Biosensors and Bioelectronics 74 (2015) 830–835

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Highly sensitive electrochemical biosensor for bisphenol A detection based on a diazonium-functionalized boron-doped diamond electrode modified with a multi-walled carbon nanotube-tyrosinase hybrid film

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ARTICLE INFO

Article history:
Received 10 May 2015
Received in revised form
22 July 2015
Accepted 23 July 2015
Available online 26 July 2015

Keywords:
Biosensor
Bisphenol A
Tyrosinase
Diamond electrode
Diazonium

ABSTRACT

A highly sensitive electrochemical biosensor for the detection of Bisphenol A (BPA) in water has been developed by immobilizing tyrosinase onto a diazonium-functionalized boron doped diamond electrode (BDD) modified with multi-walled carbon nanotubes (MWCNTs). The fabricated biosensor exhibits excellent electroactivity towards o-quinone, a product of this enzymatic reaction of BPA oxidation catalyzed by tyrosinase. The developed BPA biosensor displays a large linear range from 0.01 nM to 100 nM, with a detection limit (LOD) of 10 pM. The feasibility of the proposed biosensor has been demonstrated on BPA spiked water river samples. Therefore, it could be a promising and reliable analytical tool for on-site monitoring of BPA in waste water.

1. Introduction

Bisphenol A (BPA) is a major compound widely used in the production of polycarbonate products and epoxy resin products which are extensively used in plastic food containers, the inner surface coating of food, water bottles, baby bottles and beverage cans (Hideyuki et al., 2003; Grain et al., 2007). However, it has been demonstrated that BPA acts as an endocrine disruptor, causing abnormal hormone activity (Fan et al., 2007; Deblonde et al., 2011), thereby leading to negative health effects such as altering the development of male and female reproductive tracts and increasing the cancer risk of the mammary gland and prostate (Sonnenschein and Soto, 2010). Moreover, due to its negative impact especially on children and infants, Canada and China banned the use of BPA in baby bottles in 2010 and 2011 respectively (Wu et al., 2012; Gao et al., 2012). In January 2011 use of bisphenol A in baby bottles was forbidden in all EU-countries. In January 2015, the European Food Safety Authority (EFSA) indicated that the current Tolerable Daily Intake (TDI) level for Bisphenol A of 4 μg/kg body weight/day (EFSA Journal, 2015). BPA can be released into the environment via waste water from plastic-producing industrial landfill sites, thus causing the release of BPA into surface water. In 2010, the U.S. Environmental Protection Agency reported that over one million pounds of BPA are released into the environment annually. As a result of the ubiquitous nature and endocrine disrupting potential of BPA, a growing interest has been focused on the quantitative determination of BPA which is very important for human health and environmental protection (Erler and Novak, 2010; Grain et al., 2007; Rogers et al. 2013).

Many analytical methods have been developed for BPA measurement, harnessing high performance techniques such as liquid chromatography (Mohammad et al., 2009), gas chromatography-mass spectrometry (Zhang et al.,2011), enzyme linked immunosorbent assay (Zhao et al., 2002) and immuno-chromatographic lateral flow assay (Mei et al., 2013). These techniques are highly sensitive and have a low detection limit. However, they also require a skilled operator and time consuming pretreatment steps which do not allow rapid processing of multiple samples. In addition, they are expensive and complicated for on-site measurement (Jaffrezic-Renault and Dzyadevych, 2008). Hence, it is desirable to develop an easy and sensitive analytical method for BPA determination.

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http://dx.doi.org/10.1016/j.bios.2015.07.051
0956-5663/

