Biosensors and Bioelectronics 42 (2013) 242–247

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

PEI-coated gold nanoparticles decorated with laccase: A new platform for direct electrochemistry of enzymes and biosensing applications

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ARTICLE INFO

Article history:
Received 7 August 2012
Received in revised form
5 October 2012
Accepted 5 October 2012
Available online 2 November 2012

Keywords:
Biosensor
Laccase
Direct electron transfer
Gold nanoparticle

ABSTRACT

This paper describes the synthesis and characterization of PEI-coated gold nanoparticles (PEI-AuNP), which were applied as a new platform in the immobilization of laccase (LAC) originating from *Aspergillus oryzae*. This material (PEI-AuNP-LAC) was used in the construction of a biosensor based on a glassy carbon electrode coated with a bio-nanostructured film. The occurrence of direct electron transfer (DET) between the electroactive center of LAC and the electrode surface was observed by cyclic voltammetry (CV), suggesting that the presence of AuNP in the film acts as a bridge for electron transfer. In acetate buffer solution (pH 5.0), LAC shows a pair of well-defined redox waves with a formal potential ($E^{0\prime}$) of 0.226 V *vs.* Ag/AgCl (3 M KCl). The biosensor response indicated a surface-controlled process with an apparent electron transfer rate constant (k_s) of 0.4 s^{-1}, charge transfer coefficient (α) of 0.5, and surface coverage concentration (Γ) of 3.45×10^{-10} mol cm^{-2}. The optimized biosensor showed the following limits of detection (LOD) for the phenolic compounds tested: 0.03 μM for catechol and guaiacol; 0.14 μM for pyrogallol and 0.21 μM for hydroquinone, using square-wave voltammetry (SWV). The proposed biosensor demonstrated high sensitivity, good repeatability and reproducibility, and long-term stability (only 20% decrease in response over 90 days and after 150 measurements by SWV for each film formed). This biosensor was successfully applied to catechol quantification in spiked water samples. Furthermore, this method showed great potential for application in the development of new devices for biosensing.

1. Introduction

Recent advances in nanotechnology have provided a wide range of new materials with potential for application in the immobilization and stabilization of biomolecules (Ansari and Husain, 2012). Among the most exploited nanomaterials, gold nanoparticles (AuNP) are notable due to their intrinsic properties which include biocompatibility, low cytotoxicity and immunogenicity, good electronic conductivity and chemical stability. In addition, their synthesis and surface functionalization involve processes which are simple and easily performed (Cebrián et al., 2011; Ansari and Husain, 2012). The high surface area and tunability of AuNP provide an excellent support for the attachment of biomolecules like DNA (Castañeda et al., 2007), antibodies (Rezaei et al., 2009) and enzymes (Ansari and Husain, 2012) for application in the development of biosensors. The chemical modification of AuNP surfaces provides an efficient platform to improve the immobilization of biomolecules. For example, AuNP modified with cystamine and glutaraldehyde have been used for the immobilization of tyrosinase (Janegitz et al., 2012), and AuNP synthesized using polyethyleneimine (PEI) in ionic liquid have been applied in the immobilization of human sulfite oxidase, favoring direct electron transfer (DET) between the enzyme and the modified electrode surface (Frasca et al., 2011).

In recent years, AuNP have been widely used as effective promoters of DET between the electroactive centers of proteins and electrodes. This is important in bioelectrocatalysis, which is often exploited as a basic principal in the construction of biosensors, biofuel cells and other devices based on biomolecules (Hu et al., 2008; Dagys et al., 2010). Electrode surfaces modified with the AuNP can provide a microenvironment similar to that of the redox-proteins (e.g. oxidoreductases enzymes) in native systems, and allow more freedom in the orientation of these molecules, thus reducing the insulating effect of the protein shell around the active center and the DET can proceed through the conducting tunnels of the AuNP (Guo and Wang, 2007). Dagys et al. (2010) recently published a study that demonstrates relatively rapid DET between a laccase and AuNP-modified electrodes, enabling efficient bioelectrocatalytic oxygen reduction.

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http://dx.doi.org/10.1016/j.bios.2012.10.087

Laccases (LAC) belong to a family of multicopper-oxidase enzymes and they occur widely in fungi and less frequently in higher plants and bacteria (Mayer and Staples, 2002). These enzymes are able to catalyze the oxidation of various phenolic compounds with the concomitant reduction of oxygen. In general, LAC possess four copper atoms, which are classified in accordance with their spectroscopic characteristics as T1, T2 and T3 sites. The T1 site of the enzyme is involved in substrate binding and oxidation as well as the transfer of electrons to the T2/T3 cluster, where oxygen is reduced to water (Solomon et al., 1996; Morozova et al., 2007). Besides its use in the pulp and paper industry, the LAC has been widely applied in food industry (Minussi et al., 2002), bioremediation processes and organic synthesis (Riva, 2006). Furthermore, due to its electrocatalytic ability, LAC has been applied to the development of many biosensors (Gomes et al., 2004; Moccelini et al., 2011; Shimomura et al., 2011; Shervedani and Amini, 2012).

