enzymes [37], and rhodium compounds [38] and DNA–rhodium dimer interactions have proven to be promising tools for future Quantita-

* Corresponding author. Tel.: +55-11-3091-3837x226; fax: +55-11-3815-5579.
E-mail address: shps@iq.usp.br (S.H.P. Serrano).

0731-7085/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0731-7085(03)00296-6

tive Structure–Activity Relationship (QSAR) studies [39].

In a previous report we have shown that glassy carbon surface can be modified by adsorption of DNA degraded molecules [12] as well as the 5′-deoxyguanosine-monophosphate nucleotide [15], but no further studies have been done to evaluate if the guanine modified electrodes could be used to avoid poisoning electrode effects and/or to promote catalytic oxidation of important biological molecules, which oxidation products are able to block the electrode surface. Therefore, in this paper we describe the electroxidation of NADH, NADPH, uric acid and 8-oxoguanine using carbon paste electrodes pre-treated in guanine solutions at 1.1 V.

Carbon paste electrodes were chosen because of their low background currents, high active superficial area and mainly due to the presence of Nujol, which provides a more hydrophobic electrode surface, improving adsorption of organic molecules, than those other solid carbon electrodes.

2. Experimental

2.1. Chemicals and solutions

β-NADH, disodium salt; NADPH, tetrasodium salt (purity grade, calc. on anhydrous substance, as $\geq 95\%$), sodium nitrate; sodium hydroxide, sodium acetate, citric acid, boric acid and acetic acid were obtained from Merck (Rio de Janeiro, Brazil). Piperazine-*N*,*N′*-bis [2-ethanesulphonic acid] (PIPES) and guanine hydrochloride were obtained from Alfa AESAR. 2-Amino-6, 8 dihydroxypurine (8-oxoguanine), uric acid and Nujol oil were obtained from Aldrich Chemical Co. Graphite powder used to prepare the carbon paste electrodes was Acheson 38 from Fisher. All reagents were used without previous purification.

Stock solutions of 5.0×10^{-2} M guanine hydrochloride and 2-Amino-6,8-dihydroxypurine (8-oxoguanine) were prepared by dissolving the respective salts in 1 M NaOH.

Guanine hydrochloride and 8-oxoguanine solutions, used to modify the carbon paste electrodes, were prepared by addition of 100 μl of the respective stock solutions to the electrochemical cell containing 10.0 ml of acetate buffer, pH 4.5; PIPES buffer, pH 7.0 or universal buffer, pH 8.0. NADH and NADPH stock solutions (3.0×10^{-3} M) were prepared just prior the measurements by dissolving appropriate quantities of the salt in PIPES buffer solution. Uric acid and 8-oxoguanine solutions (3.0×10^{-3} M) were prepared dissolving appropriate quantities of salts in 0.1 M NaOH (1.0 M). Acetate buffer, pH 4.5 and PIPES buffer were prepared as described in the literature [40]. Universal buffer was prepared by addition of 2 M NaOH to a stock solution containing 0.4 M of boric acid, 0.4 M of acetic acid and 0.4 M of phosphoric acid until get pH 8.0 [41].

All solutions were prepared using purified water from a Barnstead Nanopure system.

2.2. Apparatus

All electrochemical measurements were performed using a PGSTAT 20 potentiostat/galvanostat, running with the GPES version 4.3 from Eco Chemie, Utrecht, Netherlands. Differential pulse voltammetry experiments were run with a: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s^{-1}. All pH measurements were carried out using a pH Meter Model 654 with a 6.0203.100 (OE) combined glass electrode, both from Metrohm.

2.3. Electrodes

The working electrodes were carbon paste (CPE), $A = 0.080$ cm^2, prepared from 2:1 graphite/Nujol mixture (w/w). Pt wire and a miniaturized Ag/AgCl (sat. KCl) electrodes were used as the counter and reference electrodes, respectively, all placed in a one-compartment cell.

2.4. Experimental procedure

2.4.1. Modification of carbon paste electrodes in guanine or 8-oxoguanine solutions

One hundred microlitres of guanine hydrochloride or 8-oxoguanine from the corresponding stock solutions was added to the electrochemical

cell containing 10.0 ml of buffer solution as supporting electrolyte. Potentials of 0.2, 0.4, or 1.1 V vs. Ag/AgCl were applied to the working electrode during 12 min under stirring. The modified electrodes were washed with deionized water before further utilization.

2.4.2. Modification of carbon paste electrodes in buffer solutions

Carbon paste electrodes were also modified in universal buffer solutions; pH 8.0 under the same conditions used for modification in guanine or 8-oxoguanine solutions. These electrodes were denominated as CPE and were used as a control to evaluate the role of guanine or 8-oxoguanine in the modification process. Before utilization the electrodes were washed with deionized water.

Table 1 presents the composition of the modifying solutions as well as the electrode denomination used in this paper.

