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Stable and sensitive flow-through monitoring of phenol using a carbon nanotube based screen printed biosensor

G Alarcón[1,2], M Guix[1,3], A Ambrosi[1], M T Ramirez Silva[4], M E Palomar Pardave[2] and A Merkoçi[1,5,6]

[1] Nanobioelectronics and Biosensors Group, Catalan Institute of Nanotechnology, Campus UAB, 08193 Bellaterra, Barcelona, Catalonia, Spain
[2] Departamento de Materiales, Universidad Autónoma Metropolitana, Azcapotzalco, 02200 México Distrito Federal, Mexico
[3] Chemistry Department, Autonomous University of Barcelona, Campus UAB, 08193 Bellaterra, Barcelona, Catalonia, Spain
[4] Departamento de Química, Universidad Autónoma Metropolitana Iztapalapa, 09340 México Distrito Federal, Mexico
[5] ICREA, Barcelona, Catalonia, Spain

E-mail: arben.merkoci.icn@uab.es

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Abstract

A stable and sensitive biosensor for phenol detection based on a screen printed electrode modified with tyrosinase, multiwall carbon nanotubes and glutaraldehyde is designed and applied in a flow injection analytical system.

The proposed carbon nanotube matrix is easy to prepare and ensures a very good entrapment environment for the enzyme, being simpler and cheaper than other reported strategies. In addition, the proposed matrix allows for a very fast operation of the enzyme, that leads to a response time of 15 s.

Several parameters such as the working potential, pH of the measuring solution, biosensor response time, detection limit, linear range of response and sensitivity are studied. The obtained detection limit for phenol was 0.14×10^{-6} M. The biosensor keeps its activity during continuous FIA measurements at room temperature, showing a stable response (RSD 5%) within a two week working period at room temperature.

The developed biosensor is being applied for phenol detection in seawater samples and seems to be a promising alternative for automatic control of seawater contamination. The developed detection system can be extended to other enzyme biosensors with interest for several other applications.

S Online supplementary data available from stacks.iop.org/Nano/21/245502/mmedia

1. Introduction

Phenolic compounds are important contaminants of ground and surface waters, causing problems for the living environment and showing adverse effects on animals and plants. Many analytical techniques, such as the optical method (Huili *et al* 2007), gas chromatography (Regueiro *et al* 2009), liquid chromatography (Zgoła-Grześkowiak *et al* 2009) and capillary electrophoresis (Li *et al* 2009) have been used for monitoring phenols. Therefore, the sensitive, rapid, and precise determination of phenols and its derivatives are of growing interest in environmental control and protection.

Electrochemical biosensors are considered to be the most attractive techniques for such applications. The use of electrochemical devices offers many advantages in comparison

6 Address for correspondence: Centre d'Investigació en Nanociència i Nanotecnologia Escola Tècnica, Superior d'Enginyeria (ETSE) Campus de la UAB-Edifici Q-2ªplanta, 08193 Bellaterra, Spain.

for example to optical methods. Electrochemical sensors can operate in turbid media and are more amenable to miniaturization, which is of crucial importance for monitoring purposes. In addition, the excellent compatibility of electrochemical sensors with flow injection analytical systems (FIA) increases the potential for assay automation, to which more and more attention has been paid in many fields including food analysis (Mizutani *et al* 1998) and environmental monitoring (Vazquez *et al* 2006).

One of the biggest challenges in designing a new enzyme-based biosensor is to find the optimal balance between stability and activity of the enzyme. Immobilization methods, such as cross-linking bonding (Jung and Paradiso 2009), covalent attachment (Drevon *et al* 2002), polymer inclusion (Umran and Memet 2009) and simple adsorption, have different effectiveness with regard to the stability of the signal generated, due to the fact that a higher or lower percentage of enzyme immobilized is lost during the measurement. On the other hand, better stability is generally obtained, paying the price of the loss of signal intensity related to a lower enzyme catalytic activity. A good combination of support material and immobilization method is of fundamental importance to achieve the desired performances from the sensing system.

With regard to the fabrication of enzyme-based phenol biosensors, many examples can be found in the literature, where various sources of tyrosinase and a wide variety of matrixes including graphite (Nistor *et al* 1999), carbon paste (Caruso *et al* 1999, Merkoçi *et al* 2005), conducting polymers (Adeyoju *et al* 1996, Christophe *et al* 2003), biopolymers (Aihua *et al* 2005), Nafion membrane (Furbee *et al* 1993), nanoparticles (Li *et al* 2006), silica sol–gel composite films (Li *et al* 1998) and hydrogel (Nistor *et al* 1999) have been used, with different combinations of stability and sensitivity, depending on the application.