Biosensors are gaining importance in the field of the development of systems to monitor pollutants such as phenols and their derivatives, potentially hazardous compounds which are released into the environment by a large number of industries (Shimomura et al., 2011). The determination of these pollutants compounds is necessary because of their toxicity even at low concentrations (Michałowicz and Duda, 2007). For this reason, biosensors are important analytical devices, being fast, inexpensive, accurate, sensitive and selective for the detection of potentially polluting and toxic phenolic compounds.

A novel approach to the immobilization of biomolecules and biosensing applications is proposed herein. PEI-coated gold nanoparticles (PEI-AuNP) were synthesized using PEI as a reducing agent and stabilizer simultaneously. The LAC molecules were immobilized on PEI-AuNP and thus nanoparticles decorated with the enzyme were obtained (PEI-AuNP-LAC). This material was applied in the construction of a biosensor based on a glassy carbon electrode (GCE) coated with a bio-nanostructured film, resulting in a new platform for the direct electrochemistry of enzymes. Various characterization studies were performed, and the experimental conditions were investigated in detail to obtain the best performance of the proposed method in the determination of phenolic compounds. The optimized biosensor was then successfully applied to catechol quantification in spiked water samples by square-wave voltammetry (SWV).

2. Experimental

2.1. Reagents and solutions

The LAC was purchased from Novozymes (Denmark), under the trade name Denilite® II BASE, produced by genetically modified microorganisms (*Aspergillus oryzae*). Catechol, guaiacol, pyrogallol and hydroquinone were obtained from Sigma-Aldrich. Gold(III) chloride solution ($HAuCl_4$, 30 wt% in dilute HCl) and branched polyethyleneimine (PEI, molecular weight of 25 kDa) were purchased from Aldrich and used for the preparation of PEI-AuNP. Phosphate buffer solution and acetate buffer solution (0.1 M) at various pH values were used as supporting electrolytes in the biosensor optimization studies. All reagents were of analytical grade and were used without further purification, and all solutions were prepared with distilled and deionized water.

2.2. Instrumentation and electrodes

Transmission electronic microscopy (TEM) analysis was performed with a JEOL JEM-1011 transmission electron microscope operating at 100 keV. Spectrophotometric measurements were performed on a Varian Cary 50 Bio. Atomic absorption spectroscopy (AAS) was carried out in a ContrAA® 600 graphite furnace (Analytik Jena). The voltammetric measurements were taken on an Autolab PGSTAT101 potentiostat/galvanostat (Metrohm Autolab, The Netherlands), and the data treated using NOVA software (version 1.6.013). The cyclic voltammetry (CV) and SWV measurements were carried out using a system of three electrodes: the proposed biosensor as the working electrode, a platinum plate as the counter electrode and an Ag/AgCl (3.0 M KCl) reference electrode.

2.3. Synthesis and characterization of PEI-AuNP and PEI-AuNP-LAC

The AuNP were synthesized following the procedure described by Sun et al. (2005) with some modifications. At ambient temperature with stirring, 150–1000 μL of a PEI solution, at different concentrations, was added to 5 mL of a 1 mM solution of $HAuCl_4$ to achieve a mixture of $Au:PEI_{monomer}$ in proportions of 1:3 to 1:20. After 5 min the samples were heated to 80 °C over 10 min by ramping at 5.5 °C/min and the characteristic ruby red color appeared. The temperature of 80 °C was maintained for a further 2 min when the flask was removed from the heat. The mixture was then kept under stirring until ambient temperature was reached. The bulk PEI-AuNP solutions were divided into 1 mL portions that were mixed with 50–500 μL of a 1.67×10^{-5} M LAC solution (17.1 units mL^{-1}; see supporting information) to obtain the desired PEI-AuNP-LAC nanoparticles with different enzyme concentrations. After 1 h of stirring at ambient temperature, the nanoparticles were centrifuged at 14,500 rpm, washed and redispersed in deionized water with the aid of an ultrasound bath. The best composition was identified based on the effect of the different samples on the electrode response to catechol oxidation. The nanoparticles with an $Au:PEI_{monomer}$ ratio of 1:10 and the PEI-AuNP-LAC sample with an Au:LAC ratio of 1:0.001 were subjected to quantitative analysis by TEM and AAS. Samples were prepared for TEM by diluting the nanoparticles in methanol followed by deposition on a carbon-coated copper grid. The average particle size was determined by counting approximately 300 particles using ImageJ software (Schneider et al., 2012). The AAS experiments were performed using the graphite furnace method with a high resolution continuous source and a calibration curve obtained with standard solutions. Kinetic experiments of catechol oxidation were performed to evaluate the activity of the free enzyme and the PEI-AuNP and PEI-AuNP-LAC systems (see supporting information).

