Full Paper Wiley Online Library ELECTROANALYSIS

DOI: 10.1002/elan.201400247

Graphene Paste Electrode: Analytical Applications for the Quantification of Dopamine, Phenolic Compounds and Ethanol

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Abstract: This work reports the analytical applications of a graphene paste electrode (GrPE) for the quantification of dopamine, ethanol and phenolic compounds. Dopamine was detected by differential pulse voltammetry-adsorptive stripping with medium exchange at submicromolar levels even in the presence of high excess of ascorbic acid and serotonin. The electrocatalytic activity of graphene towards the oxidation of NADH and the reduction of quinones allowed the sensitive amperometric determination of ethanol and phenols using GrPE modified with alcohol dehydrogenase/NAD^+ or polyphenol oxidase, respectively, with successful applications in real samples like alcoholic beverages and tea.

Keywords: Graphene paste electrode · Composite electrodes · Ascorbic acid · Dopamine · NADH · Enzymatic biosensors · Phenol biosensor · Ethanol biosensor · Polyphenol oxidase · Alcohol dehydrogenase

1 Introduction

Dopamine (Do) plays an important role in the function of different systems: cardiovascular, central nervous, renal, and hormonal [1]. Do is involved in drugs addiction [2] and its deficiency may cause neurological illnesses such as schizophrenia, Huntington and Parkinson diseases [3]. Several analytical methods have been proposed for the quantification of Do, high performance liquid chromatography [4], capillary electrophoresis [5], and acoustic methods [6], among others; however, such approaches depend on expensive equipments and need to be handled by trained staff. Due to their known advantages, connected with high sensitivity, possibility of miniaturization, relative low-costs, fast response and real-time detection [7], the electrochemical methods have appeared as an interesting alternative. However, the electrochemical quantification of Do presents the problem of the potential interference of electroactive compounds such as ascorbic acid (AA) and uric acid (UA), which are oxidized at potentials very close to that of Do at most of the electrodes [8]. Therefore, one of the more important challenges in bioelectroanalysis is the development of strategies that allow the selective quantification of Do in the presence of AA and UA. Several alternatives have been reported, based on the use of organic redox mediators, nanoparticles, polymers, self-assembled multilayers, CNTs, and more recently, graphene (Gr) [9–17].

Phenolic compounds are widely used in agriculture and manufacturing industries [18] and some of them present inherent toxicity to human health and environment [19]. Catechols could cause damage to the lungs, liver, kidney and genito-urinary tract after prolonged oral or dermal exposure [20]. Spectrophotometry, gas and liquid chromatography, and capillary electrophoresis have been successfully used for the quantification of phenolic compounds [21–23]; however, less expensive sensing methodologies, faster sample preparation and easier operation are still necessary. In this sense, the use of amperometric biosensors based on the immobilization of polyphenol oxidase (PPO), a copper-protein that catalyzes the oxidation of phenols to the corresponding quinones in the presence of oxygen, has received enormous attention for the detection of different phenols and catechols [24–27].

Ethanol is a compound commonly involved in clinical and forensic chemistry [28,29], control of fermentation processes and quality control of beverages and foods industries [30]. Most of the ethanol electrochemical biosensors are based on the use of alcohol dehydrogenase (ADH) and its cofactor NAD^+ [31–33] and the quantification of ethanol from the oxidation of the enzymatically generated NADH. The electrochemical detection of

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NADH presents two problems, the requirement of high overvoltages for its electrooxidation and the passivating characteristics of the oxidation product. Therefore, the development of electrodes that make possible the detection of NADH at low overvoltages without fouling the surface is highly required.

Graphene (Gr), discovered by Geim et al. in 2004 [34], is a single layer of carbon atoms with sp^2 hybridization in a closely packed honeycomb two-dimensional (2-D) sheet of carbon atoms organized in a hexagonal configuration. In view of the large specific surface, high conductivity, excellent biocompatibility, multiple possibilities of functionalization, strong accumulation ability, low production costs [35–37], and even more important, the capability to promote the electron transfer between electroactive species and electrodes, Gr has demonstrated to be an efficient electrode material for fabricating electrochemical (bio)sensors [38–40].

Different strategies have been reported for the preparation of Gr-based electrochemical (bio)sensors, by covalent attachment of biomolecules, by dispersion in different media or by creating supramolecular architectures. Since the first report of the graphene paste electrode in 2011 [41], it has been used for the development of electrochemical sensors for the quantification of chlorpromazine [41], ascorbic acid [42], paracetamol [43], heavy metals [44], phenolic compounds [45], NADH [46], acetazolamide [47], and uric acid [48]. It has been also used as platform for preparing an immunosensor for hepatitis B [49] and a DNA sensor [50].

This work is focused on the analytical application of GrPE for the highly selective quantification of Do in the presence of serotonin and AA by differential pulse voltammetry (DPV)-adsorptive stripping with medium exchange and for the development of ethanol and phenols amperometric biosensors based on the incorporation of alcohol dehydrogenase (ADH) and its cofactor NAD^+ or polyphenol oxidase (PPO), respectively, within the composite. The usefulness of the resulting biosensors for the determination of ethanol in different beverages and polyphenols in tea samples is also discussed.

2 Experimental

2.1 Reagents

Graphite powder was purchased from Fisher (Chemical Scientific grade #38). Ascorbic acid (AA) and sodium phosphates were received from Baker. Sulfuric acid and sodium hydroxide were acquired from Cicarelli while the mineral oil was obtained from Aldrich. Dopamine (Do), serotonine, catechin, catechol, hydroquinone, benzoquinone, nicotinamide adenine dinucleotide (NADH), alcohol dehydrogenase (ADH) (EC 1.1.1.1. from baker's yeast, 428 units/mg protein, 393 units/mg solid), NAD^+ and polyphenol oxidase (PPO) (EC 1.14.18.1 from mushroom 25000 U/g of solid, Catalog number T-7755) were obtained from Sigma. The reagents were analytical grade and used as received, without further purification.

