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Determination of Phenol Compounds Based on Electrodes with HRP Immobilized on Oxidized Multi-Wall Carbon Nanotubes

Arnaldo C. Pereira[1*] • Alexandre Kisner[1] • Cesar Ricardo T. Tarley[2] • Nelson Durán[1] • Lauro T. Kubota[1]

[1] Instituto de Química, Universidade Estadual de Campinas, Cx. Postal 6154, 13084-971, Campinas, SP, Brazil
[2] Departamento de Ciências Exatas, Universidade Federal de Alfenas, Unifal - MG, Alfenas/MG 37130-000, Brazil

Corresponding author: * **arnaldocsp@yahoo.com.br**

ABSTRACT

In the present work the development of an amperometric biosensor for phenol detection based on oxidized multi-wall carbon nanotubes (MWCToxi) and horseradish peroxidase (HRP) is reported. The variables that exert influence on the performance of the biosensor response, including enzyme immobilization procedure, HRP amounts, pH, and working potential were investigated. Furthermore, the feasibility of the biosensor response for various phenol compounds was also investigated. The amperometric response for cathecol using the proposed biosensor showed a wide linear response range (1 to 150 μmol L^{-1}), good sensitivity (53 μA cm^{-2} μmol L^{-1}), excellent operational stability (after 200 determinations the response remained at 97%) and very good storage stability (lifetime > 3 months). The results were compared with HRP immobilized on graphite powder, highlighting the remarkable features of MWCToxi in the biosensor performance. According to these features, it is possible to affirm that the developed biosensor is a promising tool for phenol detection due to its good electrochemical response and enzyme stabilization.

Keywords: biosensor, horseradish, multi-wall carbon nanotubes, peroxidase, phenolic compounds

INTRODUCTION

The determination of phenolic compounds and their analogues is of great importance to many areas of health and life sciences (Wang *et al*. 2003, 2004; Wang 2005), since these species are toxic and are involved in many industrial processes, such as in wastewater from oil, paint, polymer and pharmaceutical industries. Their detection and quantification in a reliable and versatile way represents a step forward to many timeless consuming and inexpensive technological systems that may be applied directly to industry. For instance, the quality of foods has been related to the presence of phenolic substances, mainly in beverages and juices (Minussi *et al*. 2007). Hence, the importance of a rapid, selective and sensitive detection of phenolic compounds is evident (Wang 2005). Several methods for phenol detection have been described in the literature, such as liquid chromatography (Csoregi *et al*. 1993; Munteanu *et al*. 1998; Rosatto *et al*. 2002; Pereira *et al*. 2003; Santos *et al*. 2003; Yu *et al*. 2003; Dugo *et al*. 2008; Im *et al*. 2008; Spacil *et al*. 2008) and UV/visible spectrophotometry (Gishen *et al*. 2005; Harnly *et al*. 2007).

Although advances have been made with these methods, all of them still require sample pre-treatment and demand time for analysis; the assay is thus inadequate for routine testing. Additionally, some of those methods present poor selectivity and/or sensitivity. On the other hand, amperometric biosensors, which combine redox enzyme reactions with electrochemical detection, have demonstrated to be rapid, sensitive and selective tools for the determination of phenolic compounds (Mailley *et al*. 2004; Kawakami *et al*. 2005; Vega *et al*. 2007; Granero *et al*. 2008). Over the last few years a lot of attention has been given for the use of amperometric biosensors based on nanostructured materials, such as carbon nanotubes (CNTs) (Wu *et al*. 2003; Katz and Willner 2004; Gong *et al*. 2005). The use of CNTs is relevant due to their metallic character, making it possible to improve the electron transfer between enzymes and electrode surfaces (Merkoçi 2006), working in this case as electron mediators, and support for enzyme immobilization (Hrapovic *et al*. 2008). Additionally, the preparations of CNT-based biosensors have demonstrated to be very simple, allowing easy adsorption of large molecules, such as organic dyes (Jeykumari *et al*. 2005), enzymes (Callegari *et al*. 2004; Luong *et al*. 2005; Wang *et al*. 2005; Santos *et al*. 2006; Chen and Dong 2007) and DNA or RNA (Wang *et al*. 2003, 2004; Wang 2005; Ye and Ju 2005). In addition, these biosensors usually are mechanically stable with low background current and reproducible electrochemical behaviour (Wang and Musameh 2003; Minussi *et al*. 2007; Im *et al*. 2008), which make them sufficiently robust for practical applications and mass production. In all cases the sensitivity is increased, attributed to the more efficient electron transfer promoted by the conductive properties of CNT. Specifically, in the case of phenolic compound determination, the use of horseradish peroxidase (HRP)-based biosensors offer some advantages in comparison to the other enzymatic systems. For example, Rosatto *et al*. (2002) showed that biosensors based on HRP immobilized are the most sensitive for a great number of phenolic molecules rather than biosensors prepared with tyrosinase (Spacil *et al*. 2005). Other approaches exploiting CNT for HRP-based biosensor construction for H_2O_2 determination were also reported (Xu *et al*. 2003; Yu *et al*. 2003; Chen and Dong 2007; Dugo *et al*. 2008), but no research group reported phenol detection using CNT and HRP, probably due to the better direct electron transfer from HRP to the electrode surfaces promoted by CNT, avoiding or even annulling the mediated electron transfer.