2.4. Biosensor preparation

Prior to the biosensor preparation, a glassy carbon electrode (GCE) of 2.0 mm in diameter was mechanically polished with an aqueous suspension of 0.05 μm alumina, and then ultrasonically cleaned for 10 min in a 1:1 ethanol:water mixture, rinsed with distilled water and dried at room temperature. The PEI-AuNP-LAC solution (2 μL) was dropped onto the GCE surface and dried in a desiccator with silica gel, under vacuum for 1–2 h. The PEI-AuNP, PEI and enzyme-modified GCE were obtained in the same way. The modified electrodes were stored in air at room temperature (around 25 °C) when not in use.

2.5. Electrochemical measurements

All of the CV and SWV measurements were carried out at room temperature (25 °C) in an electrochemical cell containing acetate buffer solution (0.1 M, pH 5.0), and successive additions of a standard solution of catechol (or other phenolic compound: guaiacol, pyrogallol or hydroquinone) or a sample of spiked water were performed using a micropipette. The CV measurements were recorded by cycling the potential between +0.7 and −0.1 V at a scan rate of

20–300 mV s^{-1}. The SWV measurements were performed at a frequency of 10–100 Hz, pulse amplitude of 10–100 mV and scan increment of 1.0–10.0 mV, after successive additions of the analyte. All potential measurements are reported *vs.* Ag/AgCl (3.0 M KCl), and a stirring time of 60 s was used to homogenize the solutions.

2.6. Preparation of spiked water samples

Different water samples (tap, mineral and urban drainage water) were transferred into volumetric flasks (25.0 mL) and spiked with a 0.5 mL catechol solution (40.0 μM). Subsequently, these flasks were vigorously stirred in order to homogenize the samples, resulting in solutions with a concentration of 0.80 μM catechol. These solutions were prepared shortly before the measurements and analyzed without any pretreatment.

3. Results and discussion

3.1. Characterization of PEI-AuNP and PEI-AuNP-LAC

The nanoparticles were synthesized at various polymer concentrations through direct reduction of the gold atoms by PEI at 80 °C. Using a stock solution of 1.67×10^{-5} M LAC, with activity of 17.1 units mL^{-1}, the enzyme was bound to the nanoparticles by adding different concentrations of LAC to the PEI-AuNP in order to obtain 16 different systems. These were subjected to catechol detection (as discussed below) and the best system chosen for further characterization. Under the above-mentioned conditions, the electrode which provided the best result was that made of PEI-AuNP-LAC synthesized using an Au:$PEI_{monomer}$ ratio of 1:10 and Au:LAC ratio of 1:0.001 (Fig. S1, in the supporting information).

As later determined by TEM, the PEI-$AuNP_{10:1}$ nanoparticles presented an average diameter of 6.7 ± 2.0 nm, which slightly increased after the addition of the enzyme to 8.5 ± 2.3 nm (Fig. 1). AAS experiments revealed a [Au]/[LAC] ratio of 21221 ± 725 gold atoms per molecule of enzyme, which means, on average, 0.89 ± 0.03 of enzyme molecules per nanoparticle synthesized. The strong binding observed could occur through electrostatic interactions between the positively charged PEI on the surface of the nanoparticle (pKa ~ 9) and the anionic LAC (pI < 4) (Hublik and Schinner, 2000) under the neutral to basic conditions of the medium used in the synthesis (pH 6–8). This hypothesis is corroborated by the fact that the activity of the bound enzyme is not completely lost. The loss of enzyme activity does occur in the absence of the polymer when the binding of sulfides causes a major change in the tertiary structure of proteins (Shang et al., 2007).