In recent years, electrochemical detection techniques have proved quite promising since they are simple, fast and cost-effective. Up to date, many electrochemical biosensors based on enzymes (Dempsey et al., 2004; Wang et al., 2014; Pan et al., 2015; Portaccio et al., 2013; Kochana et al., 2015), aptamers (Kang et al., 2011; Zhu et al., 2015; Ragavan et al., 2013; Zhu et al., 2014), antibodies (Piao et al., 2008; Rahman et al., 2007; Wang et al., 2014) and peptides (Yang et al., 2014; Qu et al., 2013; Kim et al., 2015) have been fabricated for the detection of BPA. Among them, the enzyme tyrosinase was applied to the measurement of BPA. In the presence of dissolved oxygen, tyrosinase could catalyze the oxidation of phenolic compounds to o-quinone derivatives via cathecol derivatives (Eqs. ((1) and 2)). Thus the generated o-quinone could be electrochemically reduced at low potential without any mediator. The reduction signal of o-quinone is normally used to determine the phenolic compound for the purpose of preventing interference (Eq. (3)) (Notsu et al., 2002). To enable the electrochemical detection, different types of electrodes such as glassy carbon (GC) (Yin et al., 2010; Mazzotta et al., 2013), graphene (Wu et al., 2012; Pan et al., 2015) and boron doped diamond (BDD) (Notsu et al., 2002) electrodes have been employed. Many studies of electroanalysis, particularly for the quantitative detection of trace amounts of biochemicals in the human body and harmful compounds in polluted water have been performed using a BDD electrode as it possesses many outstanding properties which make it superior to other electrode materials, like high chemical stability, a wide electrochemical potential window in aqueous solutions, low and stable capacitive background current which enables rapid measurements with sensitivity enhancement for low concentration detection, high response reproducibility and long-term response stability, excellent resistance to electrode fouling and biocompatibility (Zhou and Zhi, 2009). In the last decade, multi-walled carbon nanotubes (MWCNTs) have been used with biosensors for electrical sensing improvement in various fields (Chen et al., 2013). MWCNTs have been widely considered as a favorable platform since they were being considered as attractive materials due to their excellent electrocatalytic activity, high electrical conductivity, chemical stability and extremely high mechanical strength (Poorahong et al., 2012; Xue et al., 2013).

In this work, we developed a new biosensor for ultra-sensitive detection of Bisphenol A in water, through the construction of a MWCNTs-tyrosinase hybrid film on a diazonium-functionalized boron-doped diamond electrode. This is the first time that enzymatic detection of bisphenol A was proposed using BDD electrode. A previous study, was based on BDD electrode for the elaboration of chemical sensor for BPA detection based on direct cathodic detection (Pereira et al., 2012). Furthermore, this is the first time also that the one-step functionnalization of BDD electrode through in situ generated diazonium salt using 4-aminobenzylamine is suggested in order to develop a biosensor, by grafting of an enzymatic hybrid film. In contrast to other functionnalization method, this technique is simple and does not require any isolation or purification of diazonium salt. As well, multi-walled carbon nanotubes were directly grafted to the enzyme, then allowing direct electrical wiring. The conception of the proposed biosensor provides a high loading of enzyme, retaining its biocatalytic activity and the direct connection with MWCNTs allowing an enhanced electron transfer to the electrode (Poh et al., 2004). All these factors allow to elaborate a highly sensitive and stable biosensor for BPA detection. In addition, a successful demonstration in real samples was performed.

$$\text{Bisphenol A} + \text{tyrosinase}(O_2) \rightarrow \text{catechol} \quad (1)$$

$$\text{Catechol} + \text{tyrosinase}(O_2) \rightarrow \text{O} - \text{quinone} + H_2O \quad (2)$$

$$\text{O–quinone} + 2H^+ + 2e^- \rightarrow \text{catechol} \quad (3)$$

2. Experimental

2.1. Chemical and regeants

Tyrosinase from mushroom ($\geq$ 1000 unit/mg solid), sodium nitrite ($NaNO_2$) $\geq$ 97%, hydrogen chloride (HCl), glutaraldehyde (grade II, 25% aqueous solution), sodium phosphate dibasic, sodium phosphate monobasic, sulfuric acid, nitric acid, *N*-Hydroxysuccinimide (NHS), *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide (EDC), bisphenol A ($\geq$ 99%), phenol $\geq$ 99%, 2-nitrophenol, 4-nitrophenol, sucrose, glucose, Cd^{+2}, KNO_3, Cu^{+2}, Ni^{+2}, were purchased from Sigma Aldrich (Saint Quentin Fallavier, France) and ethanol $\geq$ 99% was purchased from Fluka. 4-aminobenzylamine was purchased from Acros Organics (France). All the solutions were prepared in distilled deionized water (resistivity no less than 18 MΩ cm) obtained from a Millipore purification system.

