Article

Electrocatalytic Detection of Glutathione - the Search for New Mediators

Denise Lowinsohn,*,a Patricia T. Lee[b] and Richard G. Compton*,b

aDepartmento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora, 36036-330 Juiz de Fora-MG, Brazil

bDepartment of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, South Parks Road, OX1 3QZ Oxford, United Kingdom

Nesse trabalho é reportada a possibilidade de usar diferentes mediadores tais como acetaminofeno, 4-aminofenol, 4-amino-2,6-diclorofenol e 4-amino-2,6-difenilfenol para detectar glutationa usando eletrodo de pasta de nanocarbono. A detecção é baseada na resposta eletrocatalítica envolvendo quinoneiminas geradas eletroquimicamente e a glutationa. O 4-aminofenol é o recomendado como sendo o mediador mais sensível visto que o limite de detecção obtido para glutationa 0,80 μmol L^{-1} foi o menor.

We report the possibility to use different mediators such as acetaminophen, 4-aminophenol, 4-amino-2,6-dichlorophenol and 4-amino-2,6-diphenylphenol to detect glutathione using a nanocarbon paste electrode. The detection is based on the electrocatalytic response involving the electrochemically generated quinoneimines and glutathione. 4-aminophenol is recommended as the most sensitive mediator in terms of giving the lowest limit detection of 0.80 μmol L^{-1}.

Keywords: nanocarbon paste electrode, acetaminophen, 4-aminophenol, 4-amino-2,6-dichlorophenol, 4-amino-2,6-diphenylphenol, glutathione

Introduction

Glutathione is the most prevalent cellular thiol and the most abundant low-molecular-weight peptide present in cells. In the human body, the antioxidant properties of glutathione are well recognized.[1] Glutathione acts as an antioxidant, participating in detoxification for xenobiotics and metabolism of numerous cellular compounds.[2,3] Changes in glutathione concentration at the cellular level have been measured and linked to diseases such as cardiovascular diseases, aging, Parkinson's disease, Alzheimer's disease, diabetes and cancer.[4-7]

Currently, the most popular method of detection of glutathione is based on high performance liquid chromatography (HPLC) because of its high sensitivity and selectivity.[8-10] However, HPLC based methods have some disadvantages such as low response, the need for sample preparation, high cost and complaints of use. Electroanalytical methods[11] have also been cited using modified electrodes[12,13] and mediators.[14-16] The purpose of this paper is to identify a range of different mediators that can be

used to detect glutathione using a nanocarbon paste electrode. One of these mediators is acetaminophen and its reaction with glutathione is based on electrocatalytic response involving the electrochemically generated *ortho*-quinone and the target (Scheme 1).[16] Similar mechanisms undergo the use of the other mediators studied in this paper.
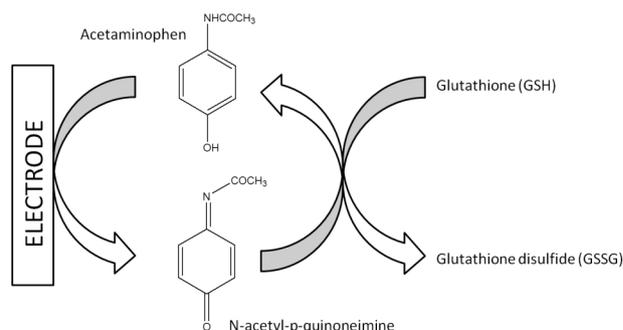
Scheme 1. Electrocatalytic reaction between quinone and glutathione.

The use of nanocarbon as an electrode material has recently been proposed offering similar advantages to other carbon electrodes but at close to zero cost.[17-20] The advantages of using nanocarbon as an alternative electrode modifier for

*e-mail: richard.compton@chem.ox.ac.uk; denise.lowinsohn@ufjf.edu.br

use in adsorptive stripping voltammetry were reported by Lo *et al.*.[17] In this case, the nanocarbon particles were cast onto a glassy carbon electrode. Lowinsohn *et al.*[18] investigated the electrochemical behaviour of nanocarbon paste electrodes prepared by mixing nanocarbon and mineral oil.