The oxidation of multi-wall CNTs and the consequent immobilization, of HRP on the side walls of CNTs could increase the protein immobilization minimizing the direct electron transfer improving the sensitivity to phenols. The development of an amperometric biosensor for the detection of typical phenolic molecules, as well as the chemical and the operational/storage stability of the biosensor prepared with oxidized CNTs are described.

Received: 1 July, 2009. Accepted: 27 October, 2009.

Original Research Paper

EXPERIMENTAL

Reagents

Horseradish peroxidase (EC 1.11.1.7) 145 U mg^{-1} was purchased from Boehringer, Mannheim, Germany. Hydrogen peroxide (30%, w/v) was purchased from Merck (Darmstadt, Germany). Multi-wall CNTs (93%) produced by thermal chemical vapor deposition were purchased from CNT Co. Ltd. Incheon, Korea. 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) was purchased from Sigma (St. Louis, MO, USA). Graphite powder (99.9%) was acquired from BDH (Poole, United Kingdom) and mineral oil (nujol from Schering-Plough, Brazil) was acquired from Sigma. All other reagents were of analytical grade.

Carboxylation of MWCTs

Oxidized multi-wall carbon nanotubes (MWCToxi) were prepared according to the published method (Xu *et al*. 2003). MWCTs were refluxed with 3 mol L^{-1} HNO_3 at 120°C for 2 h. $MCWs_{oxi}$ formed were filtered and washed with double distilled and deionized water until the pH of the filtrate became neutral then they were dried overnight at 80°C.

Biosensor preparation using MWCT

The enzyme was immobilized directly on MWCToxi by covalent bonding via EDC, employing 10 mg of MWCToxi with 100 μL of 1 mg mL^{-1} HRP solution. This mixture was homogenized and let for 15 hrs at 4°C. These materials will be hereafter designated as MWCToxi/EDC/HRP. The biosensor was prepared by mixing 10 mg of MWCToxi/EDC/HRP with 1 mg of graphite powder (99.9%) acquired from BDH (Poole, United Kingdom). After that, 30 μL of mineral oil was added and manually mixed until obtaining a homogeneous paste. This paste containing MWCToxi/EDC/HRP was placed into a cavity of a home made glass tube with dimensions of 150, 4 and 1 mm, length, internal diameter and depth, respectively. In the upper part of the cavity of tube a Pt plate was placed and connected with a nickel/chromium wire to make electrical contact.

In order to evaluate the enhancement obtained with MWCToxi employed in the biosensor preparation, a paste contained only graphite powder or MWCT as carbonaceous material was prepared. These pastes were prepared in the same way as the biosensors based on MWCToxi.

Electrochemical measurements

The electrochemical experiments were performed using a PGSTAT 30 potentiostat (EcoChemie-Autolab), interfaced with a personal computer for data acquisition and potential control. All electrochemical measurements were carried out in triplicate using a conventional three-electrode cell at room temperature, with a saturated calomel electrode (SCE) as reference, a platinum wire as counter and modified carbon paste as working electrodes.

