Determinatiom of 8-Hydroxy 2′-Deoxyguanosine Using Electrodes Modified with a Dispersion of Carbon Nanotubes in Polyethylenimine

Alejandro Gutiérrez,[a] *Silvia Gutiérrez*,[b] *Guadalupe García*,[b] *Laura Galicia*,*[a] *Gustavo A. Rivas*[c]

[a] Universidad Autónoma Metropolitana Iztapalapa. Depto. de Química. Av. Michoacán y la Purísima, Col. Vicentina. C.P. 09340, México
tel.: (555) 804-4671
[b] Departamento de Química de la Universidad de Guanajuato, Cerro de la Venada S/N.C.P. 36040, México
[c] INFIQC, Departamento de Físico Química, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. 5000 Córdoba, Argentina
*e-mail: lgl@xanum.uam.mx

Received: November 9, 2010
Accepted: November 26, 2010

Abstract
Hydroxyl radicals easily oxidize biomolecules such as proteins and DNA. The most abundant oxidative product of DNA is 8-hydroxy 2′-deoxyguanosine (8-OHdG) and this is considered a biomarker of oxidative DNA damage. This work studies the electrochemical behavior of 8-OHdG on electrodes modified with carbon nanotubes dispersed in polyethylenimine. The technique of differential pulse anodic stripping voltammetry (DPASV) enables quantification of 8-OHdG in the presence of its major interferents, such as ascorbic acid and uric acid. We obtained linear calibration plots in the range from 5.0×10^{-7} M to 3.0×10^{-5} M, with detection limit (DL) of 1.0×10^{-7} M and the quantification limit (QL) of 3.0×10^{-7} M.

Keywords: Modified electrodes, Carbon nanotubes, Polyethylenimine, Dispersion, 8-Hydroxy 2′-deoxyguanosine

DOI: 10.1002/elan.201000677

1 Introduction

Hydroxyl radicals attack DNA, inducing rupture in the molecule as a consequence of the oxidation of bases (adenine, guanine and thymine), leading to mutations [1,2] and causing several diseases [3–9]. About 20 different compounds have been identified, the 8-hydroxy 2′-deoxyguanosine (8-OHdG) being the most abundant product [10]. Therefore, 8-OHdG is considered a biomarker of oxidative damage in DNA [11], and it is eliminated through the urine [9–14]. Ascorbic acid (AA) and uric acid (UA) are the main interferents in the determination of 8-OHdG in biological fluids.

The most common methods for the determination of 8-OHdG are high performance liquid chromatography with electrochemical detection (HPLC-ECD) [15–17], and combined with solid phase extraction (HPLC-ECD-SPE) [18], capillary electrophoresis with electrochemical detection (CE-ECD) [19,20] and with UV detection (CE-UV) [21], gas chromatography-coupled mass spectrometry (GC-MS) [22], and liquid chromatography-coupled mass spectrometry (LC-MS) [23], liquid chromatography combined with electrospray ionization tandem mass spectrometry (LC-MS/MS) [24], and a specific method high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry (HPLC/ESI/MS/MS) [25].

Zhang et al. [26], reported an improved, more sensitive method of detecting 8-OHdG using liquid chromatography/positive ionization atmospheric pressure photoionization associated with mass spectrometry (LC/APPI-MS/MS). This method was used for 8-OHdG quantification recovered from biological systems in vitro and in vivo.

Recently, 8-OHdG has been detected by two methods: HPLC-ECD and ELISA [27,28] in individuals who have been exposed to toxic metals such as aluminum, chromium, nickel and arsenic. During the 8-OHdG quantification by HPLC-ECD [15,16], problems arise due to the high oxidation overpotentials applied to the working electrode (Au and glassy carbon). In addition, a gradual poisoning of the electrode occurs, with the consequent loss of signal or lack of reproducibility in the measurements.

Another example is the work of Brett et al. [29], who presented the electrochemical oxidation of 8-oxoguanine for DNA damage detection on glassy carbon. Electroanalytical determinations of this analyte were carried out and the detection limit obtained was 8×10^{-7} M.

Godínez et al. [15,30–32] reported electrostatic and covalent adsorption of poly(amidoamine) (PAMAM) dendrimers on a thiol-modified gold surface for development of dopamine and 8-OHdG sensors. Results obtained in

synthetic samples show low detection and quantification limits for 8-OHdG (1.2×10^{-9} M and 3.7×10^{-9} M, respectively), with matrix interference elimination, the sample pretreatment was avoided [15].

Another alternative is the study of electrodes modified with carbon nanotubes (CNT). The use of these materials present important advantages such as catalytic properties, a large number of reactive sites preconcentration ability, prevention of poisoning of the surface, large surface area and high conductivity.

The CNT are poorly soluble in water, and the studies have been done using solvents such as dimethylformamide (DMF) [33] and cyclohexane [34], as well as dispersions of them in different polymers. Rivas et al. [35] propose the use of polylysine as an efficient CNT dispersant, applied in the highly selective determination of uric acid (UA) in the presence of ascorbic acid (AA). Similarly, Nafion has been successfully used as a CNT dispersant [36,37]. Another CNT dispersant that has been used is the polyacrylic acid [38,39]. The resulting dispersions were used for the simultaneous determination of dopamine and uric acid in the presence of ascorbic acid. The same material has also been applied in the development of sensors for NADH. Tkac and Ruzgas [34] have shown that when CNT are dispersed in chitosan, they offer a better response for the determination of hydrogen peroxide.

Likewise, polyethylenimine (PEI) has been found to be a good CNT dispersing agent for the quantification of analytes of biological interest such as serotonin, AA and hydrogen peroxide [35,40].

This type of modified electrode provides good sensitivity in the determination of adenine, guanine [41], dopamine, ascorbic acid and serotonin [42,43], using the differential pulse voltammetry (DPV) technique.