In this paper, we explore the electrocatalytic reaction between different mediators in order to detect glutathione using unmodified and modified nanocarbon paste. The electrocatalytic reaction between catechol and glutathione, cysteine, homocysteine and ascorbic acid using nanocarbon paste electrodes were investigated in a previous paper.[21]

Experimental section

Chemicals

Acetaminophen or paracetamol ($C_8H_9NO_2$, Aldrich, 98%), glutathione ($C_{10}H_{17}N_3O_6S$, Sigma-Aldrich), potassium phosphate dibasic (K_2HPO_4, Aldrich), potassium phosphate monobasic (KH_2PO_4, Sigma), nanocarbon particles (diameter 27 ± 10 nm, Monarch 430®, Cabot Performance), mineral oil (Aldrich) were used as received without further purification. Phosphate buffer solution (PBS) was prepared using the adequate amount of K_2HPO_4 and KH_2PO_4 salts. All solutions were prepared using deionised water of resistivity not less than 18.2 MΩ cm at 25 °C (Millipore, Billerica, MA, USA). Prior to experiments, all solutions were purged through nitrogen (N_2, BOC, Surrey) to remove oxygen from the system.

Instrumental

All electrochemical experiments were conducted at (25 ± 1) °C using an Autolab (Eco Chimie, Utrecht, The Netherlands), with a standard three-electrode configuration consisting of nanocarbon or nanocarbon-acetaminophen paste as a working electrode, a graphite rod as a counter electrode and a saturated calomel electrode (SCE) as reference electrode. All experiments were performed at least three times.

Preparation of nanocarbon and nanocarbon-acetaminophen paste electrode

Nanocarbon paste: the carbon paste was prepared by hand pasting nanocarbon with mineral oil (55:45) using a pestle and mortar.[18] The pastes were kept at room temperature until used.

Nanocarbon-acetaminophen pastes: acetaminophen solution was prepared with a certain amount (ca. 10 mg) of solid acetaminophen dissolved in acetone. Acetaminophen

is not soluble in mineral oil so acetone is employed to initially dissolve it. Nanocarbon-acetaminophen paste electrodes were prepared by hand pasting nanocarbon with mineral oil and an aliquot of acetaminophen solution using a pestle and mortar.

For both pastes, unmodified and acetaminophen-modified, the material was packed into the well of the working electrode to a depth of 1 mm (Scheme 2). The surface exposed to the solution was polished using a weighing paper to give a smooth finish before use. The body of the working electrode was a Teflon tube tightly packed with the carbon paste. The electrical contact was provided by a copper wire.
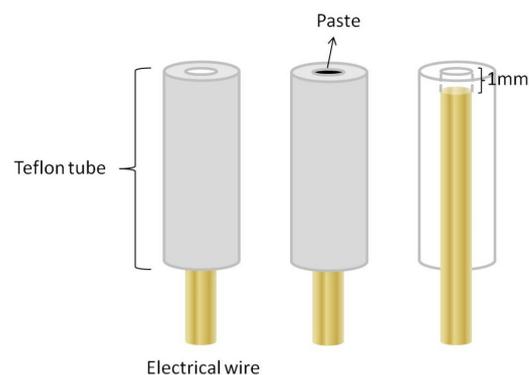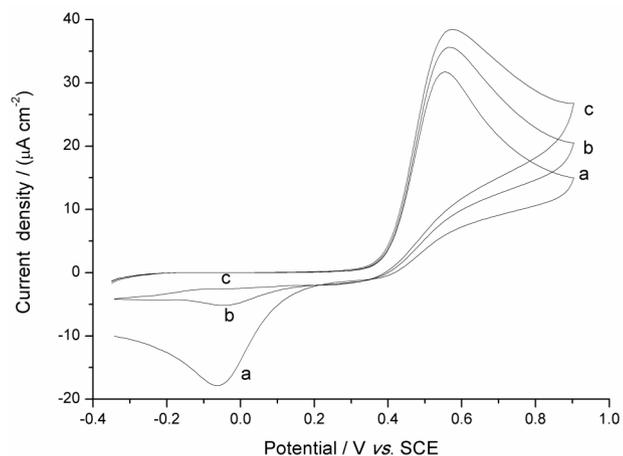

Scheme 2. Nanocarbon paste electrode.