The voltammetric and amperometric measurements were carried out in a conventional three-electrode electrochemical cell. A SCE (Corning, St. Louis, MO) and a coiled Pt wire were used as the reference and the counter electrodes, respectively. The potentiostat (Autolab-PGSTAT30, EcoChemie, Utrecht, The Netherlands) was connected to a PC microcomputer for data acquisition (software GPES 4.9, EcoChemie, Utrecht, The Netherlands) and potential control.

RESULTS AND DISCUSSION

Mechanism of the biosensor containing HRP

When peroxidase is immobilized on electrode's surface, the oxidized form of the enzyme, which is formed in the reaction with peroxide, can be reduced to its native form by direct and/or mediated electron transfer by means of electron donating compounds, such as phenol species (Wang *et al*. 2003). The enzyme mechanism that takes place in a biosensor based on peroxidase consists in the oxidation of the enzyme by hydrogen peroxide, followed by its reduction with a given phenolic compound. The reducing current observed is proportional to the phenolic compound concentration, as demonstrated below:

$$HRP\ (Fe^{3+}) + H_2O_2 \rightarrow Compound\ I + H_2O \quad \text{(Eq. 1)}$$

$$Compound\ I + AH_2 \rightarrow Compound\ II + {}^{\bullet}AH \quad \text{(Eq. 2)}$$

$$Compound\ II + AH_2 \rightarrow HRP(Fe^{3+}) + {}^{\bullet}AH + H_2O \quad \text{(Eq. 3)}$$

$$2AH^{\bullet} + 2H^{+} + 2e^{-} \rightarrow 2AH_2 \quad \text{(Eq. 4)}$$

Here, compound I and II are oxidized intermediate species of the enzyme, AH_2 is the reducer substrate (phenolic compounds) and ${}^{\bullet}AH$ is a free radical. In the first step, the enzyme is oxidized by hydrogen peroxide, in which is formed an oxidized intermediate compound from enzyme. This oxidized intermediate compound is reduced to its native form in two steps, as demonstrated in equations 2 and 3 (Wang *et al*. 2003, 2004). The electrochemical step to observe the reduction current is presented in Eq. 4.

Optimization of the biosensor preparation

Table 1 shows the effects of the immobilization matrix (graphite, carbon nanotube and oxidized carbon nanotube) on the amperometric response of biosensor for cathecol employing HRP enzyme. These measurements were carried out in 0.1 mol L^{-1} phosphate buffer at pH 7.0. The analytical curve obtained using the graphite paste electrode containing HRP presented the lowest sensitivity when a potential of –50 mV was applied in relation to SCE. However, electrodes based on MWCT/EDC/HRP and MWCToxi/EDC/HRP, in which the enzyme was linked to the support by peptide bound employing EDC as coupling agent, a considerable enhancement in the sensitivity was observed, being higher when the biosensor was developed with oxidized carbon nanotube. These results indicated that the proposed experimental procedure with MWCT oxidation is viable and this enhancement on the amount of carboxyl groups (-COOH) caused an increase in the number of the free sites on the carbon nanotube matrix to HRP immobilization, which are activated by EDC.

Due to the increase of the amount of protein immobilized (HRP), which is caused by the increase of the amount of groups –COOH on carbon nanotubes surface, the MWCToxi can decrease the probability of direct electron transfer from HRP to the electrode surface, thus amplifying sensitivity to phenol detection.

Table 1 Biosensor response for cathecol obtained for graphite/EDC/HRP, MWCT/EDC/HRP and MWCToxi/EDC/HRP. Potential applied -50 mV *vs* SCE, 0.1 mol L^{-1} phosphate buffer solution (pH 6.5). $[H_2O_2]$ = 50 μmol L^{-1}.

Electrode configuration	Sensitivity (nA cm^{-2} / μmol L^{-1})
Graphite/EDC/HRP	15.1
MWCT/EDC/HRP	32.1
MWCToxi/EDC/HRP	52.6

Effect of EDC amount on enzyme immobilization

The degree of HRP immobilizated on MWCToxi is affected by various factors, such as temperature, concentration of the coupling reagent (e.g. EDC), HRP concentration in solution, etc. Therefore, the amount of enzyme used onto MWCToxi matrix was set at 60 U and the EDC concentration was varied from 0 up to 100 mmol L^{-1}. It is well known that EDC is a way to bind the carboxyl groups on the oxidized carbon nanotube surface (MWCT-COOH) with the amino groups of the enzymes. **Table 2** shows the dependence on the immobilization efficiency of HRP on MWCToxi employing varied EDC concentrations as a function of biosensor response for cathecol, keeping the H_2O_2 concentration at

Table 2 Sensitivities of the biosensor for cathecol prepared with HRP and MWCToxi using different EDC concentrations. [H_2O_2] = 40 μmol L^{-1}, E_{apl} = - 50 mV *vs*. SCE.