The use of enzymatic electrodes is an inherently sensitive alternative for the detection of enzyme substrate. Many biosensors for phenol determination have been developed in the past using the catalytic activity of the redox enzymes such as tyrosinase, peroxidase, laccase (Duran and Esposito 2000) etc using different electrode materials, flow systems and sample pre-treatment techniques.

Some of these fabrication methods are relatively complicated and require the use of several reagents, and often the biosensor presents stability problems with usage and sometimes has a short lifetime (Dornelles and Tatsuo 2002). Currently, there is an increasing interest for the design of functional membranes for biosensing application because of their possible use for analytical applications (Liu *et al* 2005).

Biosensors based on carbon nanotubes (CNTs) have shown great potential in several fields. From the first application of CNTs as a modifier of glassy carbon electrodes for NADH detection (Wang *et al* 2002), several other interesting applications have been reported by our and other groups. The use of CNTs has become relevant due to their excellent conductivity, including the improvement of electron transfer between enzymes and electrode surfaces (Merkoçi 2006), and at the same time provides a very good matrix for enzyme immobilization (Pérez *et al* 2008). On the other hand, biosensors based on nanostructured compounds (Pérez *et al* 2005, Pumera *et al* 2006) have been demonstrated to be simple in preparation and offer great promise for developing amperometric biosensors.

In this work we propose a novel CNT based matrix for the fabrication of a stable biosensor for multiple and continuous monitoring of phenol content in seawater samples. In combination with an especially in-house-designed flow injection system, the sensing device offered excellent sensitivity with very low detection limits and also offers a concrete possibility of its implementation in automatic control systems.

2. Experimental details

2.1. Reagents

Tyrosinase (TYR, EC232-653-4, 5370 unit mg^{-1} from mushroom), phenol and glutaraldehyde (25%) were purchased from Sigma Co. (USA), and all other chemicals were analytical grade, and used without further purifications. Standard solutions of phenolic compounds were prepared by dissolving each reagent in water.

Multiwalled carbon nanotube (MWCNT) powders used were purchased from Aldrich (Stenheim, Germany) with a 95% purity, tetrahydrofuran (THF).

Potassium phosphate buffer solution (PBS) of pH 6.5 was prepared with potassium phosphate monobasic (Fluka), potassium phosphate dibasic anhydrous and potassium chloride in MilliQ water.

2.2. Preparation of the biosensor

2.2.1. Preparation of screen printed electrode (SPE). Screen printed microfabrication is based on the sequential deposition of a graphite ink, Ag/AgCl ink and insulating ink on a polyester substrate. After each layer is deposited a drying process is carried out, which consists in keeping the polyester substrate at 90 °C for 15 min.

2.2.2. MWCNT treatment. Previous chemical oxidation and thermal treatment in order to functionalize MWNCTs, and subsequently shorten and partially oxidize them, is performed as reported earlier (Pérez *et al* 2005). The chemical oxidation process consists in stirring MWCNTs in 2 M nitric acid (PanReac, Spain) at 25 °C for 24 h. The multiwalled CNTs used have a purity of 95%. After their purification, the CNT dispersion is held by sonicating a MWCNT suspension (1 mg MWCNT/1 ml THF) for 4 h. Some works have reported good CNT stability, and it has already been reported that chemical processing can improve the morphology and thermal stability of MWCNTs (Yang *et al* 2008). In addition, other works demonstrate CNT stability when they are treated with nitric acid, showing that the concentration in the dispersion after 30 days is 85% of the initial value (Peng *et al* 2009).

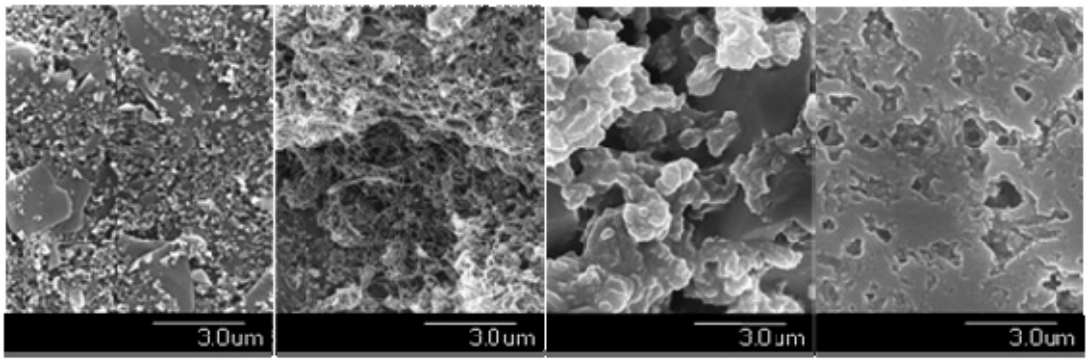

Figure 1. SEM images of the working surface area of (A) bare SPE, (B) SPE modified with MWCNTs, (C) SPE modified with MWCNT/Tyr and (D) SPE modified with MWCNT/Tyr/Glu. Images are taken at a resolution of 3 μm and an acceleration voltage of 15 kV.