2.2. Electrochemical set-up: Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS)

Electrochemical measurements were carried out in a conventional one compartment three electrode cell with an internal volume of 5 ml (Verre Equipments, Collonges au Mont d'Or, France). This electrochemical cell was designed to maintain a fixed distance between the electrodes. It was manufactured with two inlets, one for the positioning of the reference electrode and the other for BPA injection. This feature prevented further manipulation or movements of the electrodes (fixing the geometry of the cell and also ensuring the reproducibility of measurements). For this work, a saturated calomel electrode from Hach Lange (France) was used as reference electrode, a planar platinum electrode (0.59 cm^2) was used as the counter electrode and the working electrode was a boron doped diamond film (BDD) that was purchased from Neo-Coat (La Chaux-de-Fonds, Switzerland). Polycrystalline boron doped diamond (boron concentration higher than 7000–8000 ppm) 300 nm thick was grown by CVD on silicon coated with an isolating layer of silicon oxide and silicon nitride ($Si/SiO_2/Si_3N_4$) 0.5 mm thick. The active surface of the BDD working electrode, determined by an O-ring seal, was 0.07 cm^2.

The electrochemical measurements were performed using an electrochemical analyzer "VoltalabPGZ 402" (Hach Lange, France), data acquisition and processing via 'Voltamaster 4' software. A magnetic stirrer provided convective transport during electrochemical measurements after injection of BPA.

2.3. Surface characterization using AFM

Atomic Force Microscopy (AFM) was performed in air under ambient conditions using a Nano Observer (CSI Company, France), the AFM Nano-Observer, with a maximum resolution of 110 μm^2 and minimum 5 μm^2. Measurements were made using a silicon cantilevier tip (Europa, Germany). The cantilever size L:125 μm, W:35 μm and T:4.5 μm. The tip radius: $<$ 10 nm, H:14–16 μm and with a frequency of 200–400 Hz and K:25–75 N/m. Imaging was carried out using taping mode at 2.25 V amplitude 3.1 V set point, Tip DC at 596 nV and sample DC 596nV.The imaging speed was 0.25 lines per second and 1024 resolution. Samples were analyzed in a (5 μmx5 μm) area.

2.4. Fabrication of carboxyl functionalized multi-walled carbon nanotubes

Multi-walled carbon nanotubes (MWCNTs) were produced by

aerosol-assisted catalytic chemical vapor deposition (AACCVD). The growth approach was based on the catalytic decomposition of liquid hydrocarbons by pyrolysing mixed aerosols containing both the hydrocarbon and the metallic source which simultaneously and continuously fill the reactor volume (David et al., 2012). A solution, composed of 5 wt% of ferrocene dissolved in toluene, was placed inside an ultrasonic (US) aerosol generator (RBI-France), composed of several piezoelectric ceramics.Subsequently, the reactor was connected to the US aerosol generator, and an air flow of 400 mL/min was flushed through the reactor. The temperature of the tubular furnace was then raised to 820 °C for 1 h. As soon as the synthesis temperature was reached, the argon flow was increased to 1 L/min. The piezoelectric ceramic of the ultrasonic generator, operating at 850 kHz, was then activated, creating an aerosol at the gas–liquid interface that was carried by the argon flow through the heated quartz reactor. At the end of the experiment, the samples were then slowly collected and cooled to room temperature.

Afterwards, the obtained MWCNTs were functionalized through oxidation in acidic media, allowing the formation of carboxylic (–COOH) and hydroxylic (–COH) grafting groups. To that end, carbon nanotubes were treated with an acid mixture H_2SO_4/HNO_3 with a ratio of 3:1 under stirring at 90 °C, for 10 min (Cho et al., 2005; Sahoo et al., 2007). After that, the functionalized MWCNTs were collected by filtration, then washed with distilled water and dried in an oven at a temperature of 80 °C.Subsequently, the MWCNTs were added to a buffer phosphate saline (pH 7) containing 0.4 M EDC plus 0.1 M NHS and then were left to react for 90 min, thereby activating the carboxylic groups.

Fig. 1S shows the representative TEM images of a random dispersion of nanotube powder. The obtained MWNTs have the form of thin threads with lengths of several μm and an external diameter lower than 100 nm. These nanotubes are open at either end (with an inner diameter around 20 nm),leading to high porosity.

