Biosensors and Bioelectronics 26 (2010) 1768–1773

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Short communication

Enzyme entrapment by β-cyclodextrin electropolymerization onto a carbon nanotubes-modified screen-printed electrode

G. Alarcón-Ángeles[a,e,g], M. Guix[a,b], W.C. Silva[c], M.T. Ramírez-Silva[d], M. Palomar-Pardavé[e], M. Romero-Romo[e], A. Merkoçi[a,f,*]

[a] Nanobioelectronics & Biosensors Group, Catalan Institute of Nanotechnology Campus UAB, 08193 Bellaterra, Barcelona, Catalonia, Spain
[b] Chemistry Department, Autonomous University of Barcelona, Campus UAB, 08193 Bellaterra, Barcelona, Catalonia, Spain
[c] Chemistry Department, CCN, Federal University of Piaui, 64049-550 Teresina, Piaui, Brazil
[d] Chemistry Department, Autonomous Metropolitan University, Campus Iztapalapa, 02200 Mexico City, Mexico
[e] Materials Department, Autonomous Metropolitan University, Campus Azcapotzalco, 02200 Mexico City, Mexico
[f] ICREA, Barcelona, Catalonia, Spain
[g] Basic Sciences Department, Autonomous Metropolitan University, Campus Azcapotzalco, 02200 Mexico City, Mexico

ARTICLE INFO

Article history:
Received 14 April 2010
Received in revised form 31 July 2010
Accepted 18 August 2010
Available online 26 August 2010

Keywords:
Carbon nanotubes
β-Cyclodextrin
Glucose oxidase
Electropolymerization entrapment
Screen-printed electrodes
Dopamine

ABSTRACT

A novel enzyme entrapment approach based on an electropolymerization process utilizing multi-walled carbon nanotubes (MWCNT), β-cyclodextrin (β-CD) and glucose oxidase (GOx) is shown. Dopamine (DA) quantification is presented using a screen-printed electrode modified by electropolymerization of cyclodextrin with glucose oxidase, SPE/MWCNT/β-CD-GOx. In order to show the relevance of the enzyme entrapment strategy controlled by electropolymerization to develop a specific and efficient biosensor, the various parts composing the electrode: SPE, SPE/β-CD, SPE/GOx, SPE/β-CD/GOx, SPE/MWCNT/β-CD, SPE/MWCNT/GOx and SPE/MWCNT/β-CD/GOx were tested separately. It was shown that although DA determination can be achieved with all of them, the electrodes modified with MWCNT presented better analytical features that those built without MWCNT, the best being the one including all components. This biosensor displayed good reproducibility, repeatability, and prolonged life-time under cold storage conditions. Its DA limit of detection (LOD) was 0.48 ± 0.02 μA in a linear range of 10–50 μM with a sensitivity of 0.0302 ± 0.0003 μA μM^{-1} that makes it comparable or even better than many other electrodes reported in the literature. Moreover, it was also shown that using this electrode, DA quantification can be done in the presence of interfering agents such as ascorbic and uric acid. These findings demonstrate that the approach employed is feasible for enzyme entrapment and may find applications in other biosensing systems, where better sensitivity, stability and fast response are required.

1. Introduction

The loss of biological activity is one of the main problems of biosensors (Dornelles and Tatsuo, 2002). Enzyme entrapment onto electrode surfaces had been studied by using various biocompatible materials, such as natural biomaterials, biopolymers (Aihua et al., 2005; Willner and Katz, 2000), insoluble surfactants (Armstrong and Wilson, 2000; Ghindilis et al., 1997) and hydrogel polymers (Gorton et al., 1999; Lu et al., 2006; Furbee et al., 1993). These materials seem to provide a favourable microenvironment for redox proteins allowing to perform direct electrochemistry and leading to adequate biosensors designs and fabrication possibilities.