2.2.3. Modification of SPE with CNT. The working surface area of a bare SPE was modified by depositing a 7 μl drop of MWCNT suspension (1 mg MWCNT/1 ml THF) onto the working electrode surface, followed by a drying process at room temperature for 24 h. The characterization process of the working electrode surface was performed by SEM to check if a homogeneous distribution of CNT over the surface was achieved.

2.2.4. Immobilization of the enzyme. 1 mg of tyrosinase (Tyr) enzyme was dissolved in 50 μl of 0.1 M phosphate buffer (PBS) at pH 6.5 (prepared with ultra-pure water from a Millipore-MilliQ system). A 7 μl drop of Tyr solution was deposited onto the working SPE/MWCNT electrode surface and allowed to dry at room temperature for 3 h. Finally, 7 μl of glutaraldehyde (Glu) solution at 5% were cast onto the SPE/MWCNT/Tyr electrode surface and left to dry at room temperature for 4 h. The prepared SPE/MWCNT/Tyr/Glu biosensor was kept at room temperature for stability study measurements or stored, if not in use, in the refrigerator at 5 °C.

2.3. Surface characterization

SPE characterization of the surface and the profile of the working electrode were performed by scanning electron microscope (SEM). Electrodes were mounted on adhesive carbon films and then coated with gold. SEM images were recorded with an S-570 SEM (Hitachi Ltd Japan) at an accelerating voltage of 15 kV.

2.4. Electrochemical measurement procedures

An FIA setup (see figure S1 in the supporting information available at stacks.iop.org/Nano/21/245502/mmedia) that uses a home-made flow-through cell of 20 μl was built and housed the SPE/MWCNT/Tyr/Glu biosensor, ensuring a stable operational analysis. In a typical measurement a 0.1 M buffer solution of pH 6.5 was introduced for 5 min at a flow rate of 3 ml min^{-1} by using a peristaltic pump (Perimax16/3). Volumes of phenol standard solutions from 0.5 to 25 μl were injected by using an automatic injector (Hamilton 36781). The injected phenol is passed through the electrochemical flow-through cell with the integrated SPE/MWCNT/Tyr/Glu biosensor. The measurements were performed under a flow-through regime. A working potential of −100 mV was applied and chronoamperometric measurements were performed by using a model Ch-Instrument potentiostat 660A electrochemical workstation from CH Instruments Inc., Austin, TX.

Impedance analysis of the prepared SPE modified electrodes was performed by using an Autolab302 potentiostat/galvanostat/frequency-response analyser PGST30, controlled by GPES/FRA Version 4.9. All impedance measurements were conducted in the presence of 50 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) mixture in PBS (pH 7.0) as redox probe. The tested frequency range was from 10 mHz to 100 kHz with an applied potential of 240 mV. Nyquist (imaginary impedance versus real impedance) diagrams were recorded.

3. Results and discussion

3.1. Morphological studies by scanning electron microscope (SEM)

The morphology of the modified electrodes was examined by SEM. Figure 1 displays typical images of the bare (a), MWCNT (b), MWCNT/Tyr (c) and MWCNT/Tyr/Glu (d) modified SPE electrodes. CNT distribution and glutaraldehyde film were evaluated. It is observed that MWCNTs are homogeneously distributed for all the used matrices. A more sponge-like surface is observed for CNT modified electrodes in comparison to the bare SPE (figures 1(A) and (B)). This would lead to an increase of the surface-to-area ratio due to the MWCNT incorporation into the working electrode area. Regarding the glutaraldehyde film, its presence is clearly evidenced by the more planar surface (figure 1(D)).

Chemical processing can improve the morphology and thermal stability of MWCNTs (Yang *et al* 2008). We checked the presence of CNTs in the biosensing matrix after being electrochemically tested. SEM images (see figure S2 in the supporting information available at stacks.iop.org/Nano/21/245502/mmedia) show CNTs still dispersed within the glutaraldehyde film.