Results and Discussion

Electrochemical behaviour of acetaminophen in a presence of glutathione at nanocarbon paste electrode
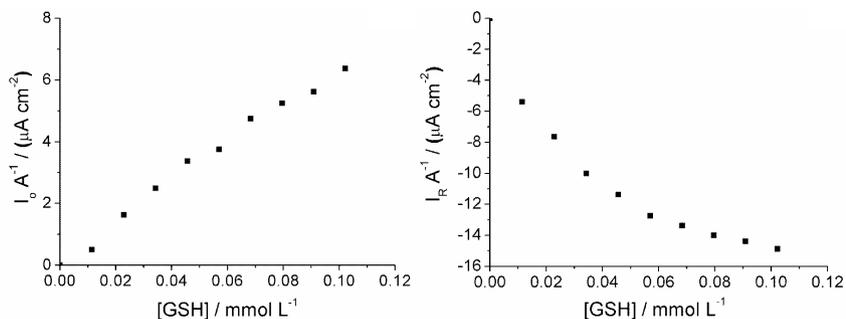
Acetaminophen (APAP) is electrochemically oxidized in a pH-dependent, 2-electron, 2-proton process to *N*-acetyl-p-quinoneimine (NAPQI) (Figure 1, curve a). In a previous paper,[18] we characterized the electrochemical behaviour of acetaminophen at nanocarbon paste electrode, obtaining the formal potentials and kinetics parameters for the acetaminophen oxidation using BASi DigiSim Simulation Software.

In order to investigate the characterization of the reaction between the electrochemically generated quinone and glutathione, an experiment was carried out at a nanocarbon paste electrode using cyclic voltammetry (100 mV s^{-1}). Figure 1 shows the cyclic voltammograms obtained in acetaminophen solution in absence (curve a) and presence of 50 µmol L^{-1} (curve b) and 100 µmol L^{-1} of glutathione (curve c). Figure 1, curve a, shows the oxidation of acetaminophen and the addition of glutathione (curves b and c) led to an increase in the height of the oxidation peak and a decrease in the magnitude of the reduction peak

Figure 1. Cyclic voltammograms of 100 μmol L^{-1} acetaminophen solution in PBS (pH 7.5) in the absence (a) and presence of 50 μmol L^{-1} (b) and 100 μmol L^{-1} (c) glutathione using nanocarbon paste electrode. Scan rate = 100 mV s^{-1}.

indicating an electrocatalytic reaction (Scheme 1).[16] In this reaction, the acetaminophen undergoes an electrochemical oxidation to form quinone, which mediates the reduction of glutathione species, RSH, to a disulfide, RSSR. An analytical curve was obtained by plotting oxidation (Figure 2a) and reduction peak (Figure 2b) current against the concentration of glutathione. The results show that it is possible to quantify glutathione in both situations, but in the case of reduction peak, the detection is limited by the initial concentration of acetaminophen.

To increase the sensitivity of glutathione detection, square wave voltammetry was utilized. The experiment was carried out in the presence of 10 μmol L^{-1} acetaminophen (PBS, pH 7.5) using frequency at 10 Hz, step potential at 15 mV and amplitude at 50 mV. Figure 3 shows the square wave voltammograms of the APAP oxidation (a) and NAPQI reduction (b) in a presence of different concentrations of glutathione at nanocarbon paste electrode.

An analytical curve was obtained by plotting oxidation and reduction peak current against the concentration of glutathione. The results obtained with square wave voltammetry are consistent with the results obtained with cyclic voltammetry. The analytical curve obtained for oxidation peak in a range between 0 to 5 μmol L^{-1} (i/A (μA cm^{-2}) = 0.0622[GSH] (μmol L^{-1})) was less sensitive that one obtained for reduction peak in a range between 0 to 7.2 μmol L^{-1} (i/A (μA cm^{-2}) = 0.14[GSH] (μmol L^{-1})), showing that the best way to quantify glutathione is to use the reduction. The limit of detection calculated using reduction peak was (0.96 ± 0.03) μmol L^{-1}.