The aim of this study is to selectively determine 8-OHdG on glassy carbon electrodes modified with carbon nanotubes dispersed in polyethylenimine in the presence of ascorbic acid and uric acid.

2 Experimental

2.1 Reagents

The 8-hydroxy-2′-deoxyguanosine, ascorbic acid, Nafion 5%, and polyethylenimine (PEI, average MW 750000, catalog number P-3143) were obtained from Sigma-Aldrich. Uric acid was purchased from Merk. The multiple wall carbon nanotubes (30 ± 15 nm in diameter and 5–20 microns in length) were from Nano Lab (USA). All solutions were prepared with ultrapure water ($\rho=18$ MΩ cm, from a Millipore-MilliQ system).

2.2 Electrode Cleaning

The glassy carbon electrode (GCE) was polished manually with aqueous suspensions of alumina of progressively smaller particle size (1.0, 0.30, and 0.05 μm) for 2 minutes on each case. After a through rinse, it was placed in an ultrasonic bath with ultrapure water for 5 seconds. Before proceeding with the surface modification, the glassy carbon electrode was cycled in a 0.050 M pH 7.40 phosphate buffer solution between 300 mV and 800 mV at a scan rate of 50 mV s^{-1} (for 5 cycles), rinsed with ultrapure water, and dried with N_2.

2.3 Preparation of CNT-PEI Dispersion

1.0 mg of CNT was dispersed in 1.0 mL of a 1.0 mg/mL PEI solution (prepared in 50:50 v/v ethanol/water), followed by sonication for 15 minutes.

2.4 Preparation of GCE/PEI Modified Electrode

A 20 μL drop of 1.0 mg/mL PEI solution (50:50 v/v ethanol/water) was placed on the GCE previously polished with alumina suspension and electrochemically cleaned.

2.5 Preparation of GCE/CNT-PEI Modified Electrode

A 20 μL aliquot of the CNT-PEI dispersion was placed on the polished GCE which was allowed to dry for 60 min. The above procedure was performed prior to the modification of the electrode to achieve a reproducible electrochemical response. Before modifying, the electrode was cycled in a phosphate buffer solution pH 7.40 between −300 and 800 mV at 50 mV s^{-1} (5 cycles).

2.6 Electrochemical System

The working electrode was glassy carbon of 3 mm diameter. A platinum wire and an Ag/AgCl were used as counter and reference electrodes, respectively. A 0.050 M (pH 7.40) phosphate buffer solution was used as supporting electrolyte. A magnetic stirrer was used for amperometric measurements.

The technique of differential pulse anodic stripping voltammetry (DPASV) consists of performing a preconcentration of 8-OHdG on GCE/CNT-PEI for a set time at a constant potential, followed by measurement by the differential pulse technique (DPV) in 8-OHdG solution. The DPV parameters are the following: scan rate of 20 mV s^{-1}, pulse amplitude of 50 mV, pulse width of 50 ms, 100 ms pulse period and a 2 s setting time. The experiments were performed with a BAS 100 B.

3 Results and Discussion

Figure 1 shows the voltammetric reponse of AA (1.0×10^{-3} M) and 8-OHdG (1.0×10^{-4} M) obtained on GCE. The peak potential for AA oxidation is 231 mV while the one for 8-OHdG oxidation is at 409 mV. The AA oxidation signal is very broad and overlaps with the 8-OHdG oxidation signal. A reduction signal at 366 mV is observed when the potential sweep is reversed towards the

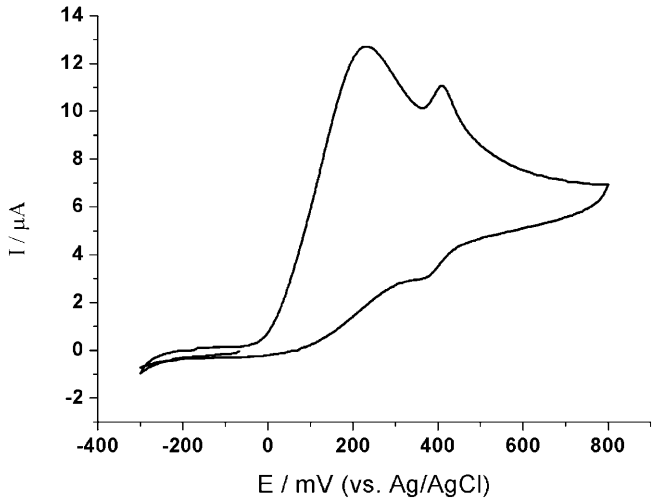

Fig. 1. Cyclic voltammogram for 1.0×10^{-3} M AA and 1.0×10^{-4} M 8-OHdG at GCE. Scan rate: 50 mV s^{-1}, supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

cathodic direction. This process could be related to the reduction of a species that is formed during the 8-OHdG oxidation which is promoted by the presence of AA. The concentrations of analytes used are similar to those found in urine and serum samples.

Figure 2 shows the current–potential curves of 8-OHdG (1.0×10^{-4} M) on A) GCE, B) GCE/PEI and C) GCE/CNT-PEI. The voltammograms show the irreversible oxidation of 8-OHdG on all the electrodes. In the case of the GCE and GCE/PEI electrodes, the oxidation peak potential is (389 ± 3) and (403 ± 4) mV, respectively, while in the case of the GCE/CNT-PEI, it is (327 ± 1) mV. It should be noted that the oxidation current for 8-OHdG at GCE/CNT-PEI increases up to (43 ± 3) μA, that is approximately 20 times higher compared to the ones obtained at GCE (2.2 ± 0.2) μA and GCE/PEI (1.8 ± 0.6) μA. This significant enhancement is mainly due to an increase in the electroactive area of the electrode, associated with the presence of CNT.

Due to the high sensitivity obtained with the electrodes modified with CNT-PEI dispersion, we evaluate the determination of analytes of interest by DPASV, in order to achieve the of 8-OHdG quantification in the presence of AA in excess.