2.5. NAD(P)H, uric acid and 8-oxoguanine detections

Several differential pulse voltammetry experiments using carbon paste modified electrodes in buffer, guanine, and 8-oxoguanine solutions, were performed in the supporting electrolyte solution until a stable baseline was achieved. Four aliquots of 25 μl, 5 aliquots of 50 μl, 1 aliquot of 550 μl and 4 aliquots of 750 μl of the analyte (3.0×10^{-3} M) stock solution were added to the electrochemical cell containing 10.0 ml of PIPES buffer, pH 7.0 and 3 consecutive differential pulse voltammograms were recorded for each concentration in the range $0.2 \leq E_{appl} \leq 0.9$ V.

Table 1
Composition of the modifying solutions and electrode denominations

Composition of the modifying solution	pH	Electrode denomination
Guanine in PIPES buffer	7.0	CPE/G pH 7.0
Guanine in acetate buffer	4.5	CPE/G pH 4.5
Guanine in universal buffer	8.0	CPE/G pH 8.0
8-oxoguanine in PIPES buffer	7.0	CPE/8-oxo pH 7.0
Universal buffer	8.0	CPE

3. Results and discussion

Carbon paste electrodes were initially pre treated in guanine solutions (pH 7.0) at 0.42 and 1.1 V. Electrochemical signals due to NADH oxidation were lower at electrodes modified at 0.42 V. An additional peak was recorded at 0.8 V, which was attributed to the oxidation products of interfacial species formed during the oxidation of guanine [42,43], Fig. 1.

According to Goyal and Dryhurst [42] guanine is oxidized to 8-oxoguanine at a PGE (pyrolytic graphite electrode) at 0.66 V vs. SCE (pH 7.0) while 8-oxoguanine is oxidized at 0.32 V (pH 7.0) to produce new products, which can also oxidized. The spike-shaped form of the peaks is an indicative of an electrode process where the electroactive species are adsorbed on the electrode surface. Therefore, by the application of a potential of 1.1 V during the pre-treatment of the carbon paste electrode in guanine solution, all 8-oxoguanine produced at the electrode surface is immediately

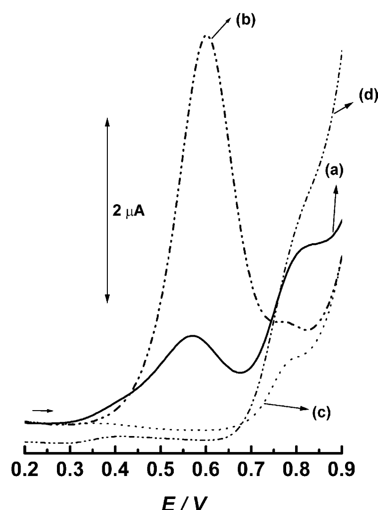


Fig. 1. Differential pulse voltammograms recorded at 4.2×10^{-4} mol l^{-1} NADH solution (curves a and b) and at PIPES solution, pH 7.0, without NADH (curves c and d) using: (a) (CPE/G; pH 7.0) modified under 0.42 V applied potential during 12 min; (b) (CPE/G; pH 7.0) modified under 1.1 V applied potential during 12 min; (c) (CPE/G; pH 7.0) modified under 1.1 V applied potential during 12 min; (d) (CPE/G; pH 7.0) modified under 0.42 V applied potential during 12 min. Experimental conditions: pulse amplitude 50 mV, pulse width 70 ms, and scan rate 5 mV s^{-1}.

oxidized and one of its oxidation products could be involved in the superficial modification of the electrode.

To get some insight on which species is responsible for the electrode superficial modification, carbon paste electrodes were also pre-treated in 8-oxoguanine solutions at 0.20 V (before 8-oxoguanine oxidation step) and at 1.1 V (after 8-oxoguanine oxidation step), Fig. 2. In both cases the NADH peak currents and corresponding peak potential were similar. However, successive voltammetric runs show a small decrease of the current peak showing superficial poisoning effect (Fig. 2a and b). Conversely, carbon paste electrodes pre-treated in guanine solution at 1.1 V showed current intensities that were almost two times higher than those obtained with electrodes pre-treated in 8-oxoguanine solution and no superficial poisoning effect was observed (Fig. 2c). Therefore, the superficial species, which is formed during the pre-treatment of the carbon paste electrode in guanine solution at 1.1 V, cannot be obtained using only 8-oxoguanine as modifying solution.

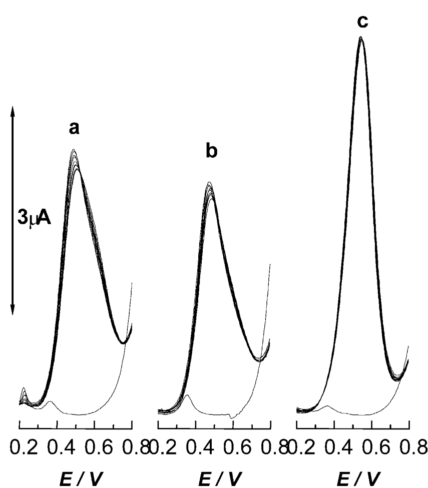
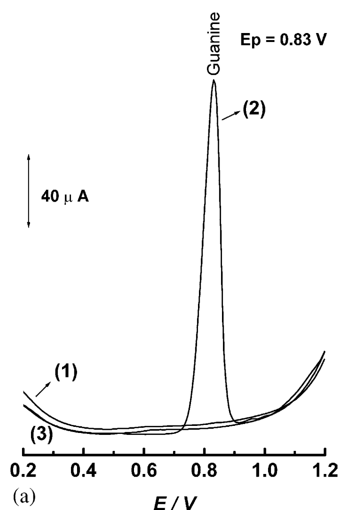