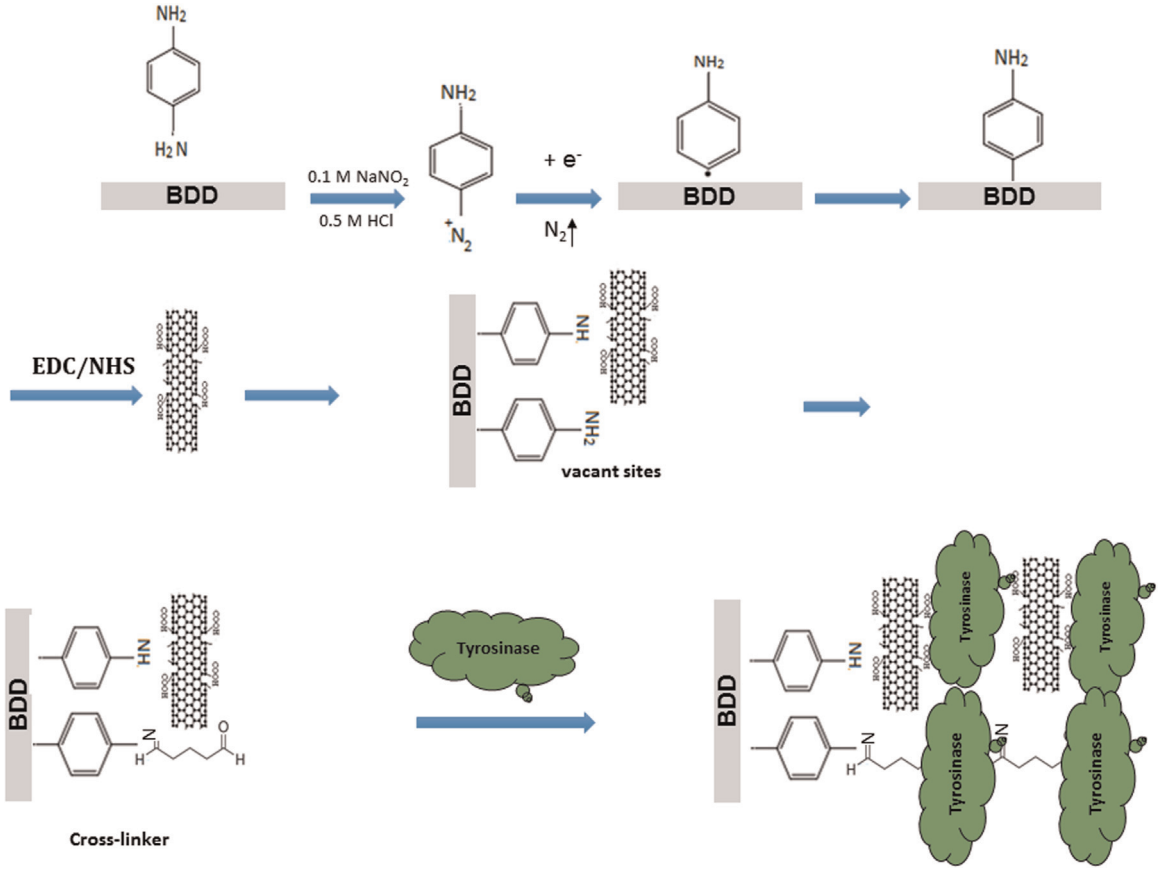
2.5. Preparation of multi-walled carbon nanotube-tyrosinase hybrid film based BDD biosensor

2.5.1. Electrochemical grafting of BDD surface

Before analysis, the BDD electrode was treated with ethanol, then thoroughly rinsed with ultrapure water and dried under nitrogen flow. Electrochemical grafting was applied to the BDD electrode in order to introduce the amino groups onto the surface of electrodes with in situ generated diazonium cations. Diazonium was synthesized by adding 0.1 M $NaNO_2$ to the electrolytic solution containing 20 mM 4ABA and 0.5 M HCl under stirring at 4 °C. Then, the mixture was left to react for about 5 min before electrochemical modification. Cyclic voltammetry were performed for the electrochemical reduction of the amino group, the voltage on the BDD electrodes was repeatedly swept from 0.6 to −1 V, and after modification the electrode was rinsed with ultrapure water.

2.5.2. Enzyme attachment

5 μL of MWCNTs (0.5 mg ml^{-1}) was added to 20 μL of tyrosinase solution (3130 unit/mg solid) and the mixture was thoroughly homogenized. After that, 10 μl of the homogenized mixture was deposited onto the sensitive surface of the modified BDD electrode. Then, the biosensor was placed in saturated glutaraldehyde vapor (cross-linker) for 10 min and was stored at 4 °C until further use. Scheme 1



Scheme 1. Schematic illustration of the functionalizing procedure and enzyme attachment on the BDD surface.