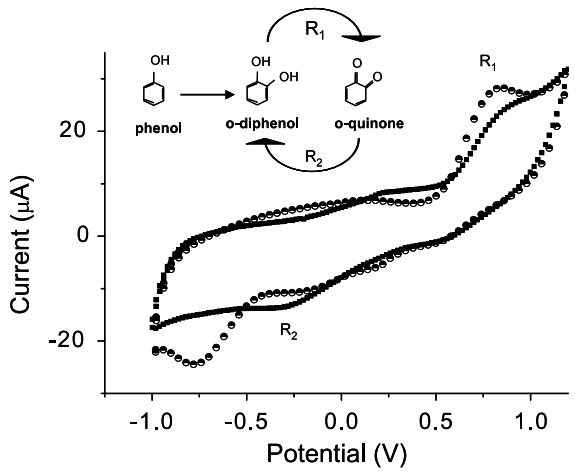

Figure 2. Cyclic voltammetry of 1 mM phenol with SPE/MWCNT (◓) and SPE/MWCNT/Tyr/Glu (■) in PBS, pH 6.5, scan rate 0.1 V s^{-1}; other experimental conditions as described in section 2 in the text.

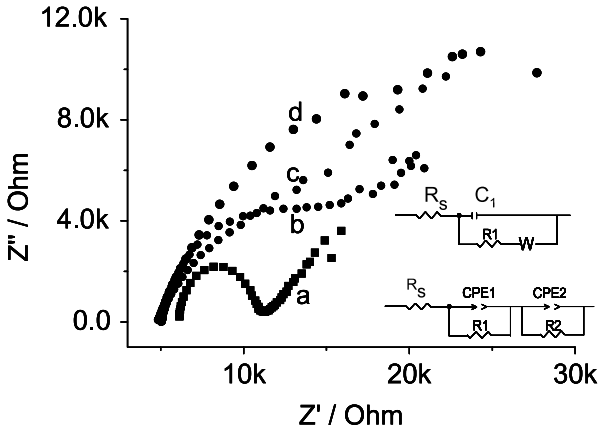

Figure 3. Typical Nyquist diagrams of electrochemical impedance analysis for (a) SPE, (b) SPE/MWCNT, (c) SPE/MWCNT/Tyr and (d) SPE/MWCNT/Tyr/Glu. A 50 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) mixture in PBS (pH 7.0) is used as redox probe. The frequency range used was from 10 mHz to 100 kHz. Applied potential was 240 mV. Corresponding circuits to bare SPE (upper part) and SPE/MWCNT/TYR/Glu (lower part) are shown. R_s is the resistance of the electrolyte solution; C_1 is the double layer capacitance of the SPE surface; W is the Warburg impedance; R_1 is the electron transference resistance and R_2, CPE1 and CPE2 are the resistance and the capacitance associated with electrode films.

3.2. Biosensor's response mechanism toward phenol detection

In order to make a biosensor selective to phenol detection tyrosinase immobilization has been used in numerous ways. Tyrosinase (Tyr) is a known copper containing protein, which uses molecular oxygen to catalyse the oxidation of phenolic compounds in two steps: the orthohydroxylation of monophenols and the two-electron oxidation of *o*-diphenols to *o*-quinones (Solomon *et al* 1996) (R_1). This reaction was firstly studied by using cyclic voltammetry of the SPE/MWCNT/Tyr/Glu biosensor (SPE modified with MWCNT and Tyr and glutaraldehyde, –Glu– as explained in section 2). In figure 2, the reduction of *o*-quinone to *o*-diphenols (R_2) is already observed to start at around 0 mV. The current increases rapidly as the applied potential moves negatively from 0 to −400 mV, achieving a steady state at around −250 mV, which can be due to the increased driving force for the fast reduction of *o*-quinone at low potential.

Figure 2 also shows, for comparison purposes, the CVs of 1 mM phenol solution obtained by using an SPE/MWCNT biosensor (SPE modified with only MWCNTs as described in section 2). By comparing the responses given by SPE/MWCNT with that given by SPE/MWCNT/Tyr/Glu it can be clearly appreciated that it is difficult to see (R_2) the reduction of quinone in the case when the Tyr is missing.

(The peak at −750 mV also appears for the pH 6.5 PBS used as a blank; see figure S3 in the supporting information available at stacks.iop.org/Nano/21/245502/mmedia.)