Fig. 2. Differential pulse voltammograms recorded at 4.2×10^{-4} mol l^{-1} NADH solutions using: (a) (CPE/8-oxo pH 7.0) modified under 0.2 V applied potential during 12 min; (b) (CPE/8-oxo pH 7.0) modified under 1.1 V applied potential during 12 min; (c) (CPE/G pH 7.0) modified under 1.1 V applied potential during 12 min. Experimental conditions similar to those of Fig. 1.

It is possible to explain the difference between the modified electrode surfaces obtained with pre-treatment in 8-oxoguanine solution (Fig. 2a and b) those obtained with pre-treatment in guanine solution (Figs. 1 and 2c) considering the model proposed by Subramanian and Dryhurst for the electrochemical oxidation of guanosine at a pyrolytic graphite electrode [44] and the report from Brett et al. [45] for oxidation of guanine at glassy carbon microelectrodes. Subramanian and Dryhurst [44] showed that the primary guanosine oxidation step involves a 1 e^{-}–1H^{+} reaction leading to a free radical with an unpaired electron located at the C (8) position. This primary radical reacts with guanosine and water to yield several other radicals that undergo a series of chemical and electrochemical reactions to give some guanine di and tri-nucleosides. In principle, these authors in reference admitted that oligomeric products are not formed in the electroxidation of guanine due to the low solubility of this compound (20–25 times less than guanosine). Low concentration of C (8) guanyl radicals should be produced to promote the formation of di- and trimer guanine species. On the other side, Brett et al. [45] showed that the 5th differential pulse voltammogram recorded in 5.0×10^{-5} M guanine solution, pH 4.5, presents three peaks: (1) at 0.55 V (due to the reversible two electron/two proton oxidation of 8-oxoguanine formed on the electrode surface); (2) at 0.8 V (due to the oxidation of guanine to 8-oxoguanine); and (3) at 0.95 V (due to the one electron transfer reversible oxidation of the guanine dimers). After transfer the electrode to another electrochemical cell containing only supporting electrolyte, successive differential pulse voltammograms were recorded and only one peak, recorded at 1.0 V (pH 4.5), which was attributed to the oxidation of guanine dimmer adsorbed on the electrode surface.

Oxidation of 5.0×10^{-5} M guanine at carbon paste electrode pre-treated at 1.1 V in buffer solution (pH 4.5) occurred at 0.83 V. After transfer the electrode to an other electrochemical cell containing acetate buffer pH 4.5 no peaks were detected, Fig. 3a. Conversely, the differential pulse voltammogram recorded in acetate buffer pH 4.5 using the carbon paste electrode pre-treated in



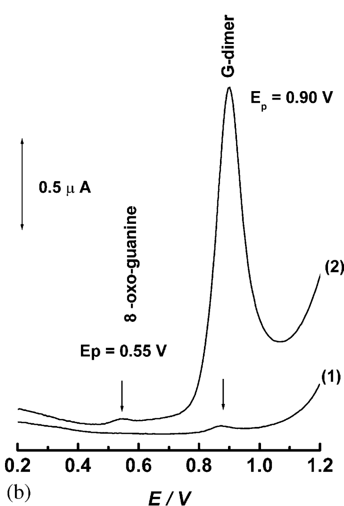


Fig. 3. (a) Differential pulse voltammograms recorded using carbon paste electrode pre-treated in acetate buffer pH 4.5 during 12 min at 1.1 V: (1) Acetate buffer, pH 4.5; (2) 50 μM of guanine solution, pH 4.5, 5th scan; (3) Acetate buffer, pH 4.5 after (2). Experimental conditions similar to those of Fig. 1. (b) Differential pulse voltammograms recorded in acetate buffer solution pH 4.5 using: (1) CPE electrode pre-treated in 5.0×10^{-5} M of guanine solution, pH 4.5, during 12 min at 1.1 V; (2) CPE electrode pre-treated in 5.0×10^{-4} M of guanine solution, pH 4.5, during 12 min at 1.1 V (CPE/G pH 4.5). Experimental conditions similar to those of Fig. 1.