Due to the slow electron transfer rates of proteins on solid surfaces, new materials had been incorporated to the modified electrodes aiming to achieve a direct electron transfer. Nanomaterials (Rosu et al., 1998; Katz et al., 2004; Merkoçi, 2006; Pérez et al., 2005, 2008; Pumera et al., 2006) have become more attractive because of their excellent properties including high surface activity, high organization and uniformity, which could provide a desirable microenvironment to immobilize proteins and facilitate direct electron transfer with the electrodes used as transducers. On the other hand, supramolecular structures such as those obtained with β-cyclodextrin (β-CD) have been used in enzyme biocatalysts (Ghanem, 2003) to increase substrate solubility, availability and enantioselectivity transformation. Reduced substrates and product inhibition are related to the host–guest interactions where β-CD is forming H-bond (Villalonga et al., 2007). The concentration of substrate in the microenvironment of active site could be increased by the formation of inclusion complexes such as β-CD-enzyme products. Higher stabilization observed for enzymes modified with

* Corresponding author at: Nanobioelectronics & Biosensors Group, Catalan Institute of Nanotechnology Campus UAB, 08193 Bellaterra, Barcelona, Catalonia, Spain. Tel.: +34 9358 12118; fax: +34 9358 12379.
E-mail address: arben.merkoci.icn@uab.es (A. Merkoçi).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.08.058

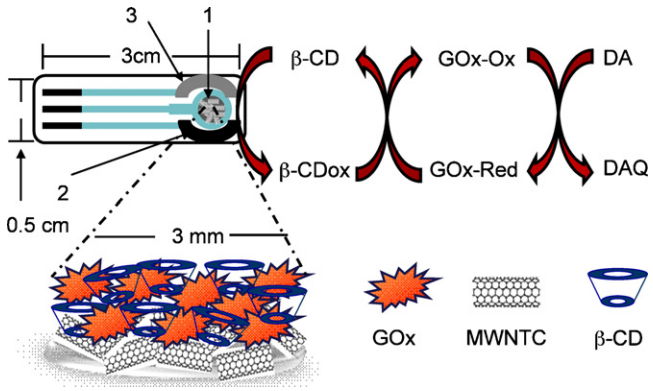

Fig. 1. Schematic representation of the biosensor and its different parts, where 1 is the working electrode, 2 is the counter electrode and 3 is the reference electrode. Also the scheme depicts the dopamine oxidation at the working electrode surface. The reactions describe the redox process of dopamine (DA) to dopamine quinone (DAQ) by GOx and using β-CD as redox mediator co-immobilized with the enzyme. SPE/MWCNT is used as transducer.

β-CD polymers can be attributed to several factors, such as covalent cross-linking with macromolecular structures, formation of new hydrogen bonds or electrostatic interactions at surface (Darias et al., 2002). Therefore, β-CD polymers constitute excellent supports for enzyme immobilization since they present several important properties: polymer matrix itself is used to entrap the enzyme, β-CD cavities may act as analyte (i.e. dopamine) host–guest centers (Alarcon-Angeles et al., 2008) and at the same time provide pores that confer permeability to the polymer. However, β-CD polymers are limited by the reproducibility of synthesis.

We present here a new way to entrap enzyme based on cyclodextrin electropolymerization onto screen-printed electrode (SPE) modified with multiwalled carbon nanotubes (MWCNT). β-CD is used as an electron-transfer mediator between enzyme and the electrode (Fig. 1). The efficiency of this methodology is proved through dopamine quantification.

2. Experimental

2.1. Apparatus and electrodes

All the electrochemical experiments were carried out on a CH-Instrument potentiostat 660A electrochemical workstation from CH Instrument Inc., Austin, TX, using a screen printed home-made electrode. A silver–silver chloride electrode (Ag/AgCl) and a graphite electrode were used as reference and counter electrode, respectively, see Fig. 1. The graphite-modified working electrode was prepared as described in Section 2.1.1.

Scanning electron microscopy (SEM) images were obtained with a Hitachi S-570 (Hitachi Ltd., Japan) instrument with a 3 μm resolution and an accelerating voltage of 15 kV.

2.1.1. Preparation of screen-printed electrode (SPE)

Screen-printing microfabrication is based on the sequential deposition of 3 graphite strips onto a polyester substrate. The central strip ends in a round shape that will be used as the working electrode. The remaining two will correspond to the counter electrode and the reference electrode, respectively. The latter is formed by applying an Ag/AgCl ink over one of the semicircles that surrounds the working electrode. The central part of the strips is finally covered by an insulating ink; see scheme S1 in the supporting information. After each layer is deposited, a drying process is held by keeping the polyester substrate at 90 °C for 15 min, is allowed.