Acetaminophen in nanocarbon paste: a reagentless approach

A possible useful alternative to the method reported above is the use of acetaminophen mixed with nanocarbon paste to create a reagentless sensor. In this case, the

Figure 2. Calibration curve: (a) oxidation peak and (b) reduction peak against glutathione concentration using a nanocarbon paste electrode. [Acetaminophen] = 100 μmol L^{-1}.
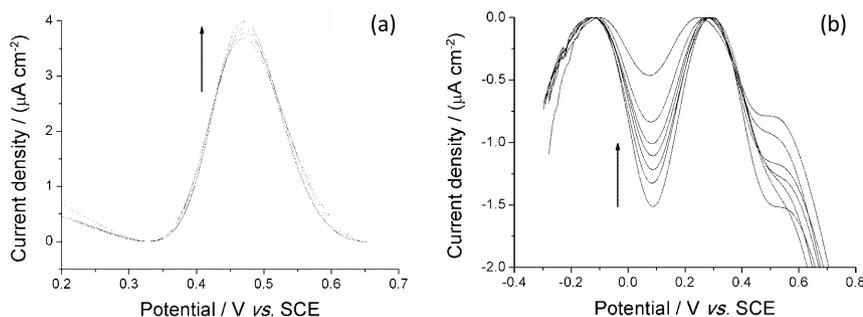
Figure 3. Square wave voltammograms of the APAP oxidation (a) and NAPQI reduction (b) in a presence of different concentrations of glutathione (a) 0-5 μmol L^{-1} and (b) 0-7.2 μmol L^{-1} at nanocarbon paste electrode. [Acetaminophen] = 10 μmol L^{-1}.

experiment requires only the presence of the target to provide a signal and after polishing the paste and washed free of analyte, the sensor is ready for reuse.

Different percentages of acetaminophen dissolved in the paste (0.3, 0.6, 1, 2, 4 and 10%) were used to make the paste electrode. Figure 4 shows cyclic voltammetric responses for 1% nanocarbon-acetaminophen paste in PBS (pH 7.5) at 100 mV s^{-1}. In this case, similar electrochemical behaviour to acetaminophen in solution was observed. Three scans with the same paste are presented in Figure 4, showing that the electrode surface area is not reproducible (standard deviation is between 51 to 66%); this is probably due to the low amount of acetaminophen in the paste generating limited homogeneity. Because of this irreproducible signal from electrode to electrode, the absolute height of the oxidation or reduction peak cannot be used (without standard additions). To solve this problem, we decided to measure the ratio between the oxidation (I_o) and reduction (I_r) peak currents instead of working with absolute current values. Using this method, the standard deviation was 3% (n = 9).
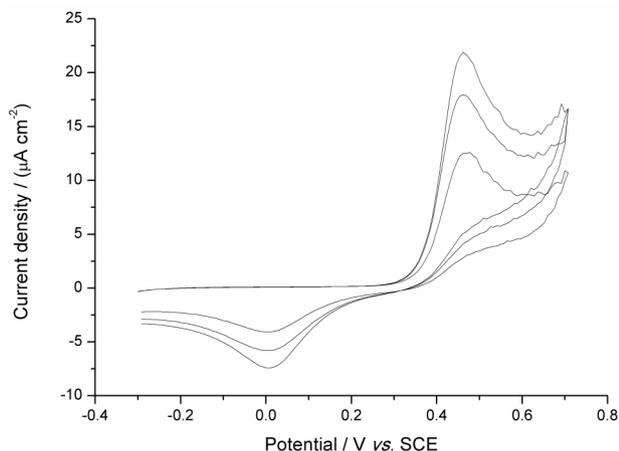
Figure 4. Cyclic voltammetric responses for 1% acetaminophen dissolved in the nanocarbon paste in PBS (pH 7.5) at 100 mV s^{-1}. Three scans with the same paste.