5.0×10^{-5} M of guanine solution (pH 4.5) during 12 min at 1.1 V presented only one small stable peak recorded at 0.88 V. Increasing the concentration of guanine in the modifying solution to 5.0×10^{-4} M (as described in Section 2), two stable peaks were detected in acetate buffer, pH 4.5: (i) at 0.55 V, due the oxidation of 8-oxoguanine; and (ii) at 0.90 V, which can be atributted to the oxidation of di- or trimer guanine structures adsorbed on the electrode surface, Fig. 3b. The latter peak potential is about 100 mV less positive than those described previously [45] and consequently the film composition and the superficial molecular arrangement obtained using macro carbon paste electrodes can be lightly different than those obtained using glassy carbon microelectrodes. Our data are not sufficient to concluded something about the immobilization of 8-oxoguanine in the film structure because it should be instantaneously oxidized at the applied potential used during the modification step and additional studies are necessary to clarify this point.

The disappearance of the PIPES oxidation peak at 1.1 V, which occurred only at the electrodes which were pre-treated in guanine solutions (Fig. 4d and e), show the total recover of the electrode surface and can be used as a control to the modification step.

The carbon paste electrode pre-treated in guanine solution at 1.1 V was then chosen for later measurements.

The pH effect on the modification process was evaluated using solutions at pH 4.5 and 8.0. As shown in Fig. 5, high current levels for NADH oxidation were obtained at pH 8.0 and these last conditions were used for further modification process.

These electrodes were used for detection of NADH, NADPH, uric acid and 8-oxoguanine. Because graphite has oxygen containing functional groups, and their number increases with anodic pre-treatment, the results were compared with those obtained using carbon paste electrodes modified only from buffer solutions. Data presented in Fig. 6 were obtained in the range $7.5 \times 10^{-6} \leq [\text{analyte}] \leq 8.1 \times 10^{-4}$ mol l^{-1}, averaged from values of two consecutive analytical curves with 14 concentration values, with three differential pulse voltammograms being recorded for each one-concentration value. Detection limits [46] and sensitivity were estimated from these data using

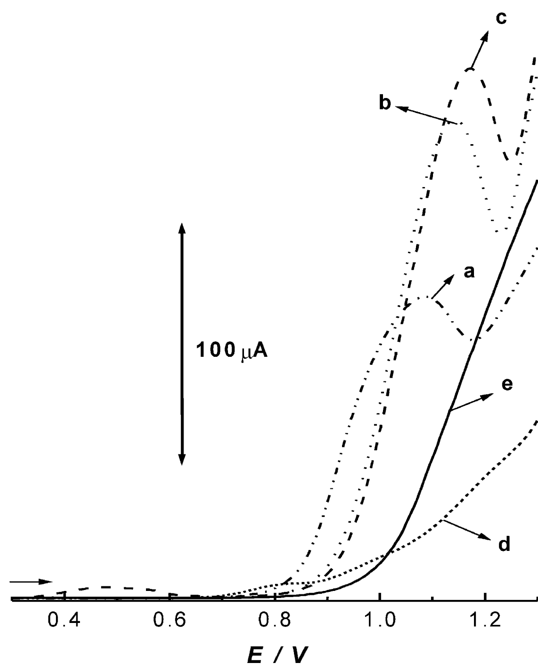


Fig. 4. Differential pulse voltammograms recorded in PIPES buffer, pH 7.0 using: (a) (CPE); (b) (CPE/8-oxo pH 7.0) modified under 0.2 V applied potential during 12 min; (c) (CPE/ 8-oxo pH 7.0) modified under 1.1 V applied potential during 12 min; (d) (CPE/G pH 7.0) modified under 1.1 V applied potential during 12 min; (e) (CPE/G pH 7.0) modified under at 0.4 V applied potential during 12 min. Experimental conditions similar to those of Fig. 1.

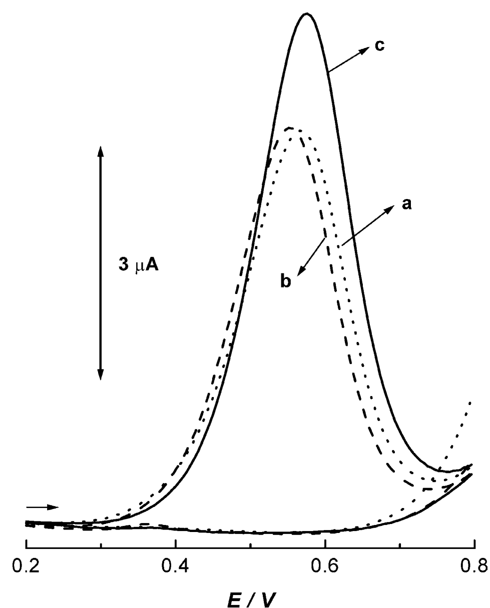


Fig. 5. Differential Pulse Voltammograms recorded from 4.2×10^{-4} mol l^{-1} NADH solutions using: (a) (CPE/G; pH 4.5) modified under 1.1 V applied potential during 12 min; (b) (CPE/G; pH 7.0) modified under 1.1 V applied potential during 12 min; (c) (CPE/G; pH 8.0) modified under 1.1 V applied potential during 12 min. Experimental conditions similar to those of Fig. 1.

values in the range $7.5 \times 10^{-6} \leq$ [analyte] $\leq 7.3 \times 10^{-5}$ mol l^{-1}, Table 2.