2.2. Reagents and solutions

Multiwalled carbon nanotubes (MWCNT) powders were purchased from Aldrich (Stenheim, Germany) with a purity of 95%. KH_2PO_4 and K_2HPO_2 were used to prepare a 0.1 mol L^{-1} phosphate buffer solution (PBS) at pH 7.44, and tetrahydrofuran (THF) was used as MWCNT dispersant agent. All of them were acquired from Fluka. Dopamine, β-cyclodextrin, glucose oxidase, uric and ascorbic acids from Aldrich, were used as received.

All aqueous solutions were prepared using analytical grade reagents and deionized water (Rs = 18 MΩ cm^{-1}) by Purelab Plus UV equipment and were bubbled with highly purified nitrogen for 10 min before each experiment, keeping this gaseous environment over the solution during the electrochemical measurements.

2.3. Methods

2.3.1. MWCNT purification

Previous chemical oxidation and heat treatment were carried out in order to purify the nanotubes, as previously described (Pérez et al., 2005). The chemical oxidation process consists on stirring MWCNT in 2 mol L^{-1} nitric acid (PanReac, Spain) at 25 °C for 24 h. After the purification step, the CNT dispersion was ensured by sonicating the MWCNT suspension (1 mg MWCNT/1 mL THF) during 4 h.

2.3.2. Modification of SPE with MWCNT

The bare SPE working electrode surface was modified by depositing 7 μL of the MWCNT suspension (1 mg MWCNT/1 mL THF) onto the working electrode surface, followed by a drying process at room temperature for 24 h. In order to check that a homogeneous distribution of MWCNT over electrode surface had been achieved, SEM characterization of the surfaces of the modified electrodes was carried out.

2.3.3. Enzyme entrapment by β-cyclodextrin electropolymerization

Electropolymerization onto the SPE/MWCNT electrode was performed by using a mixture of 0.01 mol L^{-1} β-cyclodextrin (β-CD) and 1 mg/mL of glucose oxidase enzyme (GOx) in PBS (pH 7.44). The SPE/MWCNT electrode was immersed in this solution and the potential was scanned from −0.8 to 1.3 V during 10 consecutive cycles, at 0.1 V s^{-1} sweep rate. After, the β-CD/GOx polymer was generated onto SPE/MWCNT surface. The SPE/MWCNT/β-CD/GOx electrode formed was dried using a nitrogen flow. For comparison purposes, other modified electrodes such as SPE/β-CD, SPE/GOx, SPE/MWCNT/β-CD, SPE/MWCNT/GOx were also prepared using the procedure above. All the modified electrodes were stored at 4 °C.

3. Results and discussion

3.1. Electropolymerization conditions for controlling enzyme entrapment by β-cyclodextrin

In order to optimize the enzyme entrapment by β-CD electropolymerization process during the SPE/MWCNT/β-CD/GOx biosensor preparation, several parameters such as potential window, scan rate and number of cycles were investigated. Based on this initial study, we observe that both, potential window and scan rate directly affect the response of the formed biosensor. For example, when the potential was cycled within the −1.0 to 1.3 V range, using SPE/MWCNT electrode, a film not well defined was observed. However, after applying successive scans at 0.1 V s^{-1} narrowing the potential window from −0.8 to 1.3 V, a better polymerization process giving homogeneous films that covered the whole electrode was achieved (results not shown). Furthermore, we also observed