Figure 5 shows the analytical curve for glutathione response obtained with different percentages of acetaminophen dissolved in the paste. From the results obtained, we can conclude that nanocarbon paste with percentages of acetaminophen between 0.3% and 1% can quantify glutathione up to approximately 14 μmol L^{-1} and between 2% and 10% only more than 10 μmol L^{-1}. This happens due to the limitation on the amount of acetaminophen dissolved into the paste. The limits of detection calculated for the different pastes were (1.68 ± 0.05) μmol L^{-1} (0.3%), (1.05 ± 0.05) μmol L^{-1} (0.6%), (1.09 ± 0.05) μmol L^{-1} (1%), (4.79 ± 0.19) μmol L^{-1} (2%), (1.89 ± 0.08) μmol L^{-1}

(4%) and (23.8 ± 1.2) μmol L^{-1} (10%). These results show that the nanocarbon-acetaminophen paste electrode is efficient to quantify glutathione. This modified paste electrode has the advantage that is a reagentless sensor in comparison of nanocarbon paste electrode. However the latter, using acetaminophen in solution, gives a lower limit of detection.
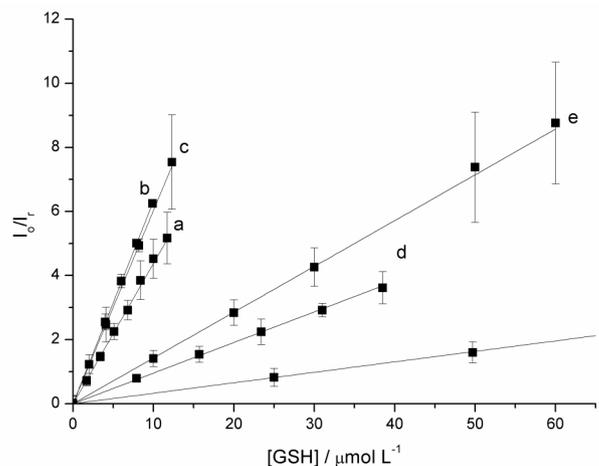
Figure 5. Calibration curve for glutathione at (a) 0.3%, (b) 0.6%, (c) 1%, (d) 2%, (e) 4% and (f) 10% acetaminophen dissolved in nanocarbon paste electrode in PBS (pH 7.5) at 100 mV s^{-1}.

Electrochemical behaviour of 4-aminophenol in the presence of glutathione at nanocarbon paste electrode

The next mediator tested was 4-aminophenol (4-AP), the primary hydrolytic degradation product of paracetamol.[22] First, cyclic voltammograms (Figure 6) were obtained in a solution containing 4-aminophenol and PBS (pH 7.5) using nanocarbon paste electrode at different scan rates (50-400 mV s^{-1}).
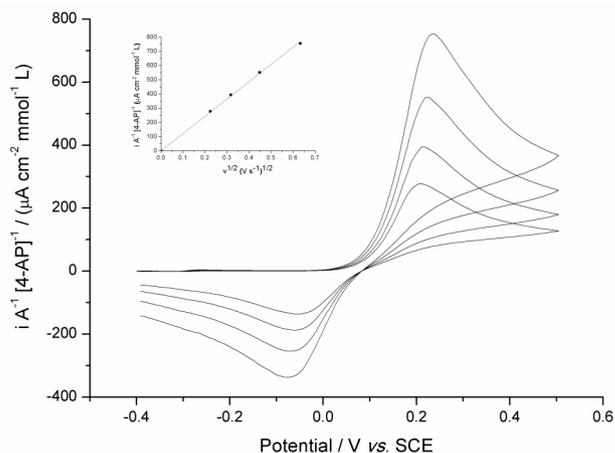
Figure 6. Cyclic voltammograms of 4-aminophenol solution in PBS (pH 7.5) using nanocarbon paste electrode at different scan rates (50-400 mV s^{-1}). [4-aminophenol] = 0.2 mmol L^{-1}.

Similar to paracetamol, the process in this case is attributed to the two-electron oxidation of 4-AP to the corresponding quinone species:
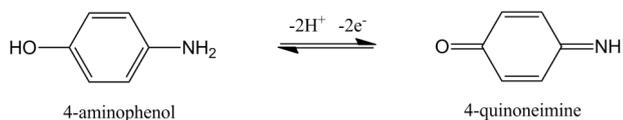
The inset in Figure 6 shows that the peak current increased linearly with the square root of scan rate, suggesting a diffusional process of 4-AP at this electrode. The 4-AP diffusion coefficient value was estimated as being 4.62×10^{-6} cm^2 s^{-1}. This is consistent with the value found in the literature (4.55×10^{-6} cm^2 s^{-1}).[23]

In order to investigate the reaction between the electrochemically generated quinone and glutathione, an experiment was carried out at a nanocarbon paste electrode using cyclic voltammetry (100 mV s^{-1}). The cyclic voltammograms obtained in 4-AP solution in absence of glutathione show only the oxidation of 4-AP. The addition of glutathione led to an increase in the height of the oxidation peak and a decrease in the magnitude of the reduction peak indicating an electrocatalytic reaction, resulting in a similar behaviour observed of the reaction with acetaminophen.