In summary, 84 differential pulse voltammograms were recorded using the same modified electrode for one analyte without any superficial cleaning between analytical curve recordings.

A large class of $NAD(P)^{+}$ dependent dehydrogenase enzymes can be used for the construction of amperometric enzyme sensors if a suitable redox mediator catalyses the electron transfer from NAD(P)H to the electrode surface. Several compounds were tested for this purpose and solid electrodes modified with cationic redox dyes [47–53] as well conducting films derived from poly-*p*-phenylenediamine [54], poly (*o*-aminophenol) and poly (*o*-phenylenediamine) [55] showed good performance for NADH oxidation at lower applied potentials, necessary condition to use the biosensors in biological fluid analysis without previous separation procedure. Sometimes, an excellent electrocatalytic property towards NADH oxidation is obtained [56], but a quite rapid degradation of the NADH electrocatalytic signal is observed if the electrochemically produced NAD^{+} is not removed. Recently Santos-Álvarez et al. [57] described an interesting paper showing the electrocatalytic determination of NADH at 50 mV (vs. Ag/AgCl, KCl saturated electrode) using a graphite electrode modified with a quinone-diimine specie derivative from the oxidation product of 2,8-dihydroxyadenine nucleotide. Analytical measurements were be done at NADH concentration levels below 1×10^{-6} M with detection limit of 1.3×10^{-7} M using electrodes with 100 h of operational life.

Data presented in Table 2 show that the carbon paste electrode pre-treated in guanine solutions at 1.1 V do not have catalytic effects towards NAD(P)H oxidation. The modified surface only avoided the poisoning effects produced by the

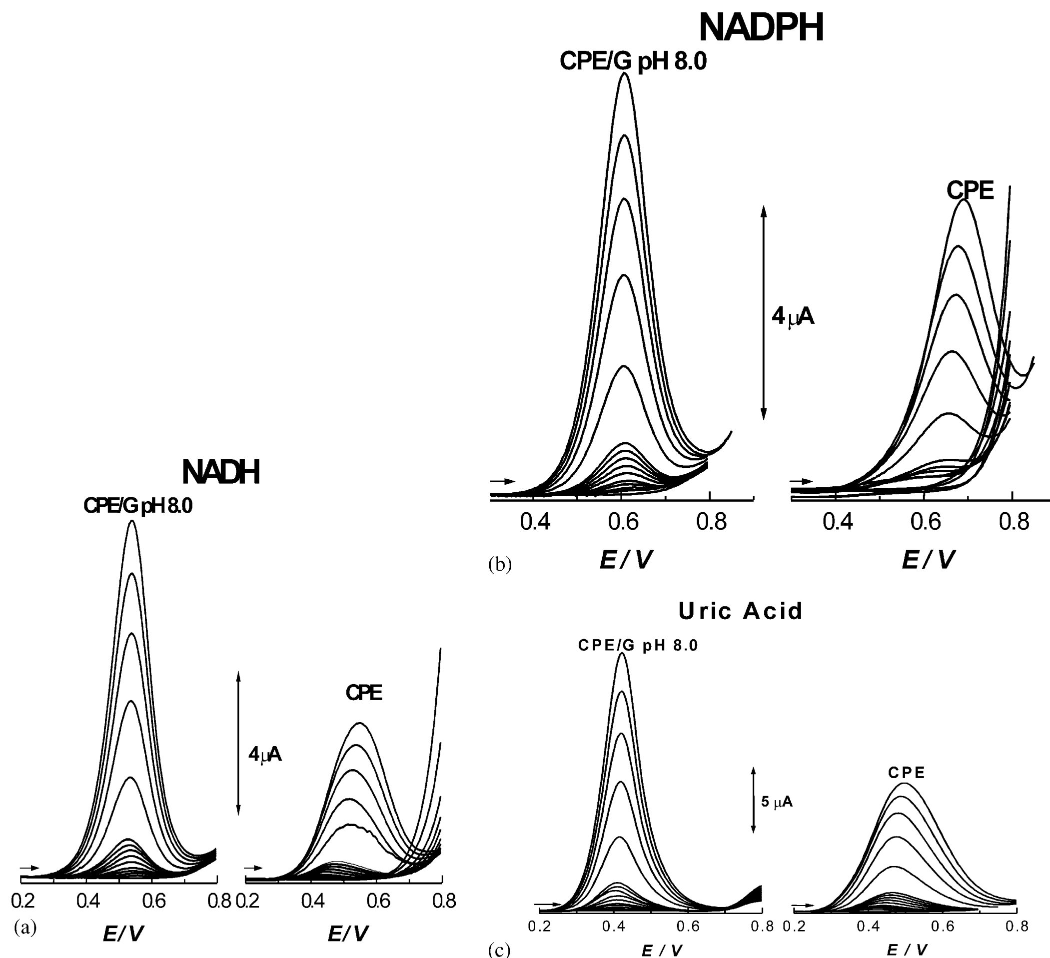


Fig. 6. (a) Differential pulse voltammograms recorded in NADH solutions and PIPES buffer, pH 7.0. Experimental conditions similar to those of Fig. 1. (b) Differential pulse voltammograms recorded in NADPH solutions and PIPES buffer, pH 7.0. Experimental conditions similar to those of Fig. 1. (c) Differential pulse voltammograms recorded in uric acid solutions and PIPES buffer pH 7.0. Experimental conditions, similar those of Fig. 1. (d) Differential pulse voltammograms recorded in 8-oxoguanine solutions with PIPES buffer, pH 7.0. Experimental conditions similar those of Fig. 1.