3.2. Direct electrochemistry of LAC immobilized on PEI-AuNP-modified electrode

Fig. 2 shows the cyclic voltammograms for different electrodes in 0.1 M acetate buffer solution (pH 5.0) at a scan rate of 100 mV s^{-1}. No redox peak was observed for the bare GCE (a), GCE-PEI (b) and GCE-PEI-AuNP (c) and thus PEI and AuNP are electroinactive within the potential window. The voltammogram for GCE-PEI-LAC (e) shows poorly defined peaks while that for GCE-PEI-AuNP-LAC (d) shows a pair of well-defined and stable redox peaks, which are attributed to the DET between the electroactive center of the immobilized LAC and the electrode surface. In this regard, another study has reported the use of PEI to immobilize LAC on GCE; however, the authors had to use a redox mediator to obtain an efficient electron transfer (Rochefort et al., 2008). In view of this, the DET obtained in electrode (d) was attributed to the presence of AuNP, which plays an important role as an efficient electron-conducting tunnel (Zhang et al., 2005). As can be seen in Fig. 2(d), the redox process is an almost reversible electrochemical process. The anodic (E_{pa}) and cathodic (E_{pc}) peak potentials are located at 0.276 V and 0.176 V (*vs.* Ag/AgCl), respectively. The formal potential ($E^{0\prime}$) for the LAC originating from *A. oryzae* was calculated from the average value of the anodic and the cathodic peak potentials, and the value obtained was 0.226 V *vs.* Ag/AgCl, in 3 M KCl (corresponding to 0.436 V *vs.* NHE). This value is quite close to the $E^{0\prime}$ values for the T1 copper of LAC (originating from *Melanocarpus albomyces* and *Rhus vernicifera*) obtained in 0.1 M phosphate buffer (pH 6.5), using CV,

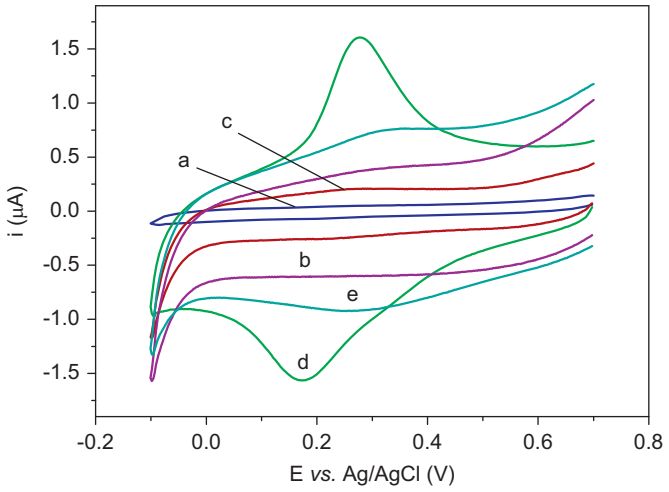

Fig. 2. Cyclic voltammograms obtained using different electrodes: (a) bare GCE, (b) GCE-PEI, (c) GCE-PEI-AuNP, (d) GCE-PEI-AuNP-LAC and (e) GCE-PEI-LAC in 0.1 M acetate buffer solution (pH 5.0) at scan rate of 100 mV s^{-1}.

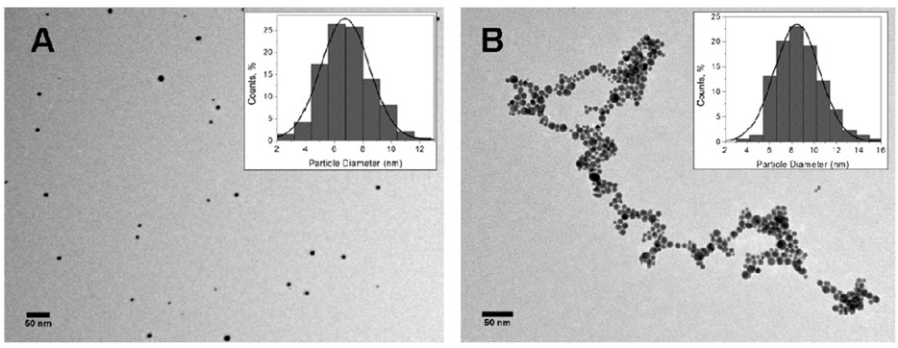

Fig. 1. TEM micrograph of the PEI stabilized AuNP (A) before and (B) after addition of LAC. The insets show the particle histogram based on approximately 300 particles.

reported by Frasconi et al. (2010), which were 0.449 V and 0.422 V *vs.* NHE, respectively. Thus, these enzymes were classified as low ($\leq$500 mV) redox potential laccases. Redox potential values for laccase of the same origin as that used in this study are not available in the literature to date.