Phosphate buffer solutions pH 7.40 (either 0.050 or 0.100 M) were employed as supporting electrolytes. Ultrapure water (resistivity = 18 MΩ cm) from a Millipore-MilliQ system was used for preparing all solutions.

2.2 Apparatus and Procedure

Raman spectra of GrO, Gr and graphite powders were obtained using a Horiba Jobin Yvon LabRam HR Raman spectrometer equipped with an argon laser. Raman spectra were collected at 514.83 nm, using a pinhole of 100 μm and no attenuator filter, accumulating 20 spectra recorded for 10 s each.

Electrochemical experiments were performed with Epsilon (BAS) and TEQ-04 potentiostats. A platinum wire and Ag/AgCl, 3 M KCl were used as counter and reference electrodes, respectively. All potentials are referred to the latter. The electrodes were inserted into the cell through holes in its Teflon cover. The amperometric experiments were carried out by applying the desired potential and allowing the transient current to reach the steady-state value prior to the addition of the analyte and the subsequent current monitoring. A magnetic stirrer and a stirring bar provided the convective transport. DPV experimental conditions were the following: step height: 4 mV; width: 50 ms; period time: 200 ms; and scan rate: 0.050 $V s^{-1}$.

Electrochemical impedance spectroscopy (EIS) measurements were performed with a Solartron 1287 FRA 1260 in the frequency range between 10 KHz and 10 mHz, with a potential perturbation of 10 mV and a working potential of −0.100 V using 1.0×10^{-3} M hydroquinone/benzoquinone. The impedance spectra were analyzed by using the Z-view program. All measurements were performed at room temperature.

2.3 Graphene Oxide and Chemically Reduced Graphene Oxide Synthesis

GrO was synthesized from graphite using the Hummers method [51,52]. Briefly, graphite, sodium nitrate and potassium permanganate were added to concentrated sulfuric acid cooled to 0 °C. After heating at 35 °C for 30 min, and when no more bubbling was observed, the reaction was carefully quenched by the slow addition of water with a pronounced temperature increase. The paste was kept at 90 °C for 15 min and after careful dilution with water it was allowed to cool down to 40 °C for 30 min, followed by the addition of hydrogen peroxide. The resulting GrO was isolated by filtration (while still warm), and the yellow-brown filter cake was washed with warm 5.0 % w/v hydrochloric acid and finally with water. The solid was solubilized in water by ultrasonication and any insoluble residue was removed by centrifugation. The resulting stable and brownish GrO aqueous solution was reduced with AA in a 1:1 GrO/AA mass ratio, at room

temperature, overnight [53,54]. The black precipitate of chemically reduced graphene oxide (CRGrO) was filtered, washed with water and dried at 100 °C for 8 h. The synthesized GrO and CRGrO were characterized by FT-IR and Raman spectroscopy before using.

2.4 Preparation of Graphene Paste Electrodes

CRGrO was manually grinded to a fine powder before preparation of the composite electrodes. The composites with ADH were obtained by mixing for 30 min in an agate mortar the corresponding mass to obtain 25.0% w/w CRGrO, 25.0% w/w graphite, 10.0% ADH, 10.0% NAD^+ and 30.0% w/w mineral oil. The composite with PPO was obtained by mixing for 30 min in an agate mortar 30.0% w/w CRGrO, 28.5% w/w graphite, 1.5% w/w PPO, and 40.0% w/w mineral oil. In the case of bare GrPE, the composition was 30.0% w/w graphite, 30.0% w/w CRGrO, and 40.0% w/w mineral oil. The classical graphite paste electrode (CPE) was obtained under similar conditions by mixing 60.0% w/w graphite powder and 40.0% w/w mineral oil. The resulting composites were firmly packed into the cavity of a Teflon tube (3 mm diameter) to obtain the corresponding electrodes. The electrical contact was established through a stainless steel screw. Prior to use, the GrPEs and CPEs were repacked and polished on a weighing paper.

3 Results and Discussion

3.1 Quantification of Do

Figure 1 A displays differential pulse voltammograms obtained at GrPE (a) and at CPE (b) in a 0.050 M phosphate buffer solution pH 7.40 after 10.0 min accumulation at open circuit potential in 1.0×10^{-5} M Do. At GrPE there is a well-defined oxidation peak at 0.060 V with an associated current of 2.4 μA. On the contrary, at CPE the current is substantially smaller (0.014 μA) and the overvoltage for the oxidation is 0.140 V more positive, clearly demonstrating the advantages of the presence of CRGrO on the adsorption and further electrooxidation of Do.

Taking into account this efficient adsorption of Do at GrPE, we developed a new strategy for the quantification of Do based on differential pulse voltammetry (DPV)-adsorptive stripping with medium exchange. Figure 1 B depicts the effect of the adsorption time at open circuit potential for 5.0×10^{-6} M Do. The DPV-adsorptive stripping signals increase fast up to 10.0 min, with a trend to saturation after that. Therefore, 10.0 min was selected as the optimum adsorption (accumulation) time for further studies. The calibration plot for Do obtained by DPV-adsorptive stripping after 10.0 min accumulation at open circuit potential and medium exchange presented a linear relationship between oxidation peak current and Do concentration from 1.2×10^{-6} to 1.0×10^{-4} M, with a sensitivity of (43.0 ± 0.4) μA mM^{-1}, a detection limit of 4×10^{-7} M (taken as $3.3\times\sigma/S$, where σ is the standard deviation of the blank signal and S the sensitivity), and a quantification limit of 1.2×10^{-6} M (taken as $10\times\sigma/S$) (not shown).