[EDC] / mmol L^{-1}	Sensitivity / nA L μ mol L^{-1} cm^{-2}
0	11.2
10	56.2
20	54.8
40	32.5
60	12.3
80	10.7
100	8.6

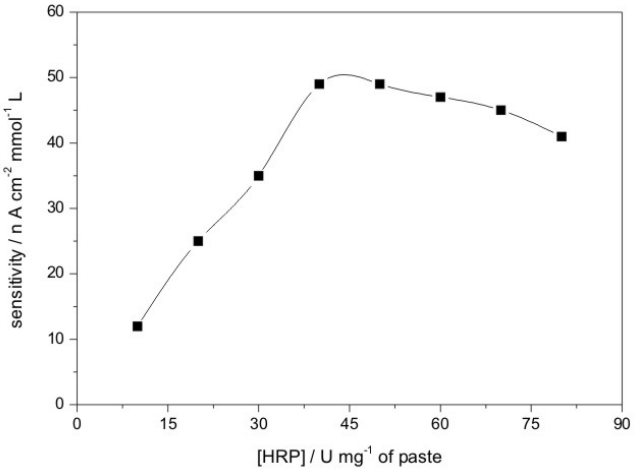

Fig. 1 The biosensor sensitivity for cathecol determination obtained with different amount of HRP loaded. Potential step to -50 mV vs. SCE, 0.1 mol L^{-1} phosphate buffer solution (pH 7.0). Sensitivity obtained by full calibration curves. [H_2O_2] = 40 μmol L^{-1}

40 μmol L^{-1}. Immobilization efficiency was enhanced when the concentration of EDC was increased from 10 to 40 mmol L^{-1}. For concentrations higher than 40 mmol L^{-1} a decrease in the immobilization efficiency was observed. **Table 2** also shows that HRP can be immobilized on MWCToxi even in the absence of EDC, although the efficiency is lower. This indicates that HRP can bind on MWCToxi through noncovalent interactions without any addition of coupling agent. However, addition of 10 mmol L^{-1} EDC in the immobilization mixture resulted in an improvement in the immobilization efficiency of HRP by approximately five times as compared to that in the absence of EDC. Therefore, subsequent immobilization experiments were performed in the presence of 10 mmol L^{-1} EDC.

The amperometric response was also examined as a function of the HRP loading on the MWCT material (**Fig. 1**). Several biosensors based on MWCToxi/EDC/HRP were prepared changing the amount of HRP over the range from 10 to 80 U of enzyme per mg of MWCToxi, whereas the amount of other components was kept constant. The sensitivity of the prepared biosensors was dependent on the amount of enzyme incorporated in MWCToxi. An increase in the sensitivity was observed from 10 to 40 U mg^{-1}, while for higher loadings no significant increase was observed. After that, further experiments were carried out using 40 U mg^{-1} of MWCToxi material.

Optimization of the biosensor response

The applied potential is a very important parameter in the response of a biosensor. Thus, the potential influence was verified in order to find an optimum operational condition for cathecol determination. As shown in **Fig. 2**, the amperometric response increases from 200 to 0 mV *vs* SCE and then remains practically constant until –100 mV *vs* SCE. It should be emphasized that in the potential range between 0 and –100 mV, the reduction of quinone species occurs, as previously discussed. For applied potentials more negative