Figure 3 shows the DPV voltammograms of 8-OHdG (1.5×10^{-5} M) (A), AA (1.0×10^{-3} M) (B), and their mixture (C). The oxidation peak potential of 8-OHdG is 320 mV and the associated current is 40 μA (Figure 3A). For the oxidation of AA (Figure 3B), we found a small oxidation signal (9 μA) at a potential of −64 mV. When the determination of 8-OHdG was carried out in the presence of AA (Figure 3C), two signals are observed, corresponding to the oxidation of AA and of 8-OHdG.

The oxidation current (44 ± 2) μA of 8-OHdG is similar to that obtained in a 0.050 M phosphate buffer solution pH 7.40 in the absence of AA (40 ± 3) μA, indicating that the important catalytic activity of CNTs towards AA oxidation makes possible the detection of 8-OHdG in the presence of a large excess of AA.

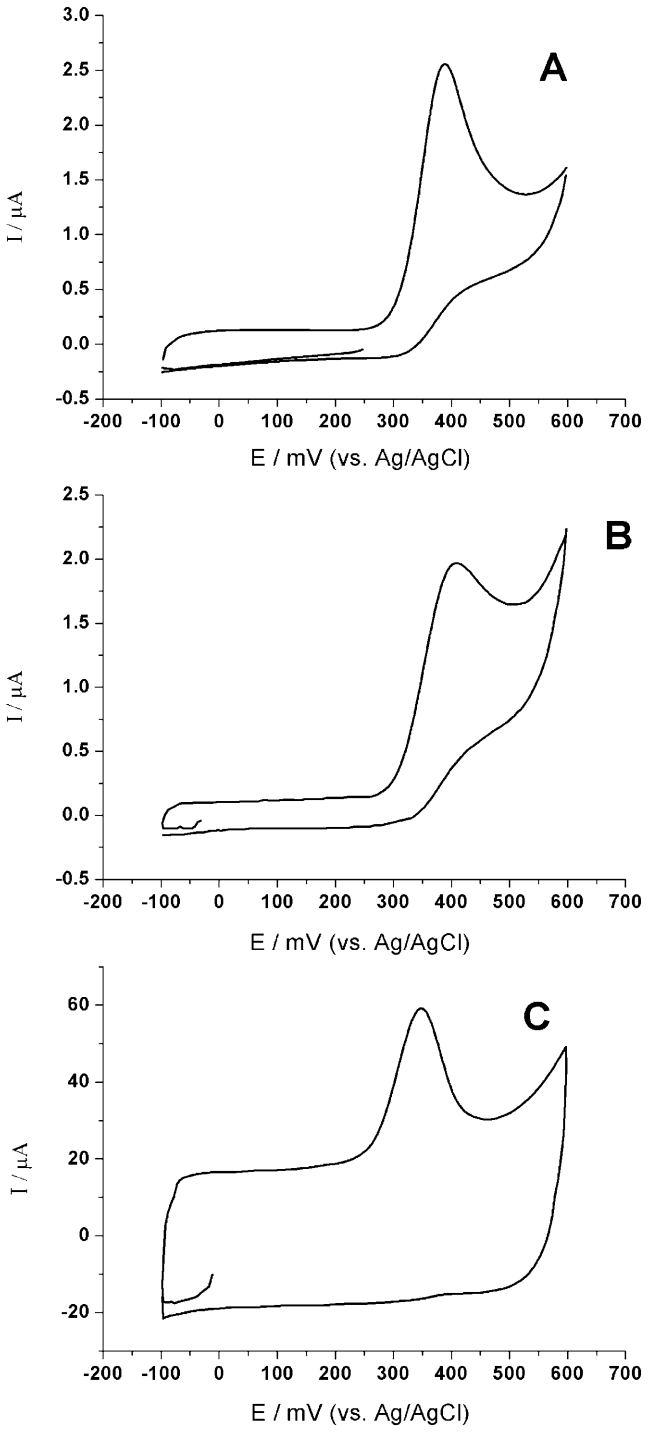

Fig. 2. Cyclic voltammogram for 1.0×10^{-4} M 8-OHdG at different electrodes: GCE (A), GCE/PEI (B), and GCE/CNT-PEI (C). Scan rate: 50 mV s^{-1}, supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

The results show the importance of CNT, since they enable obtaining a much more sensitive signal. The peak current increases from 2.2 to 44 μA compared with GCE. In addition, we obtain a significant reduction in the oxi-

dation overpotential of AA from 178 to −64 mV and an increase in the oxidation potential of 8-OHdG, enabling sensitive detection of the mixture components.

To improve the sensitivity of the modified electrode, we studied the influence of CNT concentration in the dispersion with PEI, as well as the optimum preconcentration time and potential to apply in the "stripping" technique (DPASV). Figure 4 shows variation of 8-OHdG oxidation currents obtained as a function of CNT concentration (A), preconcentration potential (B), and preconcentration time (C).

These results show that the oxidation current is 2.9 and 3.6 times when CNT concentration increases from 0.5 to 0.75 mg/mL and 0.5 to 1.0 mg/mL (Figure 4A), respectively; while it decreases for CNT concentration higher than 1.25 mg/mL. The signal decrease is probably due to blockage of CNT active sites, as their electroactivity depends mainly on the edge-plane defects of highly oriented pyrolytic graphite, located at the ends of the tubes [37]. Therefore, 1.0 mg/mL is selected as the optimum CNT concentration.

We also analyzed the influence of preconcentration potential in the range of −300 to 300 mV (Figure 4B), and the highest current was observed when the preconcentration potential was −250 mV.