Bearing in mind that AA is an important interferent in the electrochemical quantification of Do, we evaluated the adsorptive stripping response of AA at GrPE. DPV-adsorptive stripping experiments performed at GrPE at open circuit potential after 10.0 min accumulation in a 6.0×10^{-5} M Do solution containing increasing concentrations of AA from 1.00×10^{-4} M to 1.50×10^{-3} M, demonstrated that AA does not interfere in the oxidation of Do up to 1.0×10^{-3} M (within the experimental error, not shown). It is important to mention that differential pulse voltammograms obtained in phosphate buffer solution after 10.0 min accumulation at GrPE at open circuit po-

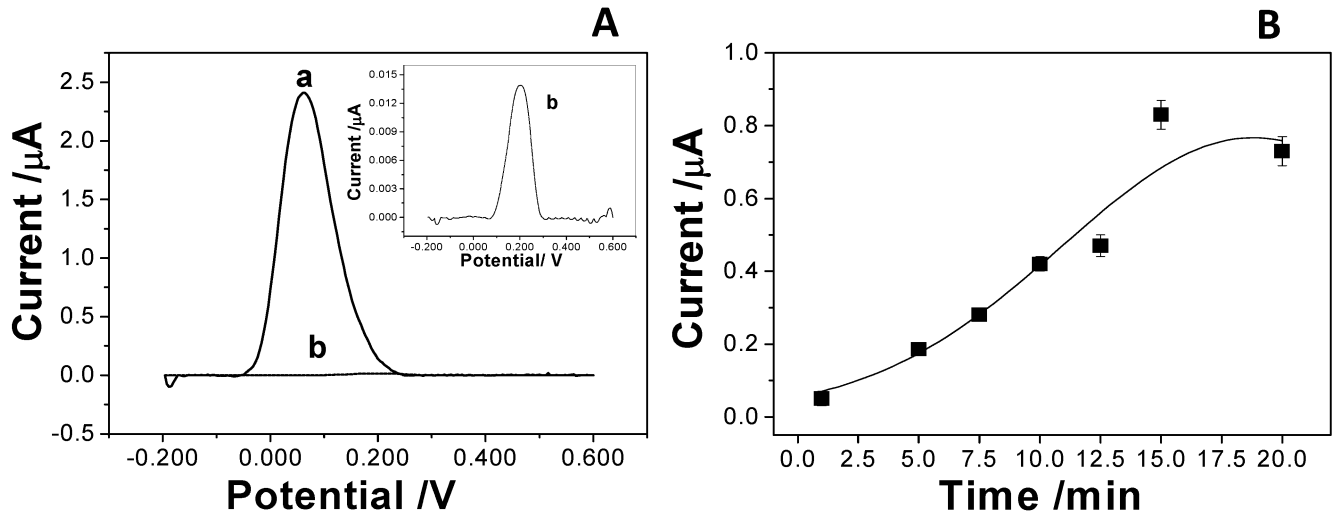

Fig. 1. Differential pulse voltammograms obtained at GrPE (a) and CPE (b) in a 0.050 M phosphate buffer solution pH 7.40 after 10.0 min accumulation at open circuit potential in 1.0×10^{-5} M Do. Inset: Response at CPE with different scale. B) Effect of the adsorption time of 5.0×10^{-6} M Do at GrPE at open circuit potential. DPV conditions: step height: 4 mV; width: 50 ms; period time: 200 ms; and scan rate 0.050 V s^{-1}. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

tential in 1.0×10^{-3} M AA solution does not show any peak, indicating that under these experimental conditions, the adsorption of AA is negligible (not shown). The calibration plot for Do in the presence of 1.0×10^{-3} M AA obtained from DPV-adsorptive stripping measurements with medium exchange under the selected conditions, gave a sensitivity of (44 ± 3) μA mM^{-1}, value very close to the one obtained for Do alone ((43.0 ± 0.4) μA mM^{-1}), confirming that GrPE allows the highly selective determination of Do even in the presence of a large excess of AA. Another interesting result is that the important electrocatalytic activity of CRGrO made also possible the selective quantification of Do not only in the presence of AA but also in the presence of serotonin (not shown). The resulting sensitivity for Do in the presence of 2.0×10^{-3} M serotonin and 1.0×10^{-3} M AA was (46 ± 3) μA mM^{-1}, indicating that Do can be selectively quantified even in the presence of large excess of both compounds.

The sensor proposed here presents detection limits similar to those obtained with GCE modified with MWCNT-β-cyclodextrin-polyluminol and gold nanoparticles [9]; better than those achieved with GCE modified with MWCNT-Fe natrolite and chitosan [10], GCE modified with SWCNT functionalized with Pt nanoparticles/ssDNA/Nafion [11], helical carbon nanotubes modified with poly(allilamine) [12], and GCE modified with Gr dispersed in dimethylformamide [13]. Other sensing protocols based on the use of GCE modified with Gr sheets mixed with MWCNT and perylene tetracarboxylic acid dispersed in ionic liquids [14], GCE modified with MWCNT-polyethylenimine-gold nanoparticles [15], Gr dispersed in β-cyclodextrin [16] or Gr functionalized with silanized EDTA and Nafion [17] reported lower detection limits; however, at variance with our sensor, in those cases the functionalization of Gr and the incorporation of additional compounds were necessary to obtain the sensing platform. In summary, the analytical platform proposed here offers a sensitive and selective detection of Do in a very easy way, without Gr functionalization and without the addition of polymeric barriers or metals nanoparticles, aspects that makes it a very competitive and useful strategy for the selective quantification of Do.