3.3. Impedance studies

The surfaces of unmodified and modified SPEs are also investigated using electrochemical impedance spectroscopy (EIS). EIS is a well known method used to study the features of surface modified electrodes. It is employed to analyse the detailed electrochemical response of the modified electrode while using individual or mixed components. The impedance features are discussed in terms of Nyquist plots so as to analyse the contributions of different components of the cell. Figure 3 shows Nyquist plots of the impedance spectrum at different stages of SPE modifications. The electrode impedance is presented as a dependence plot between two components: the imaginary $Z''(\omega)$ versus real $Z'(\omega)$ components, that originate mainly from the capacitance and the resistance of the cell. From the shape of the impedance spectrum, the electron-transfer kinetics and diffusion characteristics can be extracted. The respective semicircles of the curves correspond to the electron-transfer resistance (R) and the double layer capacity (C) nature of the modified electrode.

The EIS presented as Nyquist plots (Z'' versus Z') for the bare, MWCNT, MWCNT/Tyr and MWCNT/Tyr/Glu modified SPE respectively are shown in figure 3. On the other hand bare SPE exhibits a semicircle at high frequencies that corresponds to the electron-transfer limited process and the linear part represents the characteristics of the diffusion limited electron-transfer process on the electrode surface. Under the same conditions the MWCNT modified SPE shows an increment in the diameter of the semicircle with an interfacial resistance. The increase of the semicircle is related to the increase of the capacitance due to the presence of carbon nanotube onto the SPE surface. The EIS of the MWCNT/Tyr and MWCNT/Tyr/Glu modified SPEs shows similar behaviours also related to their composition and morphology.

In the case of the CNT matrix the study shows an especially low electron-transfer resistance. At the same time the diffusion process is improved, due to the CNT contribution not only in enhancing surface-to-area ratio but also improving an increase in the faradaic currents, leading to an improvement in the electrochemical behaviour (reflected in the analytical parameters of the biosensor). In addition, biosensor lifetime

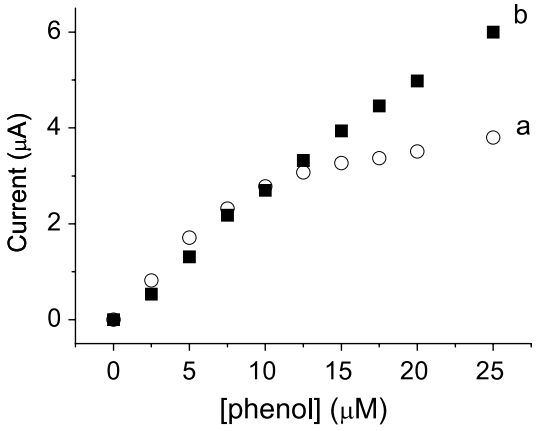

Figure 4. Effect of phenol concentration on the amperometric batch response of the biosensor: (a) SPE MWCNT/Tyr; (b) SPEMWCNT/Tyr/Glu. All measurements were made in 0.01 M phosphate buffer, pH 6.5 at 25 °C; an operating potential of −100 mV versus Ag/AgCl reference was used.

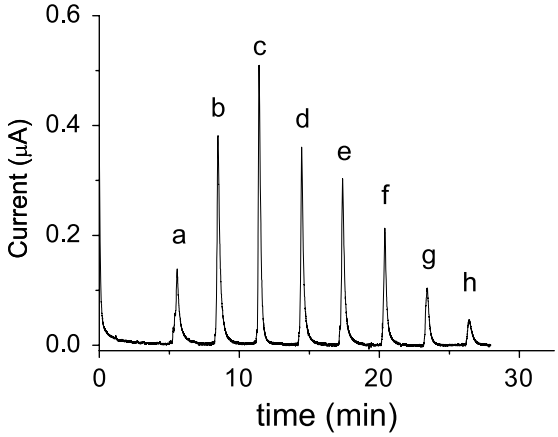

Figure 5. Amperometric responses of the SPE/MWCNT/Tyr/Glu biosensor at different pH values: (a) 4.67; (b) 5.99; (c) 6.50; (d) 7.16; (e) 7.53; (f) 8.18; (g) 8.52; (h) 8.82. Each peak corresponds to the injection of 20 μl of 10 μM of phenol solution at phosphate buffer 0.01 M, pH 6.5 at 25 °C; an operating potential of −100 mV versus Ag/AgCl was used. Other experimental conditions were as described in the text.

increases even in atmospheric conditions, making it not necessary to store them under strict environmental conditions.