3. Results and discussion

3.1. Electrochemical grafting

The functionalization of the BDD electrode was achieved by electrochemical reduction of in situ generated diazonium cations synthesized in the electrochemical cell by a reaction of 4-aminobenzylamine with $NaNO_2$ in aqueous 0.5 M HCl solution, at 4 °C. This electrodeposition method, which involves simple reagents and does not require any isolation or purification of diazonium salt, enabled the grafting of covalently bonded layers. Fig. 1 shows the voltammogram of electrochemical grafting, resulting in a reduction peak at around −0.4 V. Neither the oxidation peak no reduction peak could be observed in subsequent sweeps, indicating that the above electrochemical reduction process is irreversible and only occurs during the first reduction sweep.

3.2. Electrochemical characterization of the multi-walled carbon nanotube-tyrosinase hybrid film based BDD biosensor

3.2.1. Cyclic voltammetric characterization

Cyclic voltammetry was employed to characterize the modified electrode during the preparation process. Fig. 2S depicts the electrochemical behavior of the biosensor at different steps of the surface modification, in 8 mM solution of redox probe, $Fe(CN)_6^{3-/4-}$, with a scan rate of 50 mV/s. As can be seen in this figure, a dramatic decrease in the current occurs after electrochemical grafting of in situ generated diazonium (curve b) compared to bare electrode (curve a), and the complete disappearance of redox probe peaks after enzyme immobilization (curve c) could be attributed to the passivation of the electrode surface which therefore hindered the access of electrons to the BDD surface through the interfacial enzyme film barrier.

3.2.2. Impedimetric characterization

Electrochemical impedance spectroscopy (EIS) is a widely employed technique for the electrochemical behavior investigation of a modified electrode interface during the stepwise process. Fig. 3S shows Nyquist plots of (a) bare electrode, (b) after electrochemical grafting of diazonium and (c) after deposition of MWCNTs-Tyr on the BDD electrode in 8 mM $[Fe(CN_6)]^{3-/4-}$ solution, in a frequency range of 100 mHz–100 kHz, where the semi-circle portions corresponding to the electron transfer limited process and the straight line to a diffusion controlled process could be visualized distinctly, according to the Randles equivalent circuit (insert in Fig. 3S). Impedance spectra were modeled according to the Randles equivalent circuit using analysis program Z_{view}/Z_{plot} (Scribner Associates, USA) where R_s is the ohmic resistance for the bulk electrolyte, R_{ct} is the charge transfer resistance, C_{dl} is double layer capacitance and Z_w is the Warburg impedance.

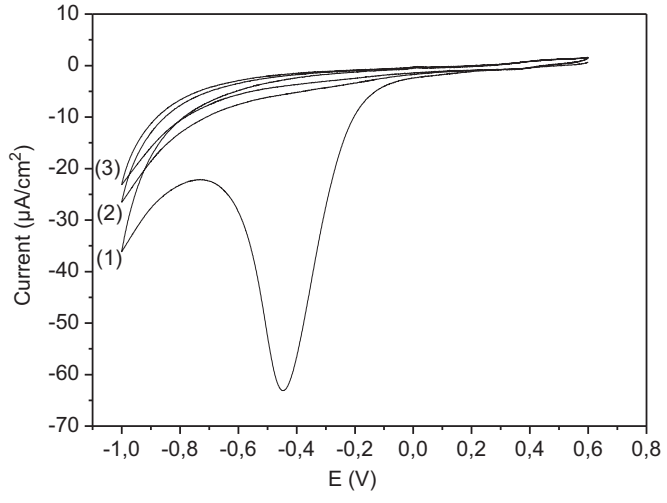


Fig. 1. Cyclic voltammograms of BDD electrode for the reduction of diazonium salt (1) first sweep, (2) second sweep, (3) third sweep. Supporting electrolyte: 0.1 M $NaNO_2$, 20 mM 4ABA in 0.5 M HCl. Scan rate: 100 mV/s.