3.2 Enzymatic Biosensors Based on GrPE

3.2.1 Phenol Biosensing Using GrPE Modified with Polyphenol Oxidase

We investigated the analytical performance of GrPE modified with PPO for the quantification of phenolic compounds. In order to select the working conditions of the biosensor, we evaluated the influence of CRGrO on the charge transfer of phenols/catechols/quinones.

Figure 2 shows cyclic voltammograms obtained at 0.100 V s^{-1} for 1.0×10^{-3} M catechol (A), hydroquinone (B), and catechin (C) at CPE (dotted line) and GrPE (solid line). Compared to CPE, the i-E profiles obtained at GrPE show higher currents and lower overvoltages for the oxidation of the different compounds, demonstrating improved electron-transfer kinetics. In fact, the overvoltages for the oxidation of catechol, hydroquinone and catechin decrease 300, 70, and 100 mV, respectively. In the case of catechol and hydroquinone, the peak potential separation (ΔE_p) decreases 190 and 88 mV, respectively; while the cathodic-to-anodic currents ratio decreases from 2.8 to 1.0, and from 1.3 to 1.1 for catechol and hydroquinone, respectively, demonstrating that, even in the presence of non-conductive mineral oil, CRGrO retains an excellent electrochemical reactivity.

The electrochemical behavior of GrPE was also evaluated by EIS. Figure 2D displays Nyquist plots obtained at CPE (empty circles) and GrPE (full circles) using 1.0×10^{-3} M hydroquinone and 1.0×10^{-3} M benzoquinone at the equilibrium potential. The solid lines represent the corresponding fitting of the data using the Randles circuit (shown in the inset). The charge transfer resistances (R_{ct}), obtained at high frequencies, are (0.95 ± 0.05) kΩ and (0.27 ± 0.06) kΩ at CPE and GrPE, respectively. The charge transfer rate constant (k°) for the system benzoquinone/hydroquinone was also obtained from the EIS experiments, using the Equation 1:

$$k^\circ = RT/n^2F^2AR_{ct}C \quad (1)$$

where R is the gas constant, T the temperature, F is the Faraday constant, n the number of electrons, A the electroactive area, and C the redox couple concentration. k° for benzoquinone/hydroquinone is 4.5×10^{-4} cm s^{-1} at CPE and 1.5×10^{-3} cm s^{-1} at GrPE. The decrease in the R_{ct} and the consequent increase in the rate constant observed at GrPE can be attributed to the high surface reactivity of CRGrO due to the presence of edge defects [37] demonstrating, once more, the advantage of doping CPE with this nanomaterial.

Figure 3 depicts the amperometric response at GrPE modified with 1.5% w/w PPO at −0.100 V to successive additions of 5.0×10^{-6} M catechol (A) and catechin (B) as well as the corresponding calibration plots (Insets). A well-defined and fast response is obtained after the addition of both analytes. The sensitivity for catechol is $(1.38\pm0.02)\times10^4$ μA M^{-1}; the detection limit, 3.4×10^{-9} M, and the quantification limit, 1.0×10^{-8} M; while for catechin the analytical parameters are the following: sensitivity of $(1.1\pm0.1)\times10^3$ μA M^{-1}; detection limit of 8.2×10^{-8} M, and quantification limit of 2.5×10^{-7} M. GrPE-PPO was used for the quantification of catechin in a tea bag without any pretreatment, being the value (1.9 ± 0.4)% w/v per bag.

The biosensor proposed here presents better detection limits for catechol than other electroanalytical platforms recently reported like GCE modified with PPO covalently attached to SWCNT dispersed in chitosan and covered by a polyaniline layer [55], ITO modified with sol-gel/gold nanoparticles and PPO [56], Au-functionalized PVC/TTF-TCNQ/PPO [57], SPE derivatized with 1-pyrenebutanoic acid, succinimidyl ester and gold nanoparticles functionalized with PPO [58], and PPO immobilized at