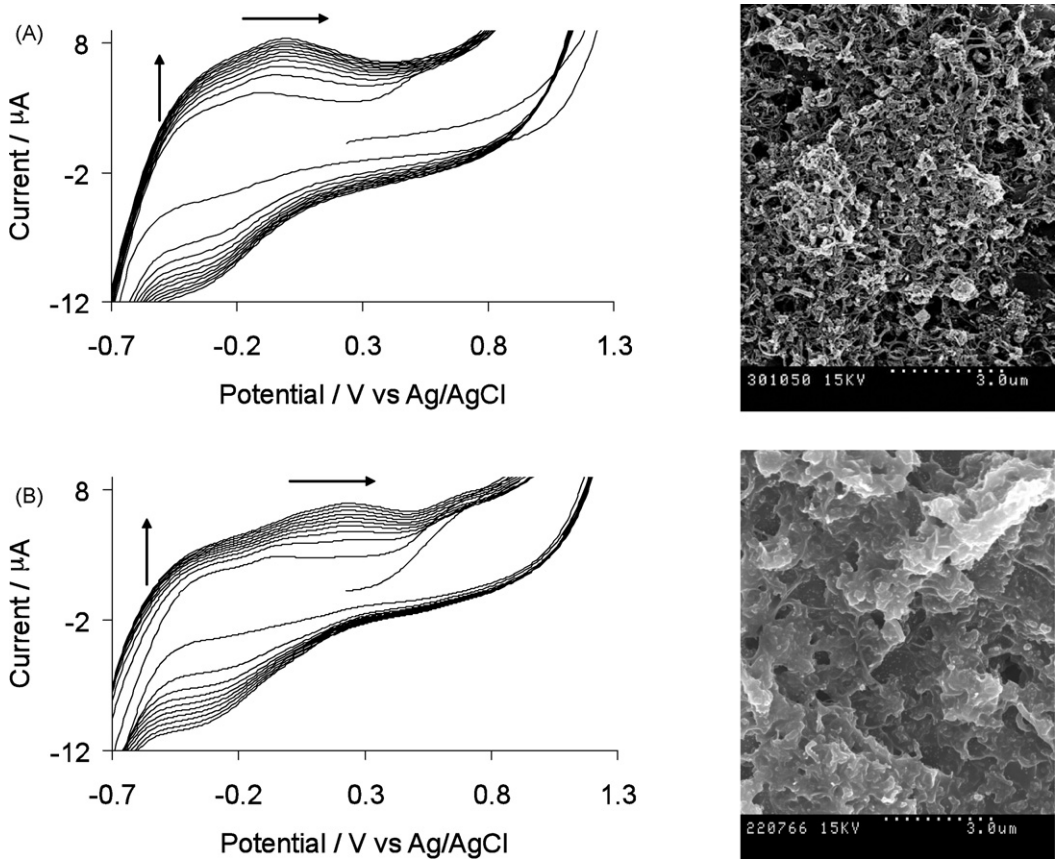

Fig. 2. Cyclic voltammograms for electrochemical polymerization of β-CD (A) and β-CD/GOx (B) in 0.1 mol L^{-1} PBS pH 7.0 onto SPE/MWCNT after 30 cycles. On the right side, the corresponding SEM images after polymerization procedure are shown.

that the incorporation of MWCNT onto the SPE surface causes an increase of the faradaic currents (see Figs. S1 and S2 in supplementary information), which facilitate the electron flow toward the electrode surface.

It is well known that the number of cycles directly affect the thickness and capacitive current of the obtained biosensor. For this reason the response for dopamine as a function of the cycle number was evaluated as illustrated in Figs. S1 and S2. The best electrochemical response for the electropolymerization process was obtained for the biosensor containing MWCNT immobilized onto SPE surface using 10 cycles. For 20 and 30 cycles, dopamine oxidation responses were similar, since the differences between the obtained faradaic currents were less that 10%.

As illustrated in Fig. 2, the growth of electropolymerized β-CD and β-CD/GOx films were followed by cyclic voltammetry in 0.1 mol L^{-1} PBS solution (pH 7.44) within the −0.8 to 1.3 V potential window and during 10 cycles. For both systems containing β-CD and β-CD/GOx the faradaic currents increase with the number of cycles indicating a successful electropolymerization process of β-CD or β-CD/GOx. The adsorbed β-CD molecules onto the SPE electrode showed two electrochemical processes at −0.1 and −0.5 V (Fig. 2A), similar to that observed in other electropolymerization processes containing β-CD species (Ghanem, 2003; Villalonga et al., 2007). However, we observe that the anodic branch of the CV for the β-CD-GOx film formation shifted to a more positive potential after incorporation of GOx within the film (Fig. 2B). This behavior can be associated to the nonconductive GOx incorporation into the polymeric β-CD species matrix, thus increasing film resistance. In fact, the SEM images of the electropolymerized films deposited onto SPE/MWCNT surfaces exhibited different morphologies (Fig. 3). After GOx entrapment by β-CD electropolymerization, the modified electrode showed a well defined