The amperometric current responses of SPE/MWCNT/Tyr and SPE/MWCNT/Tyr/Glu versus phenol concentrations up to 25 μM were also studied (see figure 4) in order to find the effect of glutaraldehyde. SPE/MWCNT/Tyr (curve (a), figure 4) shows a lower sensitivity compared to SPE/MWCNT/Tyr/Glu (curve (b), figure 4). This phenomenon is probably due to the fact that the enzyme in the case of SPE/MWCNT/Tyr is directly exposed to substrate (phenol). The sites of catalytic activity of the enzyme are more accessible, compared to the case where glutaraldehyde is covering the enzyme. As seen from curve (a) of figure 4 the saturation of the enzyme for the SPE/MWCNT/Tyr biosensor seems to occur at a phenol concentration of around 10 μM. The calculated value of apparent Michaelis–Menten constant (K_M^{app}) for the SPE with free Tyr enzyme (SPE/MWCNT/Tyr) was 25 mM. This value for the enzyme covered by glutaraldehyde (SPE/MWCNT/Tyr/Glu) was 68 mM. K_M^{app} shows the affinity of an enzyme for its substrate, so if K_M^{app} is smaller this indicates a stronger substrate binding and higher catalytic activity. This effect is probably related to glutaraldehyde acting as an enzyme cross-linker. It helps a better immobilization of tyrosinase, preserving protein conformation (Quiocho and Richards 1964) and thus maintaining its biological activity (Avrameas 1969) and preventing leakage (Satish and Mohapatra 1994).

On other hand the K_M^{app} value for SPE/MWCNT/Tyr/Glu is lower compared to other reported matrixes used for Tyr immobilization like Tyr–titania sol–gel (Yu *et al* 2003), poly(allylamine)–tyrosinase–carbon fibre (PAA-Tyr-PAA/CF) (Rijiravanich *et al* 2006), graphite–Teflon (Serra *et al* 2002) and Tyr–ZnO nanorods (Chen *et al* 2007). The obtained results show that the MWCNTs connected with glutaraldehyde provide an excellent matrix for the immobilization of tyrosinase, which retains high catalytic activity for phenol oxidation.

3.4. Optimization of experimental variables

Parameters such as working potential, pH of the measuring solution, flow rate and injection time were analysed so as to find the optimum working conditions. The effect of applied potential on the phenol response of the biosensor was studied by the amperometric technique in the range of 0 to −500 mV (see figure S4 in the supporting information available at stacks.iop.org/Nano/21/245502/mmedia). The highest signal-to-noise ratio was recorded applying a working potential of −100 mV, and therefore this potential was used for the further optimizations and studies.

The enzymatic activity is greatly influenced by the medium pH; therefore, the sensor response was investigated using pH ranging from 4.6 to 8.8. Figure 5 shows the FIA responses of the biosensor for different pH values. The biosensor response increased from pH 4.6 up to 6.5. A gradually decreased response was observed at pH values above 6.5, which can be associated with a lower tyrosinase activity. The pH value of 6.5 was selected as optimal and used in all the following measurements.

Parameters related to the FIA system were also optimized. The effect of injection time from 2 to 30 s on the amperometric response of the biosensor towards phenol was studied (see figure S5 in section S1 available at stacks.iop.org/Nano/21/245502/mmedia). A stable and reproducible response at 10 s injection time was obtained. For shorter injection times (t < 10 s) the analytical response was not reproducible. A current decrease related to the short exposure time of the enzyme to the phenol substrate was observed. For longer injection times (10 s < t < 60 s) a saturation of the signal was observed. The heights of the peaks for retention times longer than 60 s remained almost constant but broadened, and therefore not adequate in terms of the time of analysis.

The study of the flow rate effect (results not shown) shows that the response time of the biosensor decreases at flow rates