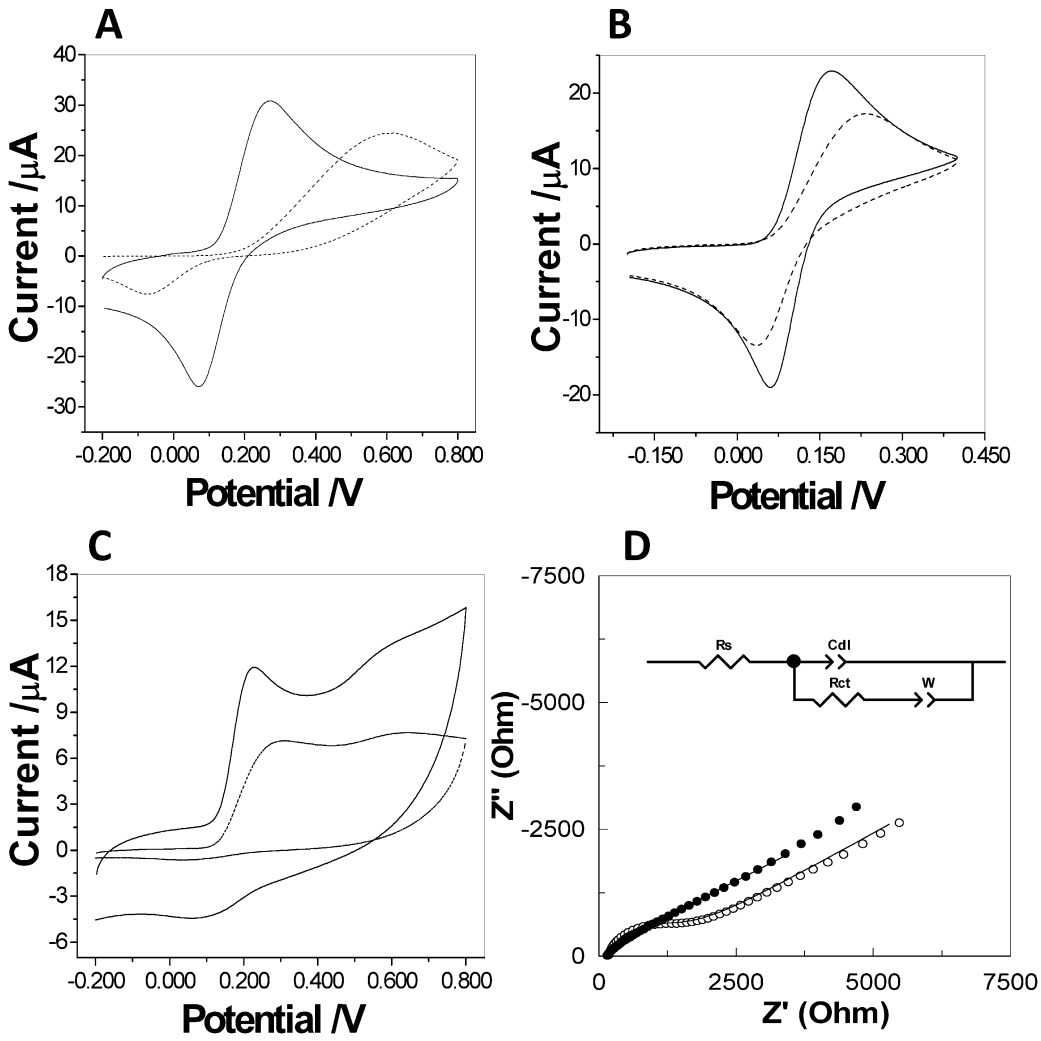

Fig. 2. Cyclic voltammograms obtained at $0.100\,V\,s^{-1}$ for 1.0×10^{-3} M catechol (A), hydroquinone (B), and catechin (C) at CPE (dotted line) and GrPE (solid line). Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. (D) Nyquist plots (EIS) obtained at CPE (○) and GrPE (●) using 1.0×10^{-3} M hydroquinone and 1.0×10^{-3} M benzoquinone. Inset: Corresponding circuit. Supporting electrolyte: 0.100 M phosphate buffer solution pH 7.40.

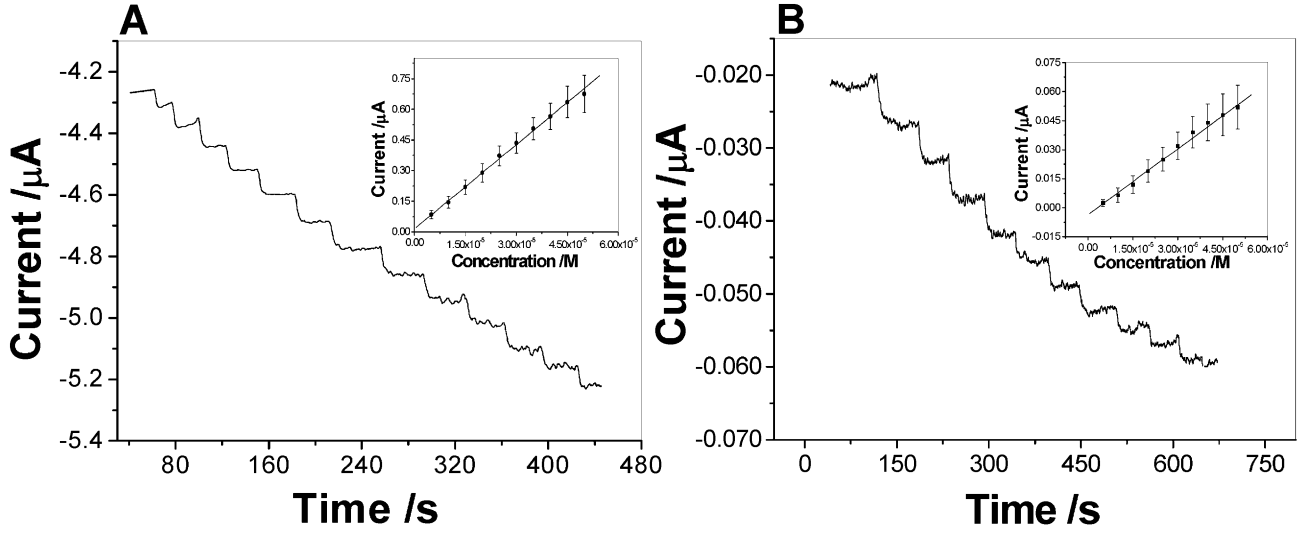

Fig. 3. Amperometric recordings for successive additions of 5.0×10^{-6} M catechol (A) and catechin (B) at GrPE-PPO (containing 1.5% w/w PPO). The insets show the corresponding calibration curves. Working potential: −0.100 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

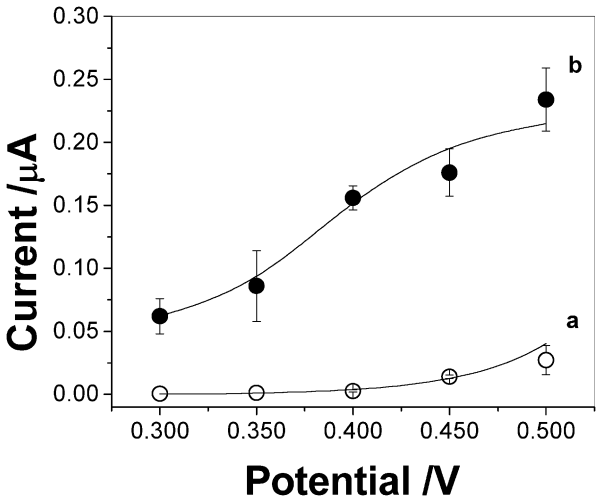

Fig. 4. Hydrodynamic voltammograms for 1.0×10^{-5} M NADH obtained at CPE (a) and GrPE(b). Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

a gold SPE modified with diazonium [59]. Catechin detection was also very sensitive, with detection limits lower than those obtained at a GCE modified with Mn hexacyanoferrate mixed-valent poly(3,4-ethylene dioxythiophene) (PEDOT) [60]. Singh et al. [61] proposed a methodology for the detection of catechin with lower detection limit although they have used a sensor obtained by entrapment of PPO and gold nanoparticles during the polymerization of pyrrole. Thus, the GrPE modified with PPO proposed here represents a very simple, fast and sensitive alternative to quantify phenolic compounds.