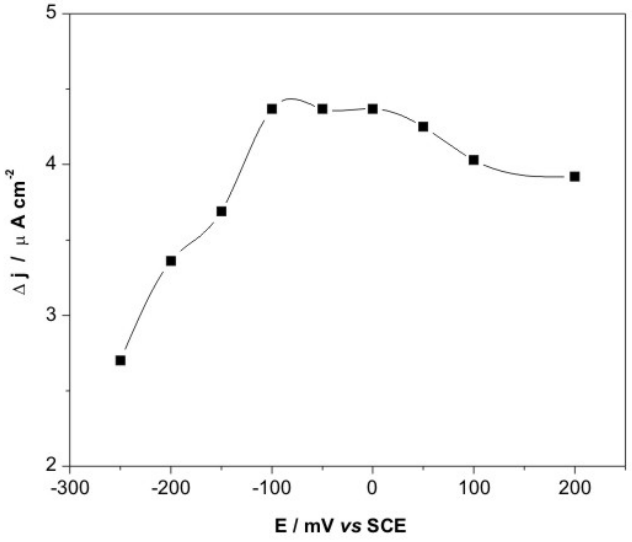

Fig. 2 Amperometric response of the MWCToxi/EDC/HRP biosensor at different applied potentials. Cathecol concentration 80 μmol L^{-1} in 0.1 mol L^{-1} phosphate buffer at pH 7.0. [H_2O_2] = 50 μmol L^{-1}.

Table 3 Biosensor response for cathecol (80 μmol L^{-1}) obtained at different H_2O_2 concentration. The experiments were carried out in 0.1 mol L^{-1} phosphate buffer solution at pH 6.5 applying a potential of -50 mV *vs* SCE.

[H_2O_2] / μmol L^{-1}	Sensitivity / nA L μmol L^{-1} cm^{-2}
10	36.1
20	48.5
40	56.2
60	56.2
80	55.1
100	50.1

than –100 mV *vs* SCE a sharp decrease in the sensitivity was verified. This was attributed to the irreversible inactivation of HRP. In fact, similar behavior was already reported by Gorton et al (Csoregi *et al*. 1993), who noticed that at applied potentials more negative than –150 mV a slow irreversible deactivation of HRP ocurred. Thus, due to its good sensitivity and in order to avoid enzyme inactivation, a potential of –50 mV *vs* SCE was set for further experiments.

It is well known that in HRP-based biosensors for phenol detection, the use of adequate amounts of H_2O_2 is very important to achieve the best sensitivity and longer lifetimes (Munteanu *et al*. 1998). Thus, the influence of the substrate concentration on the proposed biosensor response for cathecol detection was investigated. **Table 3** shows the dependence on the hydrogen peroxide concentration on the biosensor sensitivity. The sensitivity reached a maximum value when the H_2O_2 concentration was varied between 40 to 80 μmol L^{-1} in the measurement cell. Thus, when the H_2O_2 concentration was fixed at 50 μmol L^{-1}, reproducible results were obtained and the biosensor presented a long lifetime. This latter was associated with a decrease on the

Table 4 Biosensor response (nA) obtained in different solution pH. Measurements carried out in 0.1 mol L^{-1} phosphate buffer solution containing 50 μmol L^{-1} H_2O_2 and 80 μmol L^{-1} cathecol, applying a potential of -50 mV vs SCE.

pH	I / nA
5.0	1.35
5.5	2.80
6.0	4.14
6.5	4.40
7.0	4.40
7.5	3.92
8.0	2.58

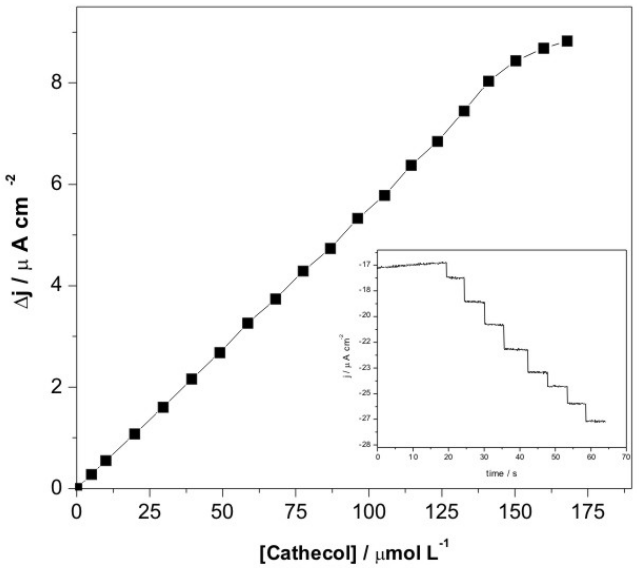

Fig. 3 Typical analytical curve obtained with the MWCToxi/EDC/ HRP biosensor. Measurements carried out in 0.1 mol L^{-1} phosphate buffer solution at pH 6.5 containing 50 μmol L^{-1} H_2O_2 , applying a potential of -50 mV vs SCE. Inset the current-time response curve.

degree of deactivating of enzyme due to the high peroxide concentrations. Thus, a concentration of 50 μmol L^{-1} in H_2O_2 was used for further experiments.