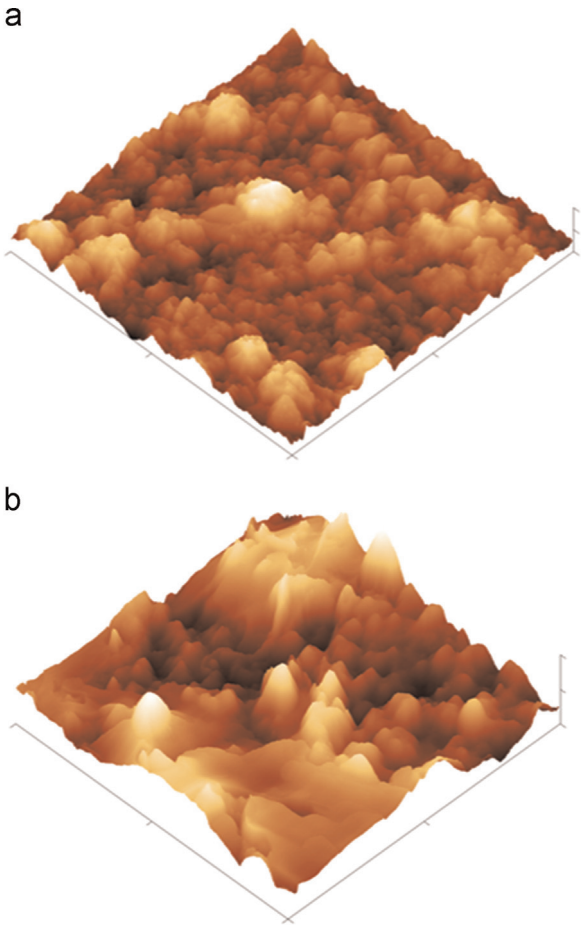


Fig. 2. AFM images of (a) bare BDD electrode, (b) multi-walled carbon nanotube-tyrosinase hybrid film based BDD sensor. Area of the images: 5 μm x 5 μm.

It is obvious from the figure that the impedance spectra of the modified electrode (curves b and c) describe a larger semi-circle than that of the bare electrode (curve a, R_{ct}=3.61 KΩ cm^2) with increased R_{ct} values after each step, indicating that the film formed on the BDD surface restricts electron transfer between the electrolyte and electrode interface (curve b, R_{ct}=12.10 KΩ cm^2). The blocking of electron transfer (curve c, R_{ct}=23.40 KΩ cm^2) is due to the passivation of the BDD electrode through tyrosinase deposition, which confirms its successful immobilization onto the electrode surface.

3.2.3. Morphological characterization of BPA biosensor

Fig. 2 displays the morphologic characteristics of the electrodes examined by AFM. The microcrystalline structure of BDD is presented in Fig. 2a, where the mean diameter of microcrystallites being 200 nm, RMS roughness was found to be 5 nm with a depth of 0.27 μm. This rough structure corresponds to the bare BDD electrode. After enzymatic membrane deposition, the surface of the BDD electrode became smooth compared to the bare electrode. Fig. 2b shows that the film of MWCNTs-tyrosinase is homogenous and dense, masking the microcrystalline structure of BDD with a RMS roughness of 3 nm.

3.3. Analytical characteristics

Fig. 3A shows the enzymatic biosensor response obtained after the injection of different aliquots of BPA in PBS (pH 7.2). CV curves present a reduction peak at −1.1 V.The amount of BPA in the solution can be quantified by monitoring the electrochemical signal whose change is proportional to the electrochemical reduction of o-quinone species liberated from the enzymatic reaction on the electrode surface.