3.2.2 Ethanol Biosensing Using GrPE Modified with ADH and NAD$^+$

GrPE was also used as platform to build an ethanol enzymatic biosensor through the incorporation of ADH and NAD$^+$ within the composite. Figure 4 shows hydrodynamic voltammograms for 1.0×10^{-5} M NADH obtained at CPE (a) and GrPE (b). At GrPE there is a significant decrease in the overvoltage for the oxidation of NADH as well as an important enhancement in the oxidation currents, clearly demonstrating that the presence of CRGrO largely improves the electrooxidation of NADH. A potential of 0.450 V was selected for further work.

Figure 5 A displays amperometric recordings at CPE-ADH(10.0% w/w)-NAD$^+$(10.0% w/w) (a) and GrPE-ADH(10.0% w/w)-NAD$^+$(10.0% w/w) (b) for successive additions of 1.0×10^{-3} M ethanol. At CPE-ADH-NAD$^+$ the oxidation current is negligible, as expected from the hydrodynamic voltammogram previously shown. At variance with this behavior, a highly sensitive response is obtained at GrPE-ADH-NAD$^+$. Figure 5 B displays the corresponding calibration plots for ethanol at both biosensors. The analytical parameters of GrPE-ADH-NAD$^+$ are the following: sensitivity (21 ± 2) μA M^{-1}, detection limit 56 μM and quantification limit 170 μM (taken as indicated above). The biosensor proposed here allows the successful quantification of ethanol in a very fast, sensitive and easy way with better detection limits than other similar biosensing strategies [30,31,62,63]. Even when there are other approaches like the association of Gr, CNTs, polymers, ionic liquids, and cross-linking agents that present lower detection limits [64,65], it is important to emphasize that in our case, we obtained an excellent analytical performance with a very simple scheme just by mixing GrPE with ADH/NAD$^+$.

The biosensor was challenged with different alcoholic beverages. The results presented in Table 1 demonstrate that our biosensor makes possible the quantification of ethanol in Mumm champagne, Bacardi gold rum and Benjamin Nieto Senetiner white wine without any treatment and with very good correlation with the values indicated on the labels.

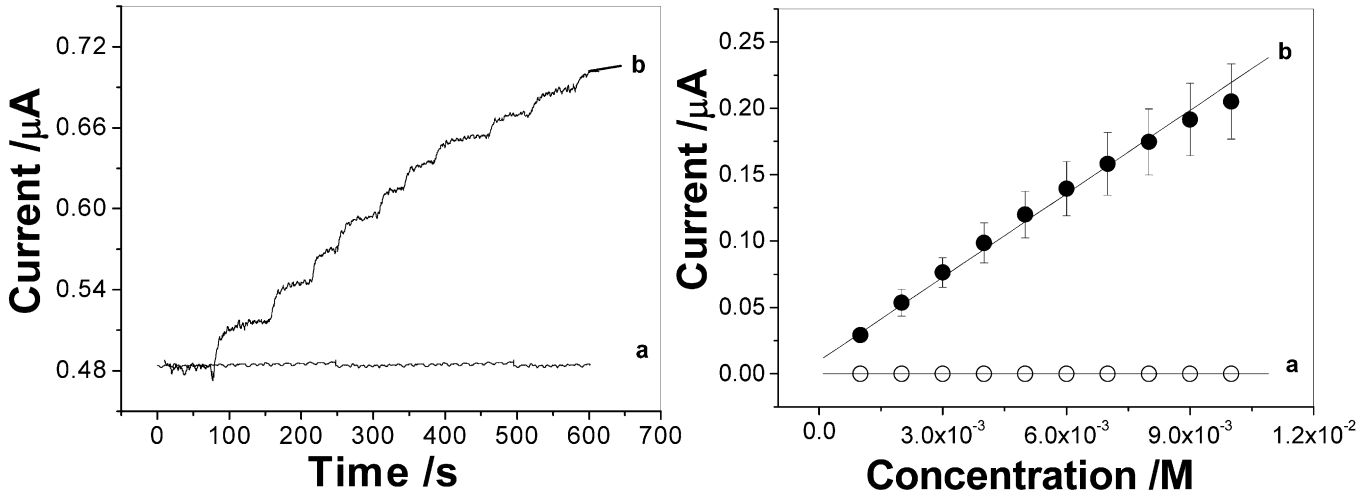

Fig. 5. A) Amperometric recordings for successive additions of 1.0×10^{-3} M ethanol at CPE-ADH(10.0% w/w)-NAD$^+$(10.0% w/w) (a) and GrPE-ADH(10.0% w/w)-NAD$^+$(10.0% w/w) (b). Working potential: 0.450 V. B) Calibration plots for ethanol obtained from the amperometric recordings shown in Figure 4 A.