The square wave voltammetry was also used. The experiment was carried out in the same conditions mentioned above. Figure 7 shows the square wave voltammograms of 4-quinoimine reduction in a presence of different concentrations of glutathione at nanocarbon paste electrode. The analytical curve obtained (inset Figure 7) in a range between 0 to 14.8 µmol L^{-1} was i/A (µA cm^{-2}) = 0.174[GSH] (µmol L^{-1}) and the limit of detection calculated was (0.80 ± 0.03) µmol L^{-1}, showing that it is also possible to use 4-AP as a mediator to detect glutathione.
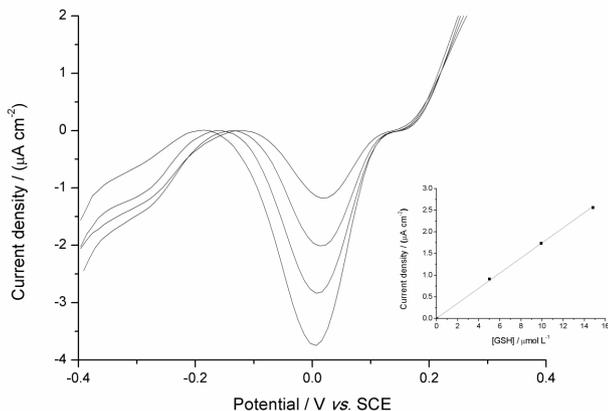
Figure 7. Square wave voltammograms of the 4-quinoneimine in a presence of different concentrations of glutathione (0-14.8 µmol L^{-1}) at nanocarbon paste electrode. Inset: analytical curve obtained. [4-aminophenol] = 20 µmol L^{-1}.

Electrochemical behaviour of 4-amino-2,6-dichlorophenol in the presence of glutathione at nanocarbon paste electrode

The mediator 4-amino-2,6-dichlorophenol was next used to detect glutathione. In order to quantify GSH, the square wave voltammetry was carried out in the same conditions mentioned above. Figure 8 shows the square wave voltammograms of different concentrations of glutathione at nanocarbon paste electrode. The analytical curve obtained (inset Figure 8) in a range between 0 to 80.0 µmol L^{-1} was i/A (µA cm^{-2}) = 0.434[GSH] (µmol L^{-1}) and the limit of detection calculated was (11.0 ± 0.4) µmol L^{-1}, demonstrating that it is also possible to use 4-amino-2,6-dichlorophenol as a mediator to detect glutathione.
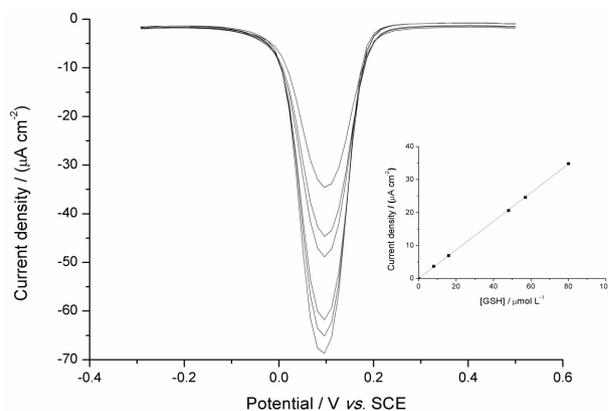
Figure 8. Square wave voltammograms of different concentrations of glutathione at nanocarbon paste electrode. Inset: analytical curve obtained. [4-amino-2,6-dichlorophenol] = 40 µmol L^{-1}.