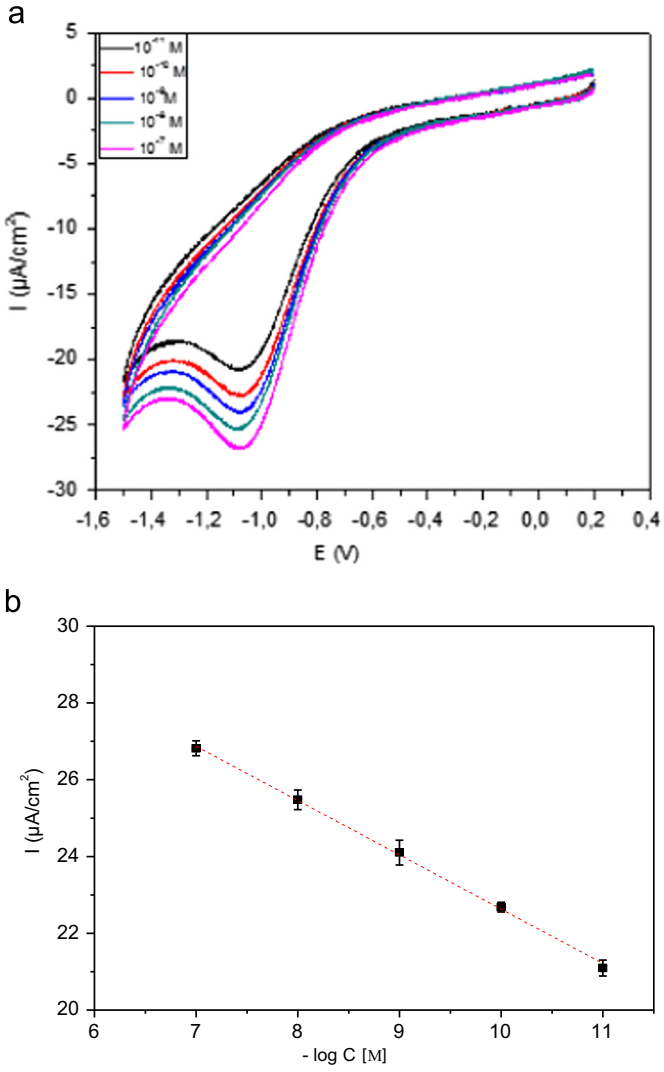


Fig. 3. (A) Cyclic voltammograms obtained after the addition of different BPA concentrations in 20 mM PBS, pH 7.2, (black curve:10^{-11} M; red curve: 10^{-10} M; blue curve: 10^{-9} M; cyan curve: 10^{-8} M; pink curve: 10^{-7} M) (B) Calibration curve for BPA detection (each point is the mean value from three experiments). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

BDD electrode modified with the MWCNTs-Tyr hybrid film exhibited a distinctive CV peak current change, mediated by the specific interaction between BPA and tyrosinase. In Fig. 3B, Data points were calculated as the average of three different electrodes. A dynamic detection limit range of the developed BPA biosensor was explored by CV analysis following the injection of BPA concentrations. It was found that the current variation showed a good linearship to the logarithmic concentration of BPA in a broad range of 10^{-11} to 10^{-7} M, with a detection limit of 10^{-11} M, at a signal-to-noise ratio of 3 (Thompson et al., 2002) and a good sensitivity of 1.81 µA µM^{-1} cm^{-2}. The reproducibility of this enzymatic biosensor was investigated for four different sensors which were prepared using the same procedure. CV studies were performed on each electrode as previously described, for the detection of 0.1 µM of BPA. The biosensor displays good reproducibility; a relative standard deviation obtained from three measurements with each biosensor was found to be 7.7%. The proposed biosensor shows better analytical performance compared to other ones, previously reported as shown in Table 1. The good biosensing performance of the biosensor based on MWCNTs-Tyr hybrid film could be attributed to different aspects such as the architecture of this hybrid film that provides a biocompatible microenvironnement for immobilized tyrosinase to retain its stability and bioactivity which can facilitate the enzymatic reaction towards BPA. Moreover, the high electric conductivity of BDD and carbon nanotubes materials that can provide a high surface matrix for the entrapment of biomolecules which increased the surface loading quantity of tyrosinase molecules and facilitate the mediation of electrocommunication between the biomolecule and the MWCNT (Poh et al., 2004). In addition, the enhanced electron transfer of o-quinone with MWCNTs. The obtained analytical results indicate that the proposed method could be an excellent platform for BPA detection and for other oxidase type enzymatic systems.

To verify the stability of the proposed biosensor, the response for 0.1 µM BPA was investigated every 7 days. Meanwhile, the biosensor was stored at 4 °C in PBS (pH 7.2). The electrochemical response of the biosensor was very stable, retaining more than 95% of its original response for 5 weeks. After that, a decrease to 80% of its original response was observed after 7 weeks. This storage time exceeds those previously reported in the literature for an electrochemical biosensor based on tyrosinase entrapped within a polythionine film (3 weeks) (Dempsey et al. 2004). This high stability can be attributed to the strong binding of MWCNTs-

Table 1
Relevant published electrochemical biosensors for BPA detection.