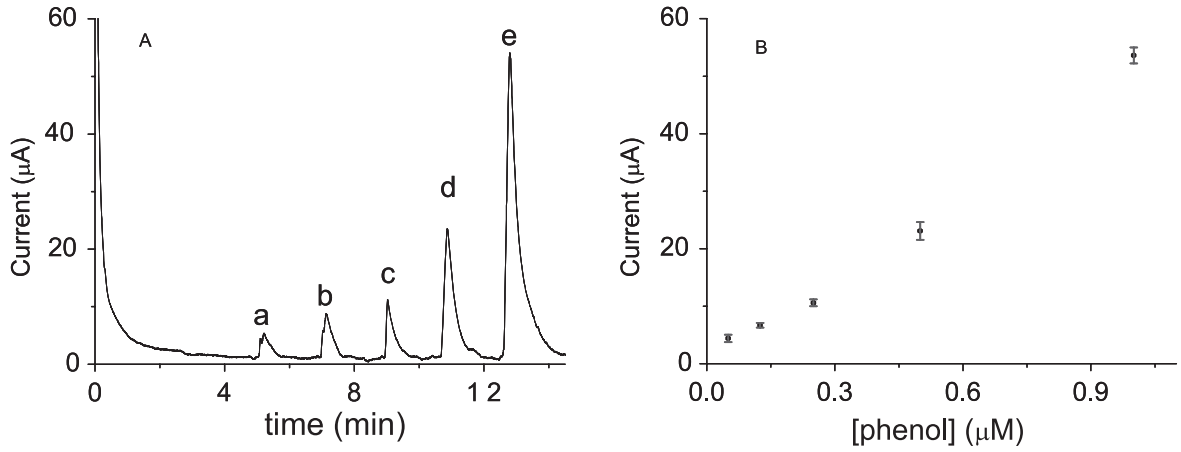

Figure 6. (A) FIA peaks obtained by chronoamperometric detection of phenol with concentrations of (a) 0.05, (b) 0.125, (c) 0.25, (d) 0.5, (e) 1 and (f) 1.25 μM. (B) Corresponding calibration curve of the current response versus phenol concentration. The error bars indicate the standard error for triplicate measurements. Conditions: temperature, 25 °C; operating potential, −100 mV versus Ag/AgCl reference; 0.01 M PBS solution at pH 6.5 is used as buffer. SPE/MWCNT/Tyr/Glu biosensor is used as detector.

lower than 2 ml min^{-1}. At higher rates the response time and the peak current increase. This is probably related to a more efficient diffusion process occurring under these conditions. This behaviour can be associated with chemical kinetics of the enzyme due to the fact that the response is better controlled by the enzymatic reaction at smaller volumes (Martinez *et al* 2008). A flow rate of 3 ml min^{-1} was chosen as the best to get a fast, stable and repeatable response.

3.5. Amperometric response of the biosensor

Electrochemical quantification of phenol using the FIA system was studied. The biosensor exhibited good response as a function of phenol concentration. The plot of the cathodic current versus phenol concentration (figure 6) in the range of 0.05–1 μM phenol shows a correlation coefficient of 0.99. The biosensor exhibits a sensitivity of 52.5 nA μM^{-1} and a detection limit of 0.14 μM phenol. The obtained detection limit is almost 500 times lower than the allowed levels of phenols in water as given by the EPA.

3.6. Stability study

The study of the analytical performance of the developed biosensor was performed in FIA conditions by following the cathodic current and using the optimal conditions obtained: applied potential (E_a) −100 mV; pH 6.5; flow rate 3 ml min^{-1}; injection time 10 s.

The typical current–time peaks were obtained for 10 μM phenol solution injections using the SPE/MWCNT/Tyr/Glu (see in figure S6 in the supporting information available at stacks.iop.org/Nano/21/245502/mmedia). Well defined peaks, virtually without any cathodic decay, were observed. A relative standard deviation (RSD) value for ten successive determinations and three replicates of 5.8% was obtained. An improved repeatability for phenol detection in comparison to other reported biosensors is obtained.

The long term stability of the biosensor (stored when not in use at room temperature in phosphate buffer 6.5) was tested for up to one month by monitoring of the cathodic current produced by injection of a 10 μM phenol solution every 3–5 days (results not shown). The biosensor shows stable response up to two weeks with an RSD of around 5%, while up to one month the RSD achieved a 25% value. The stability of this biosensor during this working period is related to the good entrapment of the enzyme within the MWCNT/Glu matrix. A remaining enzymatic activity of around 95% for a two week measuring period represents a good parameter compared to other complex matrices such as Fe_3O_4 MNPs-CNTs/Tyr (Cheng *et al* 2009) and TiO_2/CeO_2/Tyr (Njagi *et al* 2008). The storage of this biosensor at room temperature is another advantage for future technological developments and commercialization issues.

The obtained stability results probably are related to the fact that MWCNT and Glu are able to minimize surface fouling of the biosensor surface.

3.7. Phenol detection in seawater

Considering the interest for future applications of this biosensor for real seawater analysis, the recovery tests were also performed (see figure S7 in the supporting information available at stacks.iop.org/Nano/21/245502/mmedia). The recovery test in aqueous solution samples containing 5, 10 and 25 μM phenol in PBS (pH 6.5) were carried out. In addition, the recoveries of various phenol quantities added to real seawater samples (collected from the Mataro region in Barcelona, Spain, and without previous pre-treatment) were also measured.