In a parallel experiment, it was observed that 4-amino-2,6-dichlorophenol accumulated inside the paste as shown in Figure 9. This was studied by obtaining square wave voltammograms in a 40 µmol L^{-1} 4-amino-2,6-dichlorophenol solution in the same conditions mentioned above. The voltammogram in Figure 9b corresponds to the reduction of quinone on a nanocarbon paste electrode. After that, the electrode was washed and transferred to PBS solution and the voltammogram in Figure 9c was obtained, where a clear reduction signal is seen. Then, the electrode was polished and transferred to PBS solution and the voltammogram in Figure 9d was obtained, where a little reduction signal is observed. Therefore, in this case, it is not possible to reuse the same paste after one experiment.

The same behaviour was observed when we used 4-amino-2,6-diphenyphenol (Figure 10). Cyclic voltammograms were obtained in a 0.2 mmol L^{-1} 4-amino-2,6- diphenylphenol solution and the voltammogram in Figure 10b corresponds to the oxidation of 4-amino-2,6-diphenylphenol on a nanocarbon paste electrode. After that, the electrode was washed and
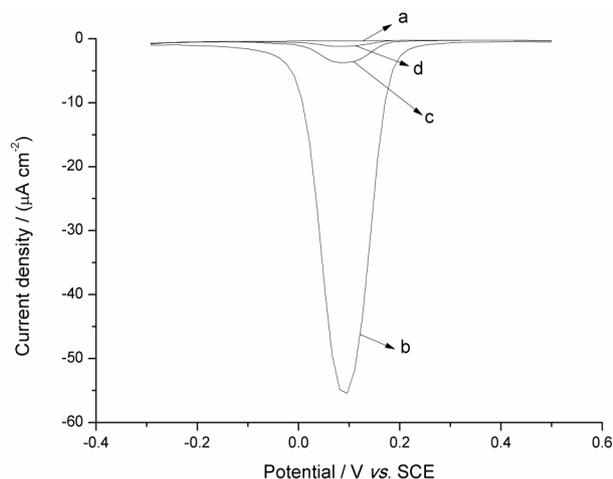
Figure 9. Square wave voltammograms in PBS (pH 7.5) (a) in the presence of 40 μmol L⁻¹ solution of 4 amino-2,6-dichlorophenol (b), after scan in 4-amino-2,6-dichlorophenol solution (c) and after polishing the electrode at nanocarbon paste electrode (d).

transferred to PBS solution and the voltammogram in Figure 10c was obtained, where a signal is seen, related to the 4-amino-2,6-diphenylphenol adsorbed into the paste. Then, the electrode was polished and transferred to PBS solution and the voltammograms in Figure 10d was obtained, where a signal was observed, also associated to 4-amino-2,6-diphenylphenol accumulated into the paste. For this reason, in this case, it is not possible to reuse the same paste after one experiment without surface renewal.
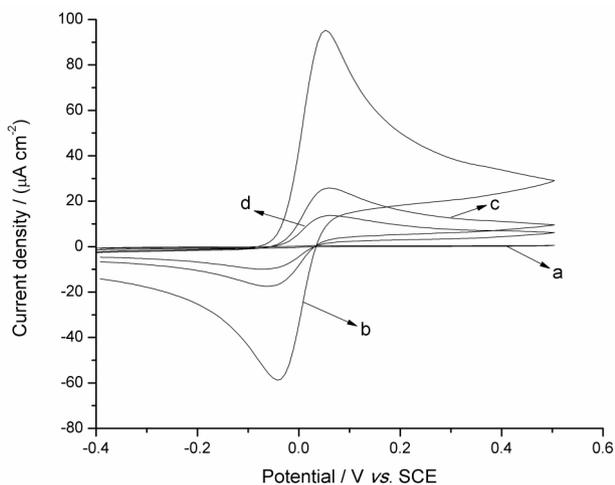
Figure 10. Cyclic voltammograms in PBS (pH 7.5) (a) in the presence of 0.2 mmol L⁻¹ solution of 4 amino-2,6-diphenylphenol (b), after scan in 4-amino-2,6-diphenylphenol solution (c) and after polishing the electrode at nanocarbon paste electrode (d).