Figure 4C shows the oxidation current behavior for 8-OHdG as a function of preconcentration time. The oxidation current increases up to 10 minutes. After this time, it remains constant, indicating saturation of available sites on the electrode surface. A preconcentration time of 5.0 min was selected as the best compromise between the sensitivity and the time required for the analysis. Thus, the selected conditions for the 8-OHdG determination by anodic stripping were: 5 minutes preconcentration of the species at −250 mV potential and at a GCE modified with 1.0 mg/mL CNT-PEI dispersion of 1.0 mg of CNT in PEI.

Figure 5A shows the DPV recordings for the anodic stripping (DPASV) of 8-OHdG at GCE/CNT-PEI in the absence and presence of a fixed AA concentration (1.0×10^{-3} M).

The 8-OHdG oxidation peak potential is 347 ± 21 mV. Figure 5B compares calibration plots for 8-OHdG in the absence (curve 1) and in the presence (curve 2) of 1.0×10^{-3} M AA. It is important to remark that every experiment was obtained with a new electrode and that each point represents the average of the currents obtained with five different electrodes. The sensitivities for 8-OHdG obtained in the presence and absence of AA are $(2.91\pm0.03)\times10^{6}$ μA M^{-1} ($r=0.99994$) and $(2.67\pm0.03)\times10^{6}$ μA M^{-1} ($r=0.99998$), respectively. In both cases, linear behavior is observed in the range between 5.0×10^{-7} M to 3.0×10^{-5} M.

The difference in sensitivities for 8-OHdG at GCE/CNT-PEI in the presence and absence of AA is just 8.2 %. Thus, it can be said that AA does not affect the 8-OHdG determination. Under these conditions, the detection limit was 1×10^{-7} M and the quantification limit was

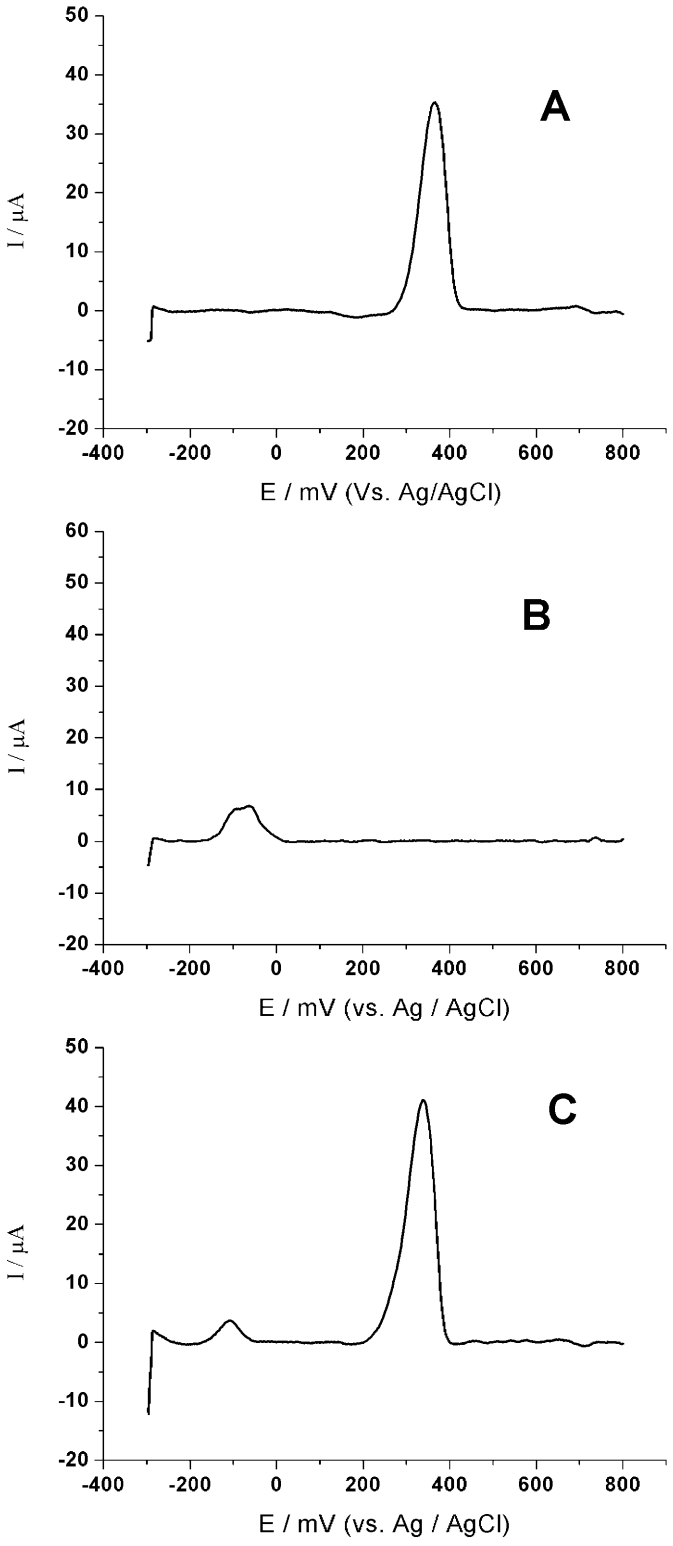

Fig. 3. Differential pulse voltammograms for: (A) 1.5×10^{-5} M 8-OHdG, (B) 1.0×10^{-3} M AA, and (C) a mixture containing 1.5×10^{-5} M 8-OHdG + 1.0×10^{-3} M AA. All of them obtained at GCE/CNT-PEI. Stripping conditions: pulse height: 50 mV. Pulse duration 100 ms. Scan rate: 20 mV s^{-1}. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

3×10^{-7} M (taking as 3.3 times the standard deviation of the blank signal/sensitivity and 10 times the standard de-