adsorption of the analyte oxidation products improving the sensitivity and the detection limits for NAD(P)H and uric acid determinations. It is possible that the superficial guanine dimeric structure avoided the formation of specie derivative from 8-oxoguanine, which, in principle, could be present catalytic properties as those, presented by 2,8-dihydroxyadenine derivative.

The application of this electrode to design new biosensor is conditioned to the utilization of enzymes (or another biological component) incorporated into the carbon paste as well the possibility of preparing a similar modified surface in the presence of an efficient electron mediator.

On the other side, there are several biological fundamental studies where ‘clean solutions’ are used and it is possible to work with high-applied potential, but it is not possible to do it with a sensor that has low lifetime. Under this viewpoint, our sensor is easy to prepare, has good stability and sensitivity. It also can be used as an electrochemical detector (ED) in High Performance

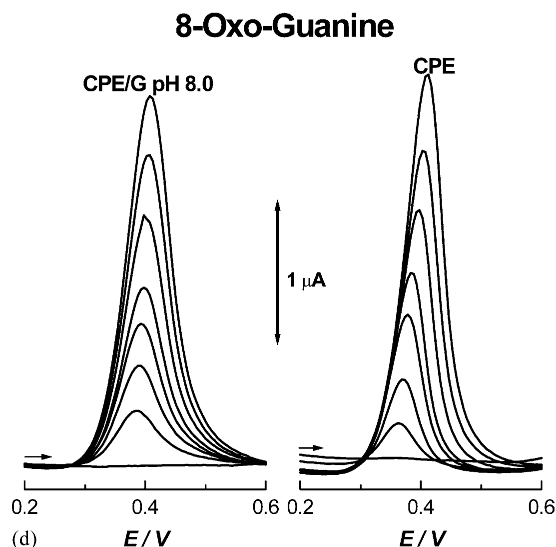


Fig. 6 (*Continued*)

Table 2
Sensitivity, detection limits and shifting on the oxidation peak potentials for two consecutive analytical curves carried out with carbon paste electrode pre-treated in buffer solution (CPE) and carbon paste electrode pre-treated in guanine solution pH 8.0 (CPE/G pH 8.0) during 12 min at 1.1 V

Analyte	Electrode	ΔEp (V)	Sensitivity ($A\ mol^{-1}\ l\ cm^{-2}$)	Detection limit ($M \times 10^6$)
NADH	CPE/G pH 8.0	0.56–0.53	0.13 ± 0.04	3.3
	CPE	0.48–0.69	0.04 ± 0.01	5.8
NADPH	CPE/G pH 8.0	0.61–0.60	0.10 ± 0.01	3.7
	CPE	0.52–0.71	0.01 ± 0.01	14
Uric acid	CPE/G pH 8.0	0.41–0.42	0.26 ± 0.06	6.6
	CPE	0.41–0.55	0.13 ± 0.11	13
8-oxoguanine	CPE/G pH 8.0	0.39–0.41	0.40 ± 0.08	2.0
	CPE	0.36–0.42	0.36 ± 0.13	3.7

Three differential pulse voltammograms were recorded for each one-concentration value in the range $7.5 \times 10^{-6} \leq [\text{analyte}] \leq 8.1 \times 10^{-4}$ M, but sensitivity and detection limits were calculated using average values in the range $7.5 \times 10^{-6} \leq [\text{analyte}] \leq 7.3 \times 10^{-5}$ mol l^{-1}.

Liquid Chromatography (HPLC). Electrochemical quantification of 8-oxoguanine, the major of modified base in DNA produced by oxidative DNA damage [58–60], as well low levels of uric acid in the brain tissues, which can be related the Alzheimer's disease [61], are an important example of an analytical and clinical problems to be solved. In this case the utilization of HPLC–ED is the most recommended method to be used.

4. Conclusions

Carbon paste electrodes pre-treated in guanine solutions, pH 8.0, under 1.1 V applied potential (CPE/G pH 8.0) showed better performance for oxidation of NADH, NADPH and uric acid than the electrodes pre-treated in universal buffer solutions, pH 8.0 (CPE), or guanine (at 0.42 V) and 8-oxoguanine (at 0.2 or 1.1 V) solutions.

Conversely to the CPE, the oxidation peak potentials at a (CPE/G pH 8.0) did not shift to more positive values during the consecutive scans at high NAD(P)H and uric acid concentrations (Table 2) and the high currents obtained with the latter electrode can be attributed to the superficial guanine di- or trimer layer that allowed the superficial analyte pre-concentration and avoided the adsorption of the analyte oxidation products on the electrode surface. Perhaps, due to superficial exclusion effects, these properties were not observed during the oxidation of 8-oxoguanine and the good results obtained with CPE towards the oxidation of this specie can be attributed to the absence of the adsorption of the oxidation products on bare electrode surface.