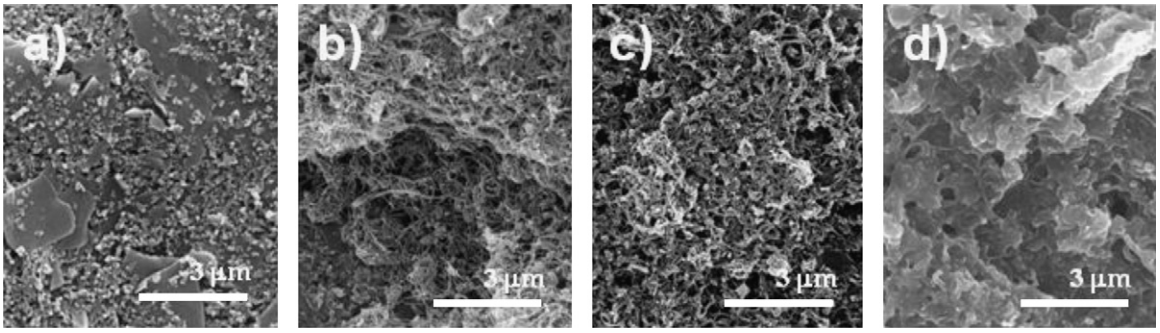

Fig. 3. SEM images of the working surface area for: (A) SPE bare, (B) SPE modified with MWCNT, (C) SPE modified with MWCNT/β-CD and (D) SPE modified with MWCNT/β-CD/GOx. The acceleration voltage was 15 kV; 3 µm for resolution was used for all the SEM images.

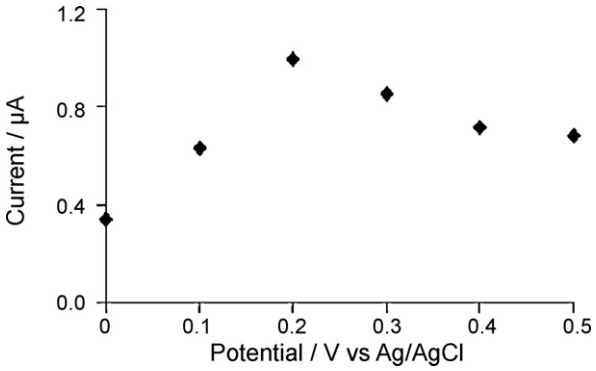

Fig. 4. Effect of applied potential for dopamine response upon addition of 50 μM, using SPE/MWCNT/β-CD/GOx. Electrolyte: 0.01 mol L^{-1} PBS at pH 7.0; T = 25 °C.

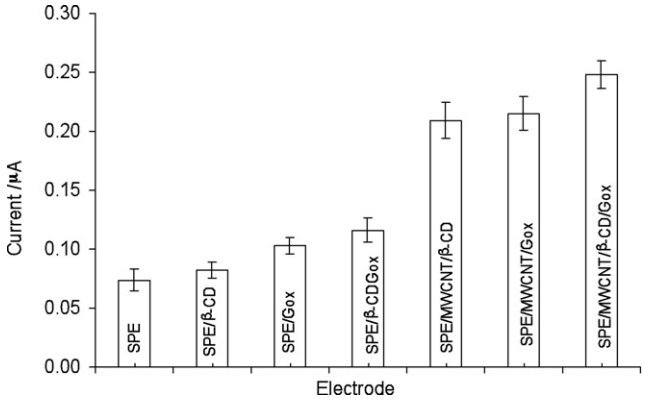

Fig. 5. Comparison of currents recorded with several SPE electrodes after the addition of 0.1 mmol L^{-1} dopamine in each case. Electrolyte: 0.01 mol L^{-1} PBS at pH 7.0; T = 25 °C and working potential, 200 mV.

globular morphology. These aspects will be discussed in the next section.

Furthermore, reproducibility of the polymeric membrane was evaluated after the polymerization using three different electrodes and measuring the average capacitive current (0.064 μA), showing a standard deviation of 0.001 μA.