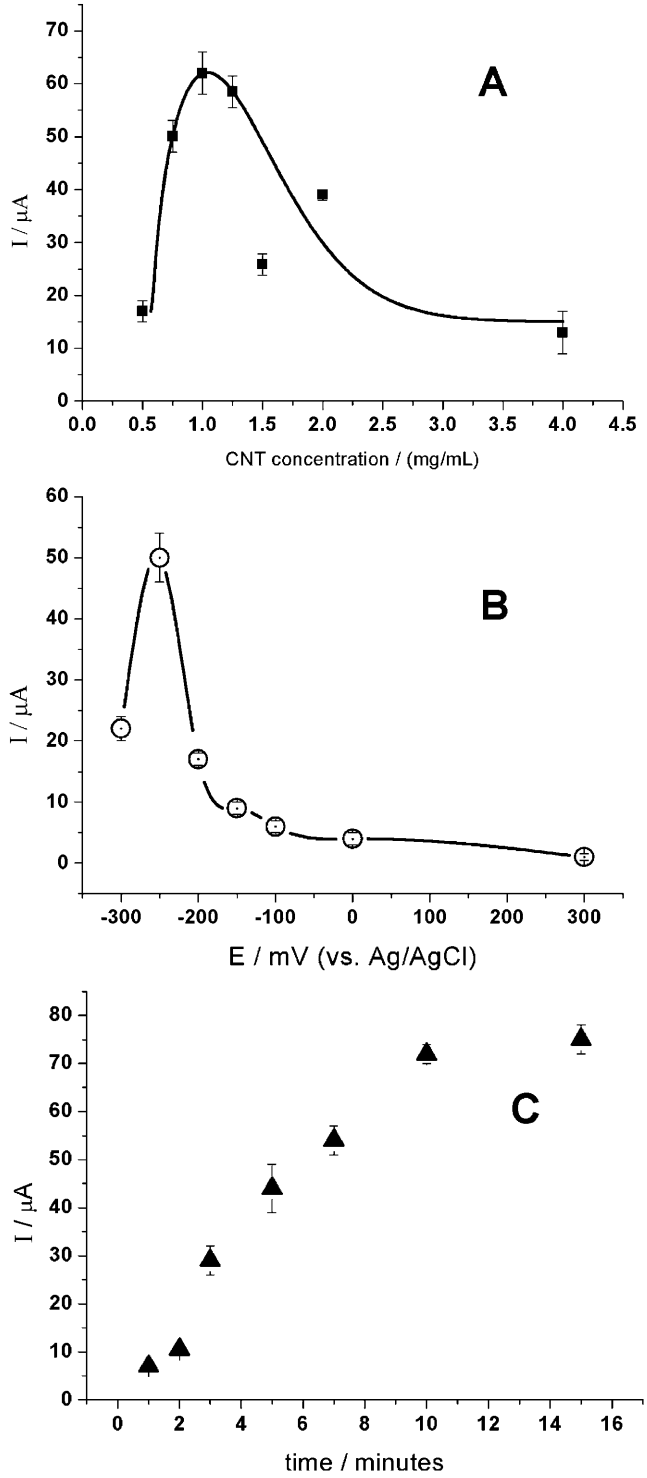

Fig. 4. Differential pulse voltammograms for 3.0×10^{-5} M 8-OHdG: (A) varying CNT concentration, 5 min of preconcentration time at −250 mV potential, (B) 2.0×10^{-5} M 8-OHdG at different preconcentration potentials and (C) 2.0×10^{-5} M 8-OHdG changing preconcentration time. All of them obtained at GCE/CNT-PEI. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

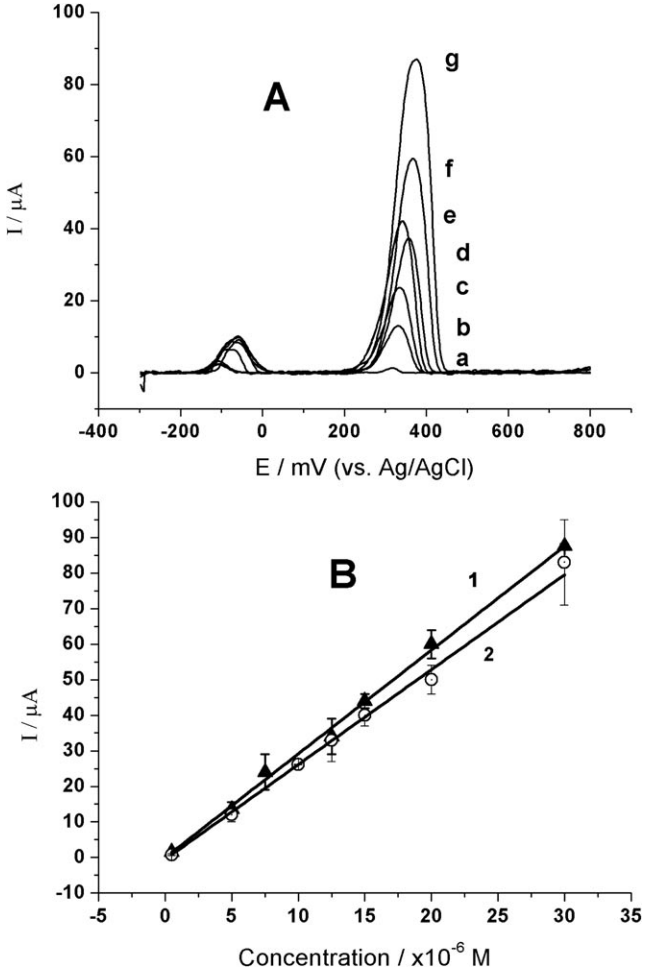

Fig. 5. (A) Differential pulse voltammograms for mixtures containing 1.0×10^{-3} M AA at different 8-OHdG concentrations: (a) 0.5, (b) 5, (c) 7.5, (d) 12.5, (e) 15, (f) 20 and (g) 30×10^{-6} M. (B) Current versus 8-OHdG concentration plot for 8-OHdG in presence (curve 1) and absence (curve 2) of AA. All of them obtained at GCE/CNT-PEI. Other conditions as in Figure 4.

viation of the blank signal/sensitivity for DL and QL, respectively). The results indicate that the CNT properties (catalytic effect and increase in area) permit us to obtain an electrochemical sensor which is highly sensitive to 8-OHdG in the presence of a high AA concentration. Our data obtained are comparable with those reported by other authors, using techniques such as HPLC-ECD [44].