CPE/G pH 8.0 did not shown electrocatalytic effects but have good stability to be used as an electrochemical detector in HPLC analysis.

Acknowledgements

Robson Pinho da Silva thanks CNPq for a fellowship; we thank FAPESP (Process number 2001 /01192-3) and CNPq for financial support, to Drs Paulo Celso Isolani, José Manuel Riveros Nigra, Paulo Teng An Sumodjo and the referees from JPBA for the helpful comments.

References

[1] G.K. Budnikov, J. Anal. Chem. 55 (2000) 1014–1023.
[2] P.I. Ortiz, P.R.A. Nader, H.A. Mottola, Electroanal. 5 (1993) 165–169.
[3] Kh.Z. Brainina, N.A. Malakhova, N. Yu, F. Stojko, J. Anal. Chem. 368 (2000) 307–325.
[4] L.D. Mello, L.T. Kubota, Food Chem. 77 (2002) 237–256.
[5] Z. Gao, A. Ivaska, Anal. Chim. Acta 284 (1993) 393–404.
[6] S. Jaenicke, R.M. Sabarathinam, B. Fleet, H. Gunasingham, Talanta 45 (1998) 703–711.
[7] R. Schrebler, M.A. Del Valle, H. Gómez, C. Veas, R.J. Cordova, J. Electroanal. Chem. 380 (1995) 219–227.
[8] G. Wittstock, A. Strubing, R. Szargan, G.J. Werner, J. Electroanal. Chem. 444 (1998) 61–73.
[9] A. Walcarius, Electroanal. 10 (1998) 1217–1225.
[10] A. Walcarius, Electroanal. 13 (2001) 701–718.
[11] A. Walcarius, Anal. Chim. Acta 384 (1999) 1–16.
[12] C.M.A. Brett, A.M. Oliveira Brett, S.H.P. Serrano, J. Electroanal. Chem. 366 (1994) 225–231.
[13] A.M. Oliveira Brett, S.H.P. Serrano, T.A. Macedo, D. Raimundo, M.H. Marques, M.A. La Scalea, Electroanal. 8 (1996) 992–995.
[14] Y.D. Zhao, D.W. Pang, Z.L. Wang, J.K. Cheng, Y.P. Qi, J. Electroanal. Chem. 431 (1997) 203–209.
[15] C.M.A. Brett, A.M. Oliveira Brett, S.H.P. Serrano, Electrochim. Acta 44 (1999) 4233–4239.
[16] A.M. Oliveira Brett, J.A.P. Piedade, S.H.P. Serrano, in: R.G. Compton, G. Hancock (Eds.), Comprehensive Chemical Kinetics, vol. 37 (Chapter 3), Elsevier, Amsterdam, 1999, pp. 91–115 (Chapter 3).
[17] Y.-D. Zhao, D.-W. Pang, S. Hu, Z.-L. Wang, J.-K. Cheng, Y.-P. Qi, H.P. Dai, B.-W. Mao, Z.-Q. Tian, J. Luo, Z.-H. Lin, Anal. Chim. Acta 388 (1999) 93–101.
[18] J. Wang, G. Rivas, X. Cai, H. Shiraishi, P.A.M. Farias, N. Dontha, D. Luo, Anal. Chim. Acta 332 (1996) 139–144.
[19] A.M. Oliveira Brett, S.H.P. Serrano, I.G.R. Gutz, M.A. La Scalea, Electroanal. 9 (1997) 110–114.
[20] A.M. Oliveira Brett, S.H.P. Serrano, I.G.R. Gutz, M.A. La Scalea, Bioelectrochem. Bioenerg. 42 (1997) 175–178.
[21] M.A. La Scalea, S.H.P. Serrano, I.G.R. Gutz, Química Nova 22 (1999) 417–424.
[22] A.M. Oliveira Brett, T.R.A. Macedo, D. Raimundo, M.H. Marques, S.H.P. Serrano, Biosensors Bioelectron. 13 (1998) 861–867.
[23] F. Lisdat, B. Ge, F.W. Scheller, Electrochem. Commun. 1 (1999) 65–68.
[24] D. Marin, R. Valera, E. De La Red, C. Teijeiro, E. Paleček, Bioelectrochem. Bioenerg. 44 (1997) 51–56.
[25] J. Wang, M. Ozsoz, X.H. Cai, G. Rivas, H. Shiraishi, D.H. Grant, M. Chicharro, J. Fernandes, E. Palecek, Bioelectrochem. Bioenerg. 45 (1998) 33–40.
[26] D. Marín, P. Pérez, C. Teijeiro, E. Paleček, Biophys. Chem. 75 (1998) 87–95.
[27] C. Teijeiro, E. De La Red, D. Marín, Electroanal. 12 (2000) 963–968.
[28] X. Lu, X. Zhou, J. Chen, J. Kang, J. Gao, Anal. Lett. 33 (2000) 193–207.
[29] J. Labuda, M. Bučková, S. Jantová, I. Štepánek, I. Surugiu, B. Danielsson, M. Mascini, Fresenius J. Anal. Chem. 367 (2000) 364–368.
[30] A. Erdem, M. Ozsoz, Anal. Chim. Acta 437 (2001) 107–114.
[31] K.M. Millan, S.R. Mikkelsen, Anal. Chem. 65 (1993) 2317–2323.
[32] V. Kertesz, N.A. Whittemore, G.B. Inamati, M. Manoharan, P.D. Cook, D.C. Baker, J.Q. Chambers, Electroanal. 12 (2000) 889–894.
[33] A. Erdem, K. Kerman, B. Meric, U.S. Akarca, M. Ozsoz, Anal. Chim. Acta 422 (2000) 139–149.
[34] A. Erdem, K. Kerman, B. Meric, M. Ozsoz, Electroanal. 13 (2001) 219–223.
[35] E.M. Boon, D.M. Ceres, T.G. Drummond, M.G. Hill, J.K. Barton, Nat. Biotechonol. 18 (2000) 1096–1100.
[36] E.M. Boon, D.M. Ceres, T.G. Drummond, M.G. Hill, J.K. Barton, Nat. Biotechonol. 18 (2000) 1318–1318.