3.2. SEM characterization of the surface morphology

With aim to examine the morphology of the modified electrodes and the influence of the supramolecular environment, several SPE modified electrodes containing MWCNT, β-CD and GOx components were investigated through SEM (Fig. 3). The surfaces of the SPE and the SPE/MWCNT electrodes (Fig. 3A and B) exhibited amorphous and fibrous morphology aspects, respectively. It was observed that β-CD-GOx supported onto the SPE/MWCNT surface showed a homogeneously distributed globular morphology over the entire surface. In addition, pores formation can be clearly visualized (Fig. 3D). In contrast, the electrodeposited film formed from β-CD exhibited only a lamellar morphology indicating the participation of β-CD molecules within the supramolecular environment (Fig. 3C). The formation of a robust supramolecular structure containing MWCNT and β-CD-GOx could be related to the enhancement of diffusive species. Film porosity induces an improvement on the electrochemical activity and stability of the immobilized enzyme, as previously reported for some other porous materials (Shan et al., 2003). In addition pores confer the required permeability on the polymer, providing a better analyte access to the active sites of the enzyme through these nanochannels.

3.3. Amperometric response of SPE/MWCNT/β-CD-GOx biosensor

In order to evaluate the optimal working potential applied to the SPE/MWCNT/β-CD-GOx biosensor, a study of the dopamine oxidation response as a function of applied potential was evaluated in the 0.0–0.5 V potential range at a 50 μM dopamine concentration. Fig. 4 shows that the anodic current increases for potential values up to 0.2 V and then decreases for higher potential values. These findings indicate that the maximum anodic current was found at 0.2 V, being this value the optimal working potential for using the biosensor. In addition, the pH effect was also evaluated in the 4–8 range with the best electrochemical responses obtained at pH 7.44, results not shown.

The effects of the presence of MWCNT, β-CD and GOx upon the biosensor response were also studied. Fig. 5 shows the dopamine current oxidation recorded using the following electrodes: SPE, SPE/β-CD, SPE/GOx, SPE/CD/GOx, SPE/MWCNT/β-CD, SPE/MWCNT/GOx, and SPE/MWCNT/β-CD/GOx immersed in a 0.1 μM DA aqueous solution containing 0.01 M PBS at pH 7.44. The higher anodic currents were recorded for the SPE electrodes containing MWCNT. In the case of SPE/MWCNT/β-CD/GOx system, the current response to DA was almost 20% higher compared to the SPE/MWCNT/β-CD and SPE/MWCNT/GOx modified electrodes, which could be due to a closer contact between the MWCNT and the building blocks. Therefore, the GOx enzyme catalyzes the dopamine oxidation giving even better responses when it is mixed with β-CD (SPE/β-CD/GOx and SPE/MWCNT/β-CD/GOx). Furthermore, for the electrodes containing MWCNT, the polymerization process was not inhibited on the active GOx sites, obtaining a current increase of about 100%. Electron transfer between β-CD/GOx and dopamine is enhanced by the increased active surface provided by MWCNT incorporation. In addition MWCNT further facilitates the direct dopamine detection. It can be seen that 0.1 mM dopamine concentration gives an analytical signal for all of them. That is to say, the analysis of dopamine can be carried out with SPE, SPE/β-CD, SPE/Gox, SPE/β-CD/Gox, SPE/MWCNT/β-CD, SPE/MWCNT/Gox and SPE/MWCNT/β-CD/Gox, however, it is noticeable that the electrodes modified with MWCNT present greater current peaks that those built without MWCNT. Thus the major effect on dopamine quantification can be ascribed to this kind of nanotubes. Notwithstanding, the electrode modified with both components: MWCNT and the enzyme display a 25% higher current than those without the enzyme.

The results obtained suggest that dopamine oxidation is facilitated when the biosensor matrix is constituted with all the mentioned components. Among the different components, β-CD polymerization leads to a better enzyme immobilization improving at the same time the efficiency of the electrooxidation step. This effect could be also attributed to the polymer permeability and its supramolecular interactions, as multipoint attachment of polymeric structures onto the enzyme surface is a well known strategy to improve the stability of biocatalysts (Villalonga et al., 2006) and contributes to the preservation of the active enzyme conformation. Therefore, as it has been observed, β-CD polymer is expected to stabilize the GOx enzyme.