The influence of solution pH on the biosensor response for cathecol is shown in **Table 4**. Higher responses are clearly observed for pH values between 5.0 and 7.0. This pH range reflects the optimum conditions for both enzymatic and mediated electrochemical reactions in the carbon nanotube electrode. Thus, the experiments were carried out at pH 6.5, in which the signal is slightly higher.

Sensor characteristics

The biosensor based on HRP immobilized onto MWCToxi showed excellent sensitivity for cathecol (**Fig. 3**) with a wide linear response range. An analytical curve was obtained from 1 to 175 μmol L^{-1} cathecol in 0.1 mol L^{-1} phosphate buffer at pH 6.5. This analytical curve was adjusted by the equation:

$$\Delta j\ /nAcm^{-2} = -\ 0.6\ (\pm\ 0.2) + 53.2\ (\pm\ 0.3)[catechol]/\mu mol\ L^{-1}$$

with a correlation coefficient of 0.9998 for n = 17. The proposed biosensor showed an excellent operational range and good sensitivity. Detection and quantification limits of 0.45 μmol L^{-1} and 1.5 μmol L^{-1} for cathecol were determined considering $3\sigma_B$/slope and $10\sigma_B$/slope, respectively. The modified electrode presented an excellent repeatability, with a relative standard deviation of 1.8% for a series of seven successive measurements of a cathecol solution at 80 μmol L^{-1}.

This biosensor was very stable, allowing *ca*. 200 measurements of cathecol, without significant change in the response. The biosensor response time was very short, reaching 95% of its maximum response in 0.6 s as can be observed in the inset of **Fig. 3**, which also shows the high signal stability as a function of time. This response time is excellent considering that it is a carbon nanotube paste electrode. All these excellent characteristics can be attributed to the CNT employed in the biosensor preparation. Furthermore, in comparison to other biosensors reported in the literature, which were constructed with pure graphite as the carbonaceous material (Rosatto *et al.* 2002; Mailley *et al*. 2004; Kawakami *et al*. 2005; Portaccio *et al*. 2006; Vega *et al*. 2007), the sensor reported here exhibits higher performance and longer lifetime for cathecol detection.

Table 5 Relative response (%) obtained with the proposed biosensor for 50 μmol L^{-1} of different phenolic compounds. [H_2O_2] = 50 μmol L^{-1}, Eapp = - 50 mV vs SCE, 0.1 mol L^{-1} phosphate buffer solution, pH = 6.5.

Analyte	Relative Response / %
Cathecol	100
Hydroquinone	83
Phenol	42
Resorcinol	23
4-chlorophenol	19

Effect of different phenolic substrates

The literature indicates that peroxidases exhibit relatively low specificity toward electron donors and various phenolic compounds can be determined (Rosatto *et al.* 2002). The relative responses obtained for different phenolic compounds are shown in **Table 5**. The best response was obtained for cathecol followed by hydroquinone and phenol. Other studied compounds showed lower responses than those observed for phenol. These results show that this device is applicable to various phenol compounds. In addition, it was found an improvement in the selectivity to these compounds in relation to others papers reported in literature (Rosatto *et al.* 2002; Mailley *et al*. 2004; Portaccio *et al*. 2006).

CONCLUSION

A MWCToxi/EDC/HRP amperometric biosensor for detection of phenol compounds was developed. The approach employed shows that the sensor was stable, sensitive and reproducible for practical uses. Moreover, it has been shown that this material is an excellent electrochemical support for construction of reproducible biosensors, with low applied potential and excellent operational stability. An unexpected and surprisingly high sensitivity of 53.2 μA cm^{-2} μmol^{-1} L for phenolic compound detection was observed. This high sensitivity was attributed to the signal amplification promoted by the efficient covalent immobilization of enzyme via EDC, which can decrease the probability of electron direct transfer between HRP and electrode surface. Finally, these results suggest that it should be straightforward to use the respective biosensor configuration as a feasible support for phenol detection.