The usefulness of the proposed sensor for the simultaneous quantification of 8-OHdG and AA in human urine was also evaluated. Figure 6 shows the DPV for a 1:5 v/v diluted urine sample obtained after adsorption at −250 mV for 5 min. There is an oxidation signal of (31 ± 2 μA) at (252 ± 5) mV (curve 1), which is attributed to the oxidation of UA [35,45]. The urine sample does not show signals of the 8-OHdG oxidation, therefore it was necessary to enrich the urine with a (1.5×10^{-5} M) solution of this species to evaluate the feasibility to perform the simultaneous determination of UA and 8-OHdG.

The DPV 2 shown in Figure 6, shows two signals that appear at very close potential. The first one at a (272 ± 5) mV is due to the UA oxidation, while the one at (354 ± 5) mV corresponds to the oxidation of 8-OHdG.

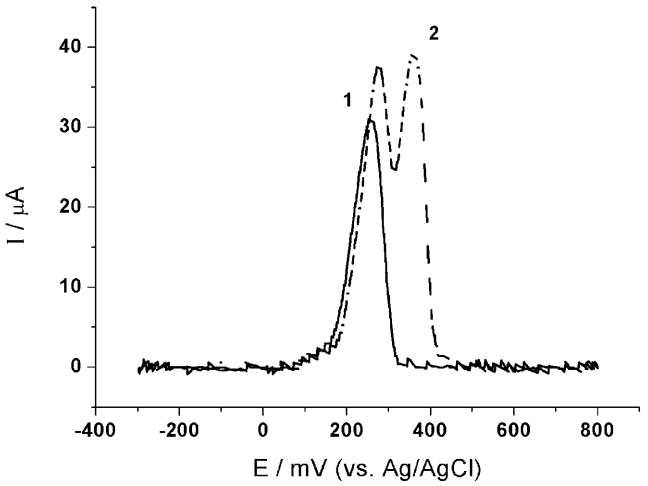

Fig. 6. Differential pulse voltammograms for diluted (1:5 v/v) urine sample before (1) and after (2) the addition of 1.5×10^{-5} M 8-OHdG. All of them obtained at GCE/CNT-PEI. Other conditions as in Figure 4.

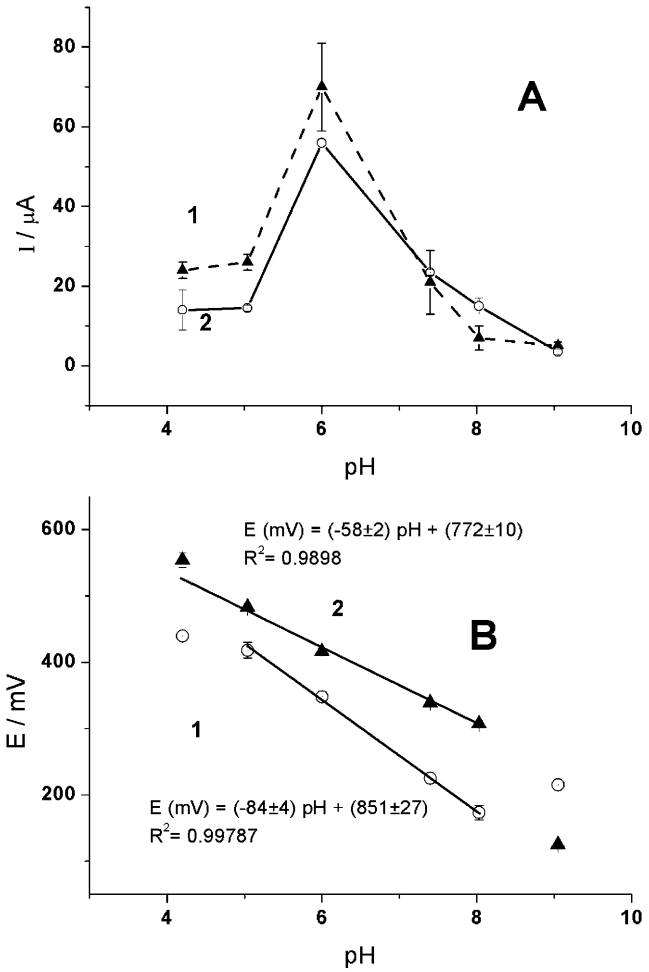

Fig. 7. (A) Current and (B) peak potential versus pH obtained of DPASV experiments: (curve 1) 1.0×10^{-4} M UA and (curve 2) 1.0×10^{-5} M 8-OHdG. All of them obtained at GCE/CNT-PEI. Other conditions as in Figure 4.

The RSD for the determination of 1.5×10^{-5} M 8-OHdG using different electrodes modified with the same dispersion was 8.2 %.

In order to separate the oxidation signals of these two species, we carried out experiments at different pHs using pure solutions of UA and 8-OHdG at levels similar to those usually present in urine samples (Figures 7 and 8).

Figure 7A shows the effect of the pH in the oxidation currents obtained by DPASV for UA (curve 1) and 8-OHdG (curve 2). We observe that the UA oxidation currents in the 4–6 pH range are higher than those for 8-OHdG. However, in the 7.4–8.0 pH range, the 8-OHdG signal (curve 2) is greater than the one for UA. At pH 9.0, the oxidation signal for both compounds is similar.