In order to clearly evaluate the MWCNT contribution on the DA response, calibration plots were obtained for the amperometric detection of dopamine using SPE, SPE/MWCNT/β-CD, SPE/MWCNT/Gox and SPE/MWCNT/β-CD/Gox (see Fig. 6) The analytical features namely: limit of detection (LOD), sensitivity and linearity range, of these electrodes during DA quantification are shown in Table 1. The best results were obtained with the SPE/MWCNT/β-CD/GOx electrode.

The amperometric detection of dopamine using SPE/MWCNT/β-CD/GOx biosensor, see Fig. S3, exhibited a remarkable response toward dopamine with a LOD of 0.48 ± 0.02 μM, with high electro-

Table 1
Analytical parameter for dopamine quantification using the different electrodes considered in this work, obtained from the corresponding calibration plots, see Fig. 6 at pH 7.4 applying a 0.2 V potential.

Electrode	LR (μM)	LOD (μM)	Sensitivity (μA μM^{-1})	r^2
SPE	10–50	4.642 ± 0.006	0.0054 ± 0.0002	0.991
SPE/MWCNT	10–50	1.682 ± 0.007	0.0119 ± 0.0001	0.999
SPE/MWCNT/β-CD	10–50	1.706 ± 0.014	0.0186 ± 0.0002	0.999
SPE/MWCNT/Gox	10–50	4.540 ± 0.031	0.0235 ± 0.0003	0.96
SPE/MWCNT/β-CD/GOx	10–50	0.48 ± 0.02	0.0302 ± 0.0003	0.999

chemical stability and fast response time (around 5 s). This LOD is 10 times lower compared to the electrode with SPE/MWCNT/β-CD showing at the same time good sensitivity and signal stability.

3.4. Reproducibility and repeatability

The repeatability of the SPE modified with MWCNT/β-CD/GOx biosensor for dopamine determination exhibited a variation coefficient of 1.3%, see Fig. S4 provided as supporting information. The reproducibility of this biosensor was also evaluated in triplicate with 3 electrodes, see Fig. S5, finding that the variation coefficient was 3%, from which it can be concluded that this biosensor displayed very good features for dopamine quantification. Moreover, when one SPE modified with MWCNT/β-CD/GOx biosensor was evaluated after nearly 8 months of storage under refrigeration at 4 °C it was found that it displayed a LOD of 1.319 and a 0.014 sensitivity. Comparing the latter with those recorded with a fresh electrode, see Table 1, it is notorious that these biosensors exhibited a significant span of life.

3.5. Analysis of the possible interferences

For any biosensor it is important to determine to what extent, the possible interfering agents will affect its performance. In case of dopamine determination, its major interferent is ascorbic acid (AA). As can be noted in Fig. S6, when the SPE/MWCNT/β-CD/GOx electrode was used to determine dopamine in the presence of ten fold AA, it was still possible to obtain sufficient data point to construct a calibration plot (r^2 = 0.987), from which DA contents can estimated with a LOD of 3.14 μM, a sensitivity of 0.0044 μA μM^{-1} and a linear range of 5–35 μM. Moreover, another important interferent, uric acid (UA) was also tested. From Fig. S7 it becomes straightforward that when UA is present at a 10 times greater concentration than DA, the peak's resolution is good enough to allow DA quantification in the presence of UA.

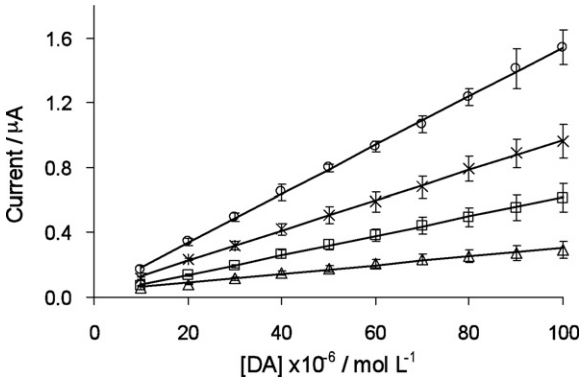

Fig. 6. Dopamine calibration plots using different electrodes: (○) SPE/MWCNT/β-CD/GOx, (×) SPE/MWCNT/β-CD, (□) SPE/MWCNT and (△) SPE: 0.01 mol L^{-1} PBS at pH 7.44; T = 25 °C; working potential, 200 mV. The solid lines were obtained from linear regression of the respective experimental points.