[37] P. Dantoni, S.H.P. Serrano, A.M. Oliveira Brett, I.G.R. Gutz, Anal. Chim. Acta 366 (1998) 137–145.
[38] E.S. Gil, L.T. Kubota, Bioelectrochemistry 51 (2000) 145–149.
[39] E.S. Gil, R. Najar, S.H.P. Serrano, E.I. Ferreira, A.C.V. Negrón, L.T. Kubota, J. Pharm. Biomed. Anal. 29 (2002) 579–584.
[40] D.D. Perrin, B. Dempsey, Buffer for pH and Metal Ion Control (Chapter 5), Chapman and Hall, New York, 1983 (Chapter 5).
[41] J. Lurie, Handbook of Analytical Chemistry, Mir Publishers, Moscow, 1975.
[42] R.N. Goyal, G. Dryhurst, J. Electroanal. Chem. 135 (1982) 75–91.
[43] A.M.O. Brett, J.A.P. Piedade, S.H.P. Serrano, Electroanal. 12 (2000) 969–973.
[44] P. Subramaniam, G. Dryhurst, J. Electroanal. Chem. 224 (1987) 137–162.
[45] A.M.O. Brett, V. Diculescu, J.A.P. Piedade, Bioelectrochemistry 55 (2002) 61–62.
[46] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry (Chapter 5), Ellis Horwood, England, 1988 (Chapter 5).
[47] B. Grundig, G. Wittstock, U. Rudel, B. Strehlitz, J. Electroanal. Chem. 395 (1995) 143–157.
[48] D.D. Shlereth, E. Katz, H.-L. Schmidt, Electroanal. 7 (1995) 46–54.
[49] A. Silber, N. Hampp, W. Schuhmann, Biosensors Bioelectron. 11 (1996) 215–223.
[50] A. Avramescu, T. Noguer, M. Avramescu, J.-L. Marty, Anal. Chim. Acta 458 (2002) 203–213.
[51] A. de, S. Santos, Lo Gorton, L. Kubota, Electroanal. 14 (2002) 805–812.
[52] D.-M. Zhou, H.-Q. Fang, H.-Y. Chen, H.-Y. Ju, Y. Wang, Anal. Chim. Acta 329 (1996) 41–48.
[53] S.M. Golabi, H.R. Zare, M. Hamzehloo, Electroanal. 14 (2002) 611–618.
[54] J.F. Rubinson, H.B. Mark, A. Galal, N.F. Atta, J. Electroanal. Chem. 384 (1995) 19–23.
[55] M.J. Lobo, A.J. Miranda, J.M. Lopez-Fonseca, P. Tunon, Anal. Chim. Acta 325 (1996) 33–42.
[56] N. Mano, A. Kuhn, Biosensors Bioelectron. 16 (2001) 653–660.
[57] N. de los Santos-Álvarez, M.J. Lobo-Castanon, A.J. Miranda-Ordieres, P. Tunon-Blanco, Anal. Chim. Acta 457 (2002) 275–284.
[58] T. Masuda, J. Ito, S. Akuzawa, K. Ishii, H. Takagi, Y. Ueno, Toxicol. Lett. 61 (1992) 9–20.
[59] J. Cadet, C. D'Ham, T. Douki, J.-P. Pouget, J.-L. Ravanat, S. Sauvaigo, Free Radical Res. 29 (1998) 541–550.
[60] N. Mei, N. Kunugita, T. Hirano, H. Kasai, Biochem. Biophys. Res. Commun. 297 (2002) 924–930.
[61] K. Hensley, M.L. Maidt, Z. Yu, H. Sang, W.R. Markesbery, Robert A. Floyd, J. Neurosci. 18 (1998) 8126–8132.