Table 1. Quantification of ethanol in different beverages using the GrPE modified with PPO. Working potential: 0.450 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

	Ethanol (%v/v)	Label (%v/v)
Champagne Mumm extra brut	12.6±0.6	12.3
Bacardi Gold (crafted rum)	40±3	40
White wine (Benjamin Nieto Senetiner)	11±2	10.5

4 Conclusions

The results presented here demonstrate that GrPE combines the advantages of the efficient electrocatalytic activity of CRGrO with the versatility of composite materials, providing a useful avenue for the electrochemical sensing and biosensing of different analytes. The preferential and efficient adsorption of Do at GrPE and the catalytic activity of Gr made possible the quantification of Do at submicromolar levels even in the presence of high excess of ascorbic acid and serotonin. The incorporation of PPO and ADH/NAD^+ within the GrPE represents a fast and simple way to design new enzymatic biosensors for the quantification of phenols and ethanol, respectively, based on the catalytic activity of Gr towards the reduction of quinones and the oxidation of NADH.

In summary, the incorporation of CRGrO within the CPE represents a very good alternative in the development of novel analytical platforms for the detection of different (bio)analytes in a simple and fast way.

Acknowledgements

The authors thank *CONICET*, *SECyT-UNC*, *ANPCyT*, *MINCyT-Córdoba* for the financial support. FG and FC acknowledge *CONICET* for the fellowships.

References

[1] L. E. Araujo, J. C. Ferreira, L. A. Tellez, X. Ren, C. W. Yeckel, *Physiol. Behavior* **2012**, *106*, 394.
[2] P. Gorwood, Y. L. Strat, N. Ramoz, C. Dubertret, J. M. Moalic, M. Simonneau, *Human Genetics* **2012**, *131*, 803.
[3] A. A. Grace, *Neuropharmacology* **2012**, *62*, 1342.
[4] P. S. Rao, N. Rujikarn, J. M. Luber, D. H. Tyras, *Chromatographia* **1989**, *28,* 307.
[5] A. Sánchez Arribas, E. Bermejo, M. Chicharro, A. Zapardiel, G. L. Luque, N. F. Ferreyra, G. A. Rivas, *Anal. Chim. Acta* **2007**, *596*, 183.
[6] M. Snejdárková, A. Otutnayová, P. Rybár, I. Lhoták, M. Himi, K. Flidrová, T. Hjanik, *Bioelectrochemistry* **2010**, *80*, 55.
[7] H. R. Kim, T. H. Kim, S. H. Hong, H. G. Kim, *Biochem. Biophys. Res. Commun.* **2012**, *419*, 632.
[8] M. Noroozifar, M. Khorasanorlagh, R. Akbari, M. B. Parizi, *Biochem. Biophys. Res. Commun.* **2011**, *419*, 632.
[9] J. Dong, D. Jianyuan, Y. Hongyan, L. Ling, X. Dan, *Talanta* **2011**, *85*, 2344.
[10] M. Noroozifara, K. M. Mozhgan, A. Reza, B. Parizi Mojtaba, *J. Electroanal. Chem.* **2011**, *28*, 56.
[11] S. S. Jyothirmayee Aravind, S. Ramaprabhu, *Sens. Actuators B, Chem.* **2011**, *155*, 679.
[12] R. Cui, X. Wang, G. Zhang, C. Wang, *Sens. Actuators B, Chem.* **2012**, *161*, 1139.
[13] Y.-R. Kima, S. Bonga, Y.-J. Kanga, Y. Yanga, R. K. Mahajanb, J. S. Kimc, H. Kima, *Biosens. Bioelectron.* **2010**, *25*, 2366.
[14] X. Niu, W. Yang, H. Guo, J. Ren, J. Gao, *Biosens. Bioelectron.* **2013**, *41*, 225.
[15] L. Jin, X. Gao, L. Wang, Q. Wu, Z. Chen, X. Lin, *J. Electroanal. Chem.* **2013**, *692*, 1.
[16] L. Tan, K. G. Zhou, Y. H. Zhang, H. Wang, X. D. Wang, Y. F. Guo, H. L. Zhang, *Electrochem. Commun.* **2010**, *12*, 557.
[17] S. Hou, M. L. Kasner, S. Su, K. Patel, R. Cuellari, *J. Phys. Chem. C* **2010**, 114, 14915.
[18] J. J. Roy, T. E. Abraham, K. S. Abhijith, P. V. Kumar, M. S. Thakco, *Biosens. Bioelectron.* **2005**, 21, 206.
[19] R. S. Freire, N. Duran, L. T. Kubota, *Anal. Chim. Acta* **2002**, *463*, 229.
[20] D. C. Topping, L. G. Bernard, J. L. O'Donoghue, J. C. English , *Foods, Chem. Toxicol.* **2007**, *45*, 70.
[21] S. Singh, D. V. S. Jairo, M. L. Singla, *Anal. Meth.***2013**, *5*, 1024.
[22] A. S. Arribas, M. Martínez-Fernández, M. Chicharro, *Tr. Anal. Chem.* **2012**, *34*, 78.
[23] X. Cetó, F. Céspedes, M. I. Pividori, J. M. Gutiérrez, M. Del Valle, *Analyst* **2012**, *137*, 349.
[24] S. G. Burton, *Catalysis Today* **1994**, *22*, 459.
[25] Z. Ori, A. Kiss, A. A. Ciucu, C. Mihailciuc, C. De Stefanescu, L. Nagy, G. Nagy, *Sens. Actuators B, Chem.* **2014**, *190*, 149.
[26] B. Wang, J. Zheng, Y. He, Q. Sheng, Y. He, Q. Sheng, *Sens. Actuators B, Chem.* **2013**, *186*, 417.
[27] Y. Qu, M. Ma, Z. Wang, G. Zhau, B. Li, X. Wang, H. Fang, H. Zhang, C. Li, *Biosens. Bioelectron.* **2013**, 44, 85.
[28] S. H. Zhen, Y. Wang, C. G. Liu, G. M. Xie, C. S. Zou, J. Zhen, Y. Zhu, *Forensic Sci. Int.* **2011**, *207*, 177.
[29] A. P. Periasamy, Y. Umasankr, S. M. Chen, *Talanta* **2011**, *83*, 930.
[30] D. W. Yang, H. H. Liu, *Biosens. Bioelectron.* **2009**, *25*, 733.
[31] H. Lidén, A. R. Vijayakumar, L. Gorton, G. Marko-Varga, *J. Pharm. Biomed. Anal.* **1998**, *17*, 1111.
[32] A. K. Wanekaya, M. Uematsu, M. Breimer, O. A. Sadik, *Sens. Actuators B, Chem.* **2005**, *110*, 41.
[33] W. H. Gao, Y. S. Chen, J. Xi, S. Y. Lin, Y. W. Chen, Y. J. Lin, Z. G. Chen, *Biosens. Bioelectron.* **2013**, *41*, 776.
[34] K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, I. V. Grigorieva, A. A. Firsov, *Science* **2004**, *306*, 666.
[35] Y. Shao, J. Wang, H. Wu, J. Liu, I. A. Aksay, Y. Lin, *Electroanalysis* **2010**, *22*, 1027.
[36] K. R. Ratinac, W. Yang, J. J. Gooding, P. Thordarson, F. Braet, *Electroanalysis* **2011**, *23*, 803.
[37] W. Yang, K. R. Ratinac, S. P. Ringer, P. Thordarson, J. J. Gooding, F. Braet, *Angew. Chem. Int. Ed.* **2010**, *49*, 2114.
[38] L. Yang, D. Liu, J. Huang, T. Yoo, *Sens. Actuators B, Chem.* **2014**, *193*, 166.
[39] C. Zhu, S. Dong, *Electroanalysis* **2014**, *26*, 14.
[40] M. Pumera, *Electrochem. Commun.* **2013**, *36*, 14.
[41] M. H. Parvin, *Electrochem. Commun.* **2011**, *13*, 366.
[42] F. Li, J. Li, Y. Feng, L. Yang, Z. Du, *Sens. Actuators B, Chem.* **2011**, *157*, 110.
[43] H. Bahramipur, F. Jalali, *J. Pharm. Pharmacol.* **2012**, *6*, 1298.