Figure 7B shows the UA (curve 1) and 8-OHdG (curve 2) oxidation potentials, as a function of pH. Linear behavior (curve 2) is observed between pH 4.2 and 8.0 According to the 8-OHdG pK_a values (pK_{a1} 8.6 and pK_{a2} 11.7) [39], under these experimental conditions, 8-OHdG is found as a neutral species.

The Nernst equation for the oxidation of 8-OHdG:

$$[E_{8-\mathrm{OHdGox}/8-\mathrm{OHdGred}}] = [E^0_{8-\mathrm{OHdGox}/8-\mathrm{OHdGred}}] + \frac{0.059}{2}\log\frac{[8-\mathrm{OHdG_{ox}}][\mathrm{H^+}]^2}{[8-\mathrm{OHdG_{red}}]} \quad (1)$$

$$[E] = [E^0] + \frac{0.059}{2}\log\frac{[8-\mathrm{OHdG_{oxid}}]}{[8-\mathrm{OHdG_{red}}]} - 0.059\ \mathrm{pH} \quad (2)$$

The slope of the straight line in curve 2 in Figure 7B gives a value of 58 mV/pH units, indicating that the 8-OHdG oxidation process is carried out through the transfer of two protons (H^+) and 2 electrons, according to the Nernst equation. The interception has a 772 mV value, which represents the standard potential of the reaction under the experimental conditions. According to the 8-OHdG pK_{a1} value of 8.5 when pH is 9 the value of the slope changes, since 8-OHdG loses a proton [16].

Based on these results, the optimum pH for 8-OHdG quantification is 8.0, since the UA signal (Figure 7A curve 1) is small in comparison to the 8-OHdG one (which is two times higher (Figure 7A curve 2). In addition, the oxidation potentials at pH 8 become separable.

Figure 8 shows the DPASV voltammogram obtained at pH 8.0 on GCE/CNT-PEI for "model" solutions containing the analytes of interest. We observe that the UA oxidation signal is lower than the 8-OHdG one, even at a 10 times higher concentration. In addition, a greater separation is observed between both oxidation potentials (136 mV). Therefore, the quantification of 8-OHdG in the presence of major interferents, AA and UA, at a pH of 8.0 is possible with a GCE/CNT-PEI electrode. Our results lead us to propose the GCE/CNT-PEI electrode as a good candidate for development of a sensor for 8-OHdG in samples of biological fluids such as urine and serum.

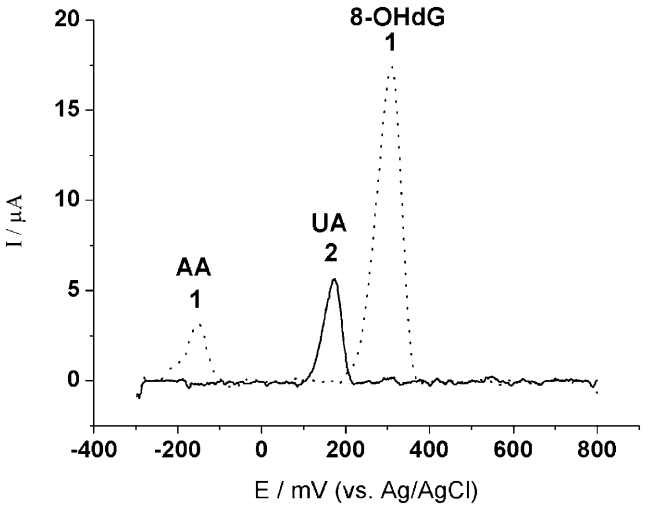

Fig. 8. Differential pulse voltammograms for (1) 8-OHdG (1.0×10^{-5} M) + AA (1×10^{-3} M) and (2) UA (1.0×10^{-4} M) obtained at GCE/CNT-PEI. Other conditions as in Figure 4.

4 Conclusions

The modification of a glassy carbon electrode with a CNT-PEI dispersion enabled 8-OHdG detection using synthetic solutions. When parameters such as working pH, CNT concentration in the dispersion, potential and preconcentration time are optimized, the GCE/CNT-PEI electrode selectively and sensitively makes possible the determination of 8-OHdG in the presence of its main interferents such as AA and UA. This is due to the electrocatalytic properties of CNT and an increase in the electroactive area.

The results obtained using the GCE/CNT-PEI electrode for the 8-OHdG can be compared with the ones reported using other analytical methods (HPLC-ECD and ELISA). We can say that this electrode is a good alternative for determining in a fast way important biological analytes.