Conclusions

In this paper, we described the possibility of using different mediators such as acetaminophen, 4-aminophenol and, 4-amino-2,6-dichlorophenol and 4-amino-2,6-

diphenylphenol to detect glutathione using a nanocarbon paste electrode. Using 4-aminophenol as a mediator in solution, we obtained the lowest limit of detection, (0.80 ± 0.03) μmol L⁻¹, for glutathione. This value is comparable with the other limit of detection obtained with different mediators such as catechol (0.94 μmol L⁻¹),[14] caffeic acid (2.2 μmol L⁻¹)[12] and 3,4-dihydroxy-cinnamic acid (0.194 μmol L⁻¹).[24] We also investigated the preliminary accumulation of 4-amino-2,6-dichlorophenol and 4-amino-2,6-diphenylphenol inside the nanocarbon paste electrode, showing the possibility to explore the modification of the electrode with theses mediators.

Acknowledgements

References

1. Deneke, S. M.; Fanburg, B. L.; *Am. J. Phys.* **1989**, *257*, L163.

2. Filomeni, G.; Rotilio, G.; Ciriolo, M. R.; *Biochem. Pharmacol.* **2002**, *64*, 1057.

3. Moran, L. K.; Gutteridge, J. M.; Quinlan, G. J.; *Curr. Med. Chem.* **2001**, *8*, 763.

4. Pastore, A.; Federici, G.; Bertini, E.; Piemonte, F.; *Clin. Chim. Acta* **2003**, *333*, 19.

5. Smeyne, M.; Smeyne, R. J.; *Free Radical Bio. Med.* **2013**, *62*, 13.

6. Traverso, N.; Ricciarelli, R.; Nitti, M.; Marengo, B.; Furfaro, A. L.; Pronzato, M. A.; Marinari, U. M.; Domenicotti, C.; *Oxi. Med. Cell. Longev.* **2013**, *2013*, 1.

7. Liu, H. L.; Wang, H.; Shenvi, S.; Hagen, T. M.; Liu, R.-M.; *Ann. N. Y. Acad. Sci.* **2004**, *1019*, 346.

8. Ferina, R.; Pavão, M. L.; Baptista, J.; *J. Chromatogr. B* **2012**, *911*, 15.

9. Squellerio, I.; Caruso, D.; Porro, B.; Veglia, F.; Tremoli, E.; Cavalca, V.; *J. Pharmaceut. Biomed.* **2012**, *71*, 111.

10. Conlan, X. A.; Stupka, N.; McDermott, G. P.; Francis, P. S.; Barnett, N. W.; *Biomed. Chromatogr.* **2010**, *24*, 455.

11. Harfield, J. C.; Batchelor-McAuley, C.; Compton, R. G.; *Analyst* **2012**, *137*, 2285.

12. Lee, P. T.; Compton, R. G.; *Electroanal.* **2013**, *25*, 1613.

13. Lee, P. T.; Ward, K. R.; Tschulik, K.; Chapman, G.; Compton, R. G.; *Electroanal.* **2014**, *26*, 366.

14. Seymour, E. H.; Wilkins, S. J.; Lawrence, N. S.; Compton, R. G.; *Anal. Lett.* **2002**, *35*, 1387.

15. White, P. C.; Lawrence, N. S.; Davis, J.; Compton, R. G.; *Electroanal.* **2002**, *14*, 89.

16. Shayani-Jam, H.; Nematollahi, D.; *Chem. Commun.* **2010**, *46*, 409.

17. Lo, T. W. B.; Aldous, L.; Compton, R. G.; *Sensor Actuat. B-Chem.* **2012**, *162*, 361.

18. Lowinsohn, D.; Gan, P.; Tschulik, K.; Foord, J. S.; Compton, R. G.; *Electroanal.* **2013**, *25*, 2435.

19. Gan, P.; Lowinsohn, D.; Foord, J. S.; Compton, R. G.; *Electroanal.* **2014**, *26*, 351.

20. Hocevar, S. B.; Ogorevc, B.; *Talanta* **2007**, *74*, 405.

21. Lowinsohn, D.; Lee, P. T.; Compton, R. G.; *Int. J. Electrochem. Sci.* **2014**, *9*, 3458.

22. Bloomfield, M. S.; *Talanta* **2002**, *580*, 1301.

23. Zhang, X.; Wang, S.; Shen, Q.; *Microchim. Acta* **2005**, *149*, 37.

24. Keyvanfard, M.; Karimi-Maleh, H.; Alizad, K.; *Chin. J. Catal.* **2013**, *34*, 1883.