[44] W. Wonsawat, S. Chuanuwatanabul, W. Dungchai, E. Punrat, S. Motomizu, O. Chailapakul, *Talanta* **2012**, *100*, 282.
[45] L. Ma, G.-C. Zhao, *Int. J. Electrochem.* **2012**, 24303.
[46] A. Gasnier, M. L Pedano, M. D. Rubianes, G. A. Rivas, *Sens. Actuators B, Chem.* **2013**, *176*, 921.
[47] V. Shakibaian, M. H. Parvin, *J. Electroanal. Chem.* **2012**, *683*, 119.
[48] Y. Yu, Z. Chen, B. Zhang, X. Li, J. Pan, *Talanta* **2013**, *112*, 31.
[49] K-J. Huang, J. Li, Y-M. Liu, X. Cao, S. Yu, M. Yu, *Microchim Acta* **2012**, *177*, 419.
[50] Y. Bo, W. Wang, J. Qi, S. Huang, *Analyst* **2011**, *136*, 1946.
[51] W. S. Hummers, R. E. Offeman, *J. Am. Chem. Soc.* **1958**, *80*, 1339.
[52] D. R. Dreyer, S. Park, C. W. Bielawski, R. S. Ruoff, *Chem. Soc. Rev.* **2010**, *39*, 228.
[53] J. Zhang, H. Yang, G. Shen, P. Cheng, J. Zhang, S. Guo, *Chem. Commun.* **2010**, *46*, 1112.
[54] V. Dua, S. P. Surwade, S. Ammu, S. R. Agnihotra, S. Jain, K. E. Roberts, S. Park, R. S. Ruoff, S. K. Manohar, *Angew. Chem.* **2010**, *122*, 2200.
[55] B. Wang, J. Zhang, Y. He, Q. Zheng, *Sens. Actuators B, Chem.* **2013**, *186*, 417.
[56] S. Singh, D. V. S. Jain, M. L. Singh, *Sens. Actuators B, Chem.* **2013**, *182*, 161.
[57] G. Sanchez-Obrero, M. Mayén, J. M. Rodrpiguez-Mellado, R. Rodríguez-Amaro, *Int. J. Electrochem. Sci.* **2012**, *7*, 10952.
[58] W. Song, D.-W. Li, Y.-T. Li, Y. Li, Y.-T. Long, *Biosens Bioelectr.* **2011**, *26*, 3181.
[59] M. Cortina-Puig, X. Muñoz-Berbel, C. Calas-Blanchard, J.-L. Marty, *Microchim. Acta* **2010**, *171*, 187.
[60] T.-H. Tsai, Y.-C. Huang, S.-M. Chen, *Int. J. Electrochem. Sci.* **2011**, *6*, 3238.
[61] S. Singh, D. V. S. Jain, M. L. Singh, *Anal. Meth.* **2013**, *5*, 1024.
[62] E. Hua, L. Wang, X. Jing, Ch. Chen, G. Xie, *Talanta* **2013**, *111*, 163.
[63] S. N. Liu, C. X. Cai, *J. Electroanal. Chem.* **2007**, *602*, 103.
[64] K. Guo, K. Quian, S. Zhang, J. Kong, Ch. Yu, B. Liu, *Talanta* **2011**, 85, 1174.
[65] Ch. Shan, H. Yang, D. Han, Q. Zhang, A. Ivaska, L. Niu, *Biosens. Bioelectron.* **2010**, 25, 1504.

Received: May 15, 2014
Accepted: June 7, 2014
Published online: July 18, 2014