Acknowledgement

A. G. is grateful to *CONACYT* for the financial support provided to make this research possible.

References

[1] A. P. Breen, J. A. Murphy, *Free Rad. Biol. Med.* **1995**, *18*, 1033.
[2] Y. Mizushima, S. Kan, S. Yoshida, S. Sasaki, S. Aoyama, T. Nishida, *Geriatrics Gerontol. Internat.* **2001**, *1*, 52.
[3] Y. Yang, Y. Tian, C. Yan, X. Jin, J. Tang, X. Shen, *Environ. Toxicol.* **2001**, *1*, 52.
[4] M. Chuma, S. Hige, M. Nakanishi, K. Ogawa, M. Natsuizada, Y. Yamamoto, M. Asaka, *J. Gastroenterol. Hepatol.* **2008**, *23*, 1431.
[5] J. Musarat, J. Arezina-Wilson, A. A. Wani, *Eur. J. Cancer* **1996**, *32*, 1209.
[6] S. Asami, H. Manabe, J. Miyake, Y. Tsurudome, T. Hirano, R. Yamaguchi, H. Itoh, H. Kasai, *Carcinogenesis* **1997**, *18*, 1763.
[7] V. L. Wilson, B. G. Taffe, P. G. Shields, A. C. Povey, C. C. Harris, *Environ. Health Perspect.* **1993**, *99*, 261.
[8] E. S. Fiala, C. C. Conaway, J. E. Mathis, *Cancer Res.* **1989**, *49*, 5518.
[9] M. Erhola, S. Toyokuni, K. Okada, T. Tanaka, H. Hiai, H. Ochi, K. Uchida, T. Osawa, M. M. Nieman, H. Alho, P. Kellokumpu-Lehtinen, *FEBS Lett.* **1997**, 409.
[10] D. C. Tarng, T. P. Huang, Y. H. Wei, T. Y. Lin, H. W. Chen, T. Wen Chen, W. C. Yang, *Am. J. Kidney. Dis.* **2000**, *36*, 934.
[11] H. Kasai, *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **1997**, *387*, 147.
[12] J. Y. Kim, S. Mukherjee, L. Ngo, D. C. Christiani, *Environ. Health Perspect.* **2004**, *112 (6)*, 666.
[13] C. Y. Lu, Y. C. Ma, J. M. Lin, C. Y. Chuang, F. C. Sung, *Environ. Res.* **2007**, *103*, 331.
[14] A. L. Liu, W. Q. Lu, Z. Z. Wang, W. H. Chen, *Environ. Health Perspect.* **2006**, *114*, 673.
[15] A. Gutiérrez, S. Osegueda, S. Gutiérrez-Granados, A. Alatorre, M. G. García, L. A. Godínez, *Electroanalysis* **2008**, *20*, 2294.
[16] T. H. Li, W. L. Jia, H. S. Wang, R. M. Liu, *Biosens. Bioelectron.* **2007**, *22*, 1245.
[17] K. Tamae, K. Kawai, S. Yamasaki, K. Kawanami, M. Ikeda, K. Takahashi, T. Miyamoto, N. Kato, H. Kasai, *Cancer Sci.* **2009**, *100(4)*, 715.
[18] S. Koide, Y. Kinoshita, N. Ito, J. Kimura, K. Yokoyama, I. Karube, *J. Chromatogr. B* **2010**, *878*, 2163.
[19] S. D. Arnet, D. M. Osbourn, K. D. Moore, S. S. Vandaveer, C. E. Lunte, *J. Chromatogr. B* **2005**, *825*, 16.
[20] S. W. Zhang, C. J. Zou, N. Lou, Q. F. Weng, L. S. Cai, C. Y. Wu, J. Xing, *Chin. Chem. Lett.* **2010**, *21*, 85.
[21] V. Kvasnicová, E. Samcová, A. Jursová, I. Jelinék, *J. Chromatogr. A* **2003**, *985*, 513.
[22] J. L. Ravanat, P. Guicherd, Z. Turce, J. Cadet, *Chem. Res. Toxicol.* **1999**, *12*, 802.
[23] S. Mei, Q. Yao, C. Wu, G. Xu, *J. Chromatogr. B* **2005**, *827*, 83.
[24] B. Crow, M. Bishop, K. Kovalcik, D. Norton, J. George, J. A. Bralley, *Biomed. Chromatogr.* **2008**, *22*, 394.
[25] J. Hu, W. Zhang, H. Ma, Y. Cai, G. Sheng, J. Fu, *J. Chromatogr. B* **2010**, *878*, 2765.
[26] F. Zhang, W. T. Stott, A. J. Clark, M. R. Schisler, J. J. Grundy, B. B. Gollapudi, M. J. Bartels, *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3949.
[27] S. Kinamura, H. Yamauchi, Y. Hibino, M. Iwamoto, K. Sera, K. Ogino, *Basic Clin. Pharmacol. Toxicol.* **2006**, *98*, 496.
[28] Y.-Y. Han, M. Donovan, F.-C. Sung, *Chemosphere* **2010**, *79*, 942.
[29] A. M. O. Brett, J. A. P. Piedade, S. H. P. Serrano, *Electroanalysis* **2000**, *12*, 969.
[30] J. Ledesma-García J. Manríquez, S. Gutiérrez-Granados, L. A. Godínez, *Electroanalysis* **2003**, *15*, 659.
[31] E. Bustos, J. Manríquez, L. Echegoyen, L. A. Godínez, *Chem. Commun.* **2005**, 1613.
[32] E. B. Bustos, Ma. G. G. Jiménez, B. R. Diaz-Sánchez, E. Juaristi, T. W. Chapman, L. A. Godínez, *Talanta* **2007**, *72*, 1586.
[33] Q. Shen, X. Wang, *J. Electroanal. Chem.* **2009**, *632*, 149.
[34] J. Tkac, T. Ruzgas, *Electrochem. Commun.* **2006**, *8*, 899.
[35] M. C. Rodríguez, J. Sandoval, L. Galicia, S. Gutiérrez, G. A. Rivas, *Sens. Actuators B* **2008**, *134*, 559.
[36] J. Wang, M. Musameh, Y. Lin, *J. Am. Chem Soc.* **2003**, *125*, 2408.

[37] A. Gutiérrez, S. Gutiérrez, G. García, L. Galicia, G. A. Rivas, *ECS Trans.* **2010**, *29*, 369.
[38] A. Liu, I. Honma, H. Zhou, *Biosens. Bioelectron.* **2007**, *23*, 74.
[39] A. Liu, T. Watanabe, I. Honma, J. Wang, H. Zhou, *Biosens. Bioelectron.* **2006**, *22*, 694.
[40] M. C Rodríguez, M. D. Rubianes, G. A. Rivas, *J. Nanosci. Nanotechnol.* **2008**, *18,* 6003.
[41] Q. Shen, X. Wang, *J. Electroanal. Chem.* **2009**, *632*, 149.
[42] M. D. Rubianes, G. A. Rivas, *Electrochem. Commun.* **2007**, *9*, 480.
[43] C. E. Banks, R. G. Compton, *Analyst* **2006**, *131*, 15.
[44] T. Hofer, L. Möller, *Chem. Res. Toxicol.* **1998**, *11*, 882.
[45] A. Liu, I. Honma, H. Zou, *Biosens. Bioelectron.* **2007**, *23*, 74.