ACKNOWLEDGEMENTS

The authors acknowledge FAPESP, FAPEMIG, CNPq and National Network Research in Carbon Nanotubes for financial support. A.C.P. and A. K. are indebted to FAPESP for the fellowships.

REFERENCES

Callegari A, Cosnier S, Marcaccio M, Paolucci D, Paolucci F, Georgakilas V, Tagmatarchis N, Vázquez E, Prato M (2004) Functionalised single wall carbon nanotubes/polypyrrole composites for the preparation of amperometric glucose biosensors. *Journal Materials Chemistry* **14**, 807-810

Chen H, Dong S (2007) Direct electrochemistry and electrocatalysis of horseradish peroxidase immobilized in sol–gel-derived ceramic–carbon nanotube nanocomposite film. *Biosensors and Bioelectronics* **22**, 1811-1815

Csoregi E, Jonsson-Pettersson G, Gorton L (1993) Mediatorless electrocatalytic reduction of hydrogen peroxide at graphite electrodes chemically modified with peroxidases *Journal Biotechnology* **30**, 315-337

Dugo P, Cacciola F, Kumm T, Dugo G, Mondello L (2008) Comprehensive multidimensional liquid chromatography: Theory and applications. *Journal Chromatography A* **1184**, 353-368

Gong K, Yan Y, Zhang M, Su L, Xiong S, Mao L (2005) Electrochemistry and electroanalytical applications of carbon nanotubes: A review. *Analytical Sciences* **21**, 1383-1393

Granero AM, Fernandez H, Agostini E, Zon MA (2008) An amperometric biosensor for trans-resveratrol determination in aqueous solutions by means of carbon paste electrodes modified with peroxidase basic isoenzymes from *Brassica napus*. *Electroanalysis* **20**, 858-864

Gishen M, Dambergs RG, Cozzolino D (2005) Grape and wine analysis – enhancing the power of spectroscopy with chemometrics. A review of some applications in the Australian wine industry. *Australian Journal Grape and*

Wine Research **11**, 296-305

Hrapovic S, Male KB, Liu Y, Luong JHT (2008) Preparation of polymer–carbon nanotube composite materials and their applications for enzyme entrapment. *Analytical Letters* **41**, 278-288

Harnly JM, Bhagwat S, Lin LZ (2007) Profiling methods for the determination of phenolic compounds in foods and dietary supplements. *Analytical and Bioanalytical Chemistry* **389**, 47-61

Im HW, Suh BS, Lee SU, Kozukue N, Ohnisi-Kameyama M, Levin CE, Friedman M (2008) Analysis of phenolic compounds by high-performance liquid chromatography and liquid chromatography/mass spectrometry in potato plant flowers, leaves, stems, and tubers and in home-processed potatoes. *Journal of Agricultural and Food Chemistry* **56**, 3341-3349

Jeykumari DRS, Kumar SS, Narayanan SS (2005) Immobilization of redox mediators on functionalized carbon nanotube: A material for chemical sensor fabrication and amperometric determination of hydrogen peroxide. *PRAMANA – Journal of Physics* **65**, 731-738

Katz E, Willner I (2004) Biomolecule-functionalized carbon nanotubes: Applications in nanobioelectronics. *ChemPhysChem* **5**, 1084-1104

Kawakami M, Akamatsu N, Koya H, Amada K (2005) Amperometric detection of phenol with cytochrome c-modified gold electrode using dual working electrode system. *Analytical Letters* **38**, 549-561

Lee YM, Kwon OY, Yoon YJ, Ryu K (2006) Immobilization of horseradish peroxidase on multi-wall carbon nanotubes and its electrochemical properties. *Biotechnology Letters* **28**, 39-43

Luong JHT, Hrapovic S, Wang D (2005) Multiwall carbon nanotube (MWCNT) based electrochemical biosensors for mediatorless detection of putrescine. *Electroanalysis* **17**, 47-53

Mailley P, Cummings EA, Mailley S, Cosnier S, Eggins BR, McAdams E (2004) Amperometric detection of phenolic compounds by polypyrrole-based composite carbon paste electrodes. *Bioelectrochemistry* **63**, 291-296