Biosensor configuration	Linear range (M)	Detection limit (M)	References
Tyr– AuNPs /graphite oxide	2.5×10^{-9} –3×10^{-6}	10^{-9}	Pan et al. (2015)
Tyr/TiO_2-MWCNTs-PDDA- Nafion/ graphite	2.8×10^{-7}–4.5×10^{-5}	6.6×10^{-8}	Kochana et al. (2015)
Tyr–Au–Thioctic NH_2 /Au	3.99×10^{-7}– 2.34×10^{-4}	1.33×10^{-7}	Wang et al. (2014)
peptide (CKSLENSYC)/ Au	1×10^{-9} –5×10^{-6}	7×10^{-10}	Yang et al. (2014)
Tyr–silk peptide–graphene/GCE	2×10^{-9} –5.48×10^{-6}	7.2×10^{-10}	Qu et al. (2013)
Laccase–thionine–carbon black/GCE	5×10^{-7}–5×10^{-6}	2×10^{-7}	Portaccio et al. (2013)
Tyr–AuNPs/ SPCE	4.2×10^{-8}–3.6×10^{-5}	1×10^{-8}	Alkasir et al. (2010)
Tyr– NiNPs/ SPCE	9.1×10^{-7}–4.8×10^{-5}	7.1×10^{-9}	Alkasir et al. (2010)
Tyr– Fe_3O_4/ SPCE	2.2×10^{-8}–4.0×10^{-5}	8.3×10^{-9}	Alkasir et al. (2010)
Polyglutamic acid–MWCNT–NH_2/ GCE	1×10^{-7}– 1×10^{-5}	2×10^{-8}	Lina et al. (2014)
Arginine– graphene/GCE	5×10^{-9}–40×10^{-6}	1.1×10^{-9}	Zhang et al. (2012)
Pd–loaded TiO_2–SiC/GCE	1×10^{-8}–2×10^{-4}	4.3×10^{-9}	Yang et al. (2014)
MoS_2 nanoflower–chitosanAuNPs/GCE	5×10^{-8}–1×10^{-4}	5×10^{-9}	Huang et al. (2014)
Tyr-diazonium-MWCNTs/ BDD	1×10^{-11}–1×10^{-7}	10^{-11}	This work

GCE: Glassy carbon electrode, Au: gold electrode, CPE: Carbon paste electrode, SPCE: Screen-printed electrode, SWCNT: Single- walled carbon nanotube.

Table 2
Analysis of real samples spiked with BPA.

Added C [M]	*Recovered C* [M]	*Recovery RSD%* ($n=3$) (%)
10 pM	10.3 pM	103 2.7
0.1 nM	0.095 nM	95 5.7
0.1 μM	0.103 μM	103 2.7

tyrosinase hybrid film with the diazonium functionalized BDD electrode and the high chemical and electrochemical stability of the BDD-diazonium bond. In order to evaluate the selectivity of this biosensor, the influence of some interfering substances was examined in PBS (pH 7.2) for the measurement of 0.1 μM BPA. It was found that 2-nitrophenol, with a concentration 20 fold higher, does not have any interfering effect. Likewise, Ni^{+2}, Cd^{+2}, KNO_3, Cu^{+2} and organic compounds like sucrose and glucose with a concentration 100 fold higher, do not interfere with BPA determination. This high degree of selectivity makes this BDD biosensor based on MWCNT-Tyr hybrid film a good analytical tool for BPA determination. For 0.1 μM of phenol, the voltammetric response is 23 $\mu A/cm^2$, an important response for this compound, which shows the limited selectivity of this biosensor.

3.4. Application of the biosensor to real samples

To study the practical performance of the proposed method, the biosensor was employed to determine trace amounts of BPA in spiked water samples. For the analysis, samples from a river-La Chaudanne-located in Lyon, were filtered, then spiked with various concentrations of BPA and analyzed according to the previously described procedure. The results are shown in Table 2 The recoveries were in the range of 95 to 103%, indicating good accuracy and validating the potential utility of the present biosensor for BPA detection in natural water samples.

4. Conclusion

In this study, a sensitive and simple electrochemical detection method has been proposed for the detection of BPA in water. The present BPA sensor based on a diazonium- MWCNTs- tyrosinase/BDD electrode displayed a wide linear range from 10^{-11} M to 10^{-7} M with a low detection limit of 10^{-11} M which is lower compared to others reported in the literature. Moreover, it presented very good reproducibility, selectivity and stability. The successful application of this sensor in water samples proves its utility for BPA determination in waste water.

Acknowledgments

The authors thank the EC for financed support through Marie Curie NANODEV Grant no. 318524.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.07.051.

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