Recovery values of around 94, 98 and 90% in PBS and 74, 99 and 98% in seawater samples respectively were obtained (see table 1 in the supporting information available at stacks.iop.org/Nano/21/245502/mmedia). The obtained results show good promise for further applications of the developed biosensor in automatic control systems with interest for the environment.

Table 1. Summary of the most relevant recent works regarding phenol detection using amperometric sensors. (GCE, glassy carbon electrode; CPE, carbon paste electrode; SPE, screen printing electrode; MWCNTs, multiwalled carbon nanotubes; SWCNTs, single-walled carbon nanotubes; PDDA, poly(dimethyldiallylammonium) chloride; PBS, phosphate buffer solution; Tyr, tyrosinase from mushroom; FIA, flow injection analysis; chit, chitosan.)

Electrode				Enzyme		Phenol detection			
Transductor	Modification	Stability	Stored	Enzyme/loading	Immobilization technique	Mode/technique	LOD (μM)	Sample	Reference
SPE/pasta carbon	No	—	—	Tyr (2590 units mg^{-1})	Cross-linking with glutaraldehyde	Batch/amperometric	0.410	Wastewater samples	Solná *et al* (2005)
GCE	No	75% after 30 days	PBS (pH 7.4) at 4 °C	Tyr mushroom (5370 units mg^{-1})	Composite MWNTs and cobalt phthalocyanine (CoPc)	Batch/amperometric	0.030	Bisphenol in plastic products	Yin *et al* (2010)
Pt	No	—	—	Tyr (4200 units mg^{-1})	Electropolymerized poly-amphiphilic pyrrole matrix or cross-linked with glutaraldehyde	Batch/amperometric	—	—	Stanca and Pospescu (2004)
CPE	SWCNTs	90% after 30 days	Dry at 4 °C	Tyr (3566 units mg^{-1})	Incorporated in the CPE	Batch/amperometric	0.020	Bisphenol	Mita *et al* (2007)
GCE	MWCNTs and TiO_2	89% after 10 days	PBS (pH 7.0). at 4 °C	Tyr (4400 unit mg^{-1})	Nafion film	Batch/amperometric	0.090	—	Lee *et al* (2007)
GCE	MWNTs–chit	93% after 10 days	PBS (pH 7.0) at 4 °C	Tyr (5370 units mg^{-1})	Glutaraldehyde	Batch/amperometric	0.005	Coliforms represented by *Escherichia coli*	Cheng *et al* (2008)
GCE	SWCNT	—	PBS (pH 7.0) at 4 °C	Tyr (3216 units mg^{-1})	Nafion film	Batch/amperometric	0.020	—	Wang (2005)
GCE	MNPs were absorbed CNTs wrapped with cationic polyelectrolyte PDDA	—	—	Tyr (5370 units mg^{-1})	Cross-linking with glutaraldehyde	FIA/amperometric	0.005	—	Cheng *et al* (2009)
ITO	No	—	4 °C	Tyr (25 000 unit, 0.3 mg)	Mixed chitosan, tyrosinase, and hydroxyl group-functionalized MWNTs	Batch/amperometric	—-	Commercial wine	Yang *et al* (2009)
SPE	3-aminopropyl	30 days	PBS (pH 7.0) at 4 °C	Tyr 2000 units mg^{-1}	Cross-linking with glutaraldehyde	FIA/amperometric	0.056	Pharmaceutical formulation	Martinez *et al* (2008)
SPE	MWCNTs	30 days 75%	Room temperature	Tyr (5370 unit mg^{-1})	Cross-linking with glutaraldehyde	FIA/amperometric	0.140	Seawater samples	Present work

4. Conclusions

A novel biosensor that uses MWCNTs and glutaraldehyde as the immobilization matrix of tyrosinase is designed and applied for phenol analysis in a FIA system. The used MWCNTs and glutaraldehyde not only immobilize the enzyme but also increase the long term stability of the biosensor.

The developed biosensor exhibited relatively fast response time (15 s) and good performance in terms of sensitivity and detection limit when the Tyr enzyme is entrapped within the MWCNT/glutaraldehyde matrix. The proposed immobilization matrix is easy to prepare and cheaper than other materials like nanorods (Gu *et al* 2009), nanoparticles, fibres (Wang and Hasebe 2009) and polymers (Lee *et al* 2007). The detection limit is comparable to other reported complex matrices (see table 1) and is below the value established by EPA criteria. The developed SPE/MWCNT/Tyr/Glu biosensor seems to be an interesting alternative for phenol detection in water including its integration into automatic control systems for pollution control in water samples.

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