Merkoçi A (2006) Carbon nanotubes in analytical sciences. *Microchimica Acta* **152**, 157-174

Minussi RS, Rossi M, Bologna L, Rotilio D, Pastore GM, Durán N (2007) Phenols removal in musts: Strategy for wine stabilization by laccase. *Journal of Molecular Catalysis B: Enzymatics* **45**, 102-107

Munteanu FD, Lindgren A, Emneus J, Gorton L, Ruzgas T, Csoregi E, Ciucu A, van Huystee RB, Gazaryan IG, Lagrimini LM (1998) Bioelectrochemical monitoring of phenols and aromatic amines in flow injection using novel plant peroxidases. *Analytical Chemistry* **70**, 2596-2600

Pereira AC, Santos AS, Kubota LT (2003) Electrochemical behaviour of riboflavin immobilized on different matrices. *Journal of Colloid and Interface Science* **265**, 351-358

Portaccio M, Martino S Di, Maiuri P, Durante D, Luca P De, Lepore M, Bencivenga U, Rossi S, Maio A De, Mita DG (2006) Biosensors for phenolic compounds: The catechol as a substrate model. *Journal of Molecular Catalysis B: Enzymatics* **41**, 97-102

Rosatto SS, Sotomayor PT, Kubota LT, Gushikem Y (2002) SiO_2/Nb_2O_5 sol–gel as a support for HRP immobilization in biosensor preparation for phenol detection. *Electrochimica Acta* **47**, 4451-4458

Santos AA, Pereira AC, Duran N, Kubota LT (2006) Amperometric biosensor for ethanol based on co-immobilization of alcohol dehydrogenase and Meldola's Blue on multi-wall carbon nanotube. *Electrochimica Acta* **52**, 215-220

Santos AS, Freire RS, Kubota LT (2003) Highly stable amperometric biosensor for ethanol based on Meldola's blue adsorbed on silica gel modified with niobium oxide. *Journal of Electroanalytical Chemistry* **547**, 135-142

Spacil Z, Novakova L, Solich P (2008) Analysis of phenolic compounds by high performance liquid chromatography and ultra performance liquid chromatography. *Talanta* **76**, 189-199

Vega D, Agui L, Gonzalez-Cortes A, Yanez-Sedeno R, Pingarron JM (2007) Electrochemical detection of phenolic estrogenic compounds at carbon nanotube-modified electrodes. *Talanta* **71**, 1031-1038

Wang J (2005) Nanomaterial-based amplified transduction of biomolecular interaction. *Small* **1**, 1036

Wang J, Liu G, Jan MR, Zhu Q (2003) Electrochemical detection of DNA hybridization based on carbon-nanotubes loaded with CdS tags. *Electrochemical Communication* **5**, 1000-1004

Wang J, Li M, Shi Z, Li N, Gua Z (2004) Electrochemistry of DNA at single-wall carbon nanotubes. *Electroanalysis* **16**, 140-144

Wang J, Li Y-B, Qiu JD, Sun DC, Xia XH (2005) Biocomposites of covalently linked glucose oxidase on carbon nanotubes for glucose biosensor. *Analytical and Bioanalytical Chemistry* **383**, 918-922

Wang J, Musameh M (2003) Carbon nanotube/teflon composite electrochemical sensors and biosensors. *Analytical Chemistry* **75**, 2075-2079

Wu K, Fei J, Hu S (2003) Simultaneous determination of dopamine and serotonin on a glassy carbon electrode coated with a film of carbon nanotubes. *Analytical Biochemistry* **318**, 100-106

Xu HZ, Zhu JJ, Wu Q, Hu Z, Chen HY (2003) An amperometric biosensor based on the coimmobilization of horseradish peroxidase and methylene blue on a carbon nanotubes modified electrode. *Electroanalysis* **15**, 219-224

Yu X, Chattopadhyay D, Galeska I, Papadimitrakopoulos F, Rusling JF (2003) Peroxidase activity of enzymes bound to the ends of single-wall carbon nanotube forest electrodes. *Electrochemical Communications* **5**, 408-411

Ye Y, Ju H (2005) Rapid detection of ssDNA and RNA using multi-walled carbon nanotubes modified screen-printed carbon electrode. *Biosensors and Bioelectronics* **21**, 735-741