As can be noted in the supporting Table S1 the SPE/MWCNT/β-CD/GOx electrode display comparable or even better analytical features towards Dopamine quantification than many other electrodes reported in the literature.

4. Conclusions

The electropolymerization of β-CD molecules allowed optimal GOx enzyme entrapment, leading to a novel and reproducible enzyme immobilization approach. β-CD polymerization showed a homogeneous distribution over the whole electrode surface, providing at the same time an improvement over the activity and stability of the enzyme response for dopamine. Therefore, supramolecular interaction between MWCNT and β-CD matrix combined with electropolymerization technique provides an excellent biocompatible microenvironment for GOx immobilization. Analytical parameters had been studied, showing a good sensitivity and low LOD for dopamine detection, even in the presence of interfering agents such as ascorbic and uric acid.

Acknowledgments

We acknowledge funding from the Spanish Ministry of Science and Innovation (MICINN, Madrid, Spain) for the project MAT2008-03079/NAN. G. Alarcón thanks CONACyT for the postdoctoral fellowship (105024). M. Guix thanks the *Ministerio de Ciencia e Innovación* (MICINN, Madrid, Spain) for the pre-doctoral fellowship (BES-2009-023939) associated to the research project MAT2008-03079. W.C. Silva gratefully acknowledges the postdoctoral fellowship from Brazilian Agency (CNPq – process number 200515/2009-8). MTRS, MPP and MRR like to thank Red SIATA, SEP and SNI CONACYT.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.08.058.

References

Aihua, L., Itaru, H., Haoshen, Z., 2005. Electrochem. Commun. 7, 233–236.
Alarcon-Angeles, G., Pérez-López, Palomar-Pardave, M., Ramírez-Silva, M., Alegret, S., Merkoçi, S., 2008. Carbon 46, 898–906.
Armstrong, F.A., Wilson, G.S., 2000. Electrochim. Acta 45, 2623–2645.
Darias, R., Herrera, I., Fragoso, A., Cao, R., Villalonga, R., 2002. Biotechnol. Lett. 24, 1665–1668.
Dornelles, M.L., Tatsuo, K.L., 2002. Food Chem. 77, 237–256.
Furbee, J., Thomas, C., Kelly, R., Malachowski, M., 1993. Anal. Chem. 65, 1654–1657.
Ghanem, A., 2003. Org. Biomol. Chem. 1, 1282–2129.
Ghindilis, A.L., Atanasov, P., Wilkins, E., 1997. Electroanalysis 9, 661–674.
Gorton, L., Lindgren, A., Larsson, T., Munteanu, F.D., Ruzgas, T., Gazaryan, I., 1999. Anal. Chim. Acta 400, 91–108.
Katz, E., Willner, I., Wang, J., 2004. Electroanalysis 16, 19–44.
Lu, X.B., Hu, J.Q., Yao, X., 2006. Biomacromolecules 7, 975–980.
Merkoçi, A., 2006. Microchim. Acta 152, 155–156.
Pérez, B., Sola, J., Alegret, S., Merkoçi, A., 2008. Electroanalysis 20, 603–610.
Pérez, B., Pumera, M., Del Valle, M., Merkoçi, A., Alegret, S., 2005. J. Nanosci. Nanotechnol. 5, 1694–1698.

Pumera, M., Merkoçi, A., Alegret, S., 2006. Sens. Actuators B 113, 617–622.
Rosu, R., Iwasaki, R., Shimizu, N., Doisaki, N., Yamae, Y., 1998. J. Biotechnol. 66, 51–59.
Shan, D., Mousty, C., Cosner, S., Mu, S., 2003. Electroanalysis 15, 1506–1512.
Villalonga, R., Tachibana, S., Cao, R., Ramirez, H.L., Asano, Y., 2006. Biochem. J. 30, 26–32.
Villalonga, R., Cao, R., Fragoso, A., 2007. Chem. Rev. 107, 3088–3116.
Willner, I., Katz, E., 2000. Angew. Chem. Int. Ed. 39, 1180–1218.