The effect of the scan rate on the electrochemical response of the immobilized LAC at the biosensor GCE-PEI-AuNP-LAC is shown in Fig. S4A. With an increase in the scan rate the E_{pa} of LAC shifted to a more positive value and the E_{pc} shifted in the negative direction. The anodic (i_{pa}) and cathodic (i_{pc}) peak currents increased linearly with an increase in the scan rate (v) from 20 to 300 mV s^{-1} (Fig. S4B) but not with the square root of the scan rate ($v^{1/2}$) (Fig. S4C). This indicates that the process is controlled by the redox species immobilized on the electrode surface (Bard and Faulkner, 2001). Fig. S5 shows that E_{pa} and E_{pc} are linearly dependent on the logarithm of the scan rate (log v), which is in agreement with the Laviron theory for the case of surface-confined electroactive species (Laviron, 1979a). Based on this theory, the values of the charge transfer coefficient (α) and the apparent electron transfer rate constant (k_s) were estimated to be 0.5 and 0.4 s^{-1}, respectively (see supporting information). The value obtained for k_s is in close agreement with that of 0.5 s^{-1} previously reported by Dagys et al. (2010) for the DET between LAC and the surface of an AuNP-modified GCE and lower than the value of 1.94 s^{-1} observed for LAC immobilized on a Nafion/LAC-BP2000/GCE (Wang et al., 2012). An approximate value of the surface coverage of the electrode also can be calculated according to the Laviron equation (Laviron, 1979b; Bard and Faulkner, 2001) as 3.45×10^{-10} mol cm^{-2} (see supporting information). This value is much higher than the value of 1.3×10^{-11} mol cm^{-2} previously reported by Wang et al. (2012), indicating that more LAC was immobilized on the PEI-AuNP due to large surface area of this matrix. In addition, the effect of pH on the electrochemical process occurring at the surface of the GCE-PEI-AuNP-LAC biosensor was also studied by CV (see supporting information).

3.3. Film composition and detection principle of the proposed biosensor

Fig. 3A shows the components used in the formation of the bio-nanostructured film on the GCE surface, which include the LAC and AuNP coated with PEI. This film provides an appropriate environment for the immobilization of the LAC, without denaturation of this biomolecule. Furthermore, the enzymatic immobilization through electrostatic interaction between the positively charged PEI on the surface of the nanoparticle and the anionic LAC and, principally, the presence of AuNP in the film improve the electron transfer between the electroactive center of LAC and the electrode surface.

Fig. 3B shows a scheme of the reaction involved in the oxidation of catechol catalyzed by LAC on the electrode surface. Initially, catechol in contact with LAC, in the presence of molecular oxygen, is oxidized to the corresponding *o*-quinone. Subsequently, the quinone produced is electrochemically reduced on the biosensor surface at a potential of +0.25 V *vs.* Ag/AgCl. The resultant current obtained in the electrochemical reduction of *o*-quinone is proportional to the catechol concentration and was used for the monitoring/quantification of this analyte. The same process can be extended to many other phenolic compounds (e.g., guaiacol, pyrogallol, and hydroquinone), changing only the peak potential.

3.4. Study on the contribution of the modifiers used in the biosensor construction

A study using different electrodes was carried out to evaluate the contribution of each modifier material employed in the biosensor construction to the analytical response of these sensors. Fig. 4 shows the square-wave voltammograms obtained using the (a) bare GCE, (b) GCE-PEI, (c) GCE-PEI-AuNP and (d) GCE-PEI-AuNP-LAC (biosensor) in 0.1 M acetate buffer solution (pH 5.0) with 5.0 μM of catechol. As can be observed, the use of PEI on GCE (sensor "b") does not modify significantly the response of the sensor, but the presence of AuNP in film (sensor "c") increases the response by around a factor of two in relation to the bare GCE (a), due to the intrinsic characteristics of noble metal NP, such as high conductivity and catalytic capacity. An important gain in the response was obtained with the use of immobilized enzyme (PEI-AuNP-LAC) in the construction of electrode "d" (biosensor), which showed an approximately 5-fold increase in the peak current for catechol compared to the unmodified electrode. This finding can be attributed to the high catalytic activity of LAC in the oxidation of this phenolic compound, as well as the facilitation of the electron transfer between the enzyme and the electrode surface provided by the presence of AuNP in the film. Thus, this investigation confirms the advantages of the use of modifier materials, such as PEI-AuNP and LAC, in the development of biosensors with high analytical performance.

3.5. Optimization of the biosensor construction and experimental conditions

In order to optimize the biosensor construction and the experimental conditions of the proposed method, some parameters were investigated using SWV. Initially, the effect of the amount of PEI-AuNP-LAC solution used for the film formation, in the range of 1–6 μL, was studied. The best analytical response of the biosensor in 0.1 M acetate buffer solution (pH 5.0) containing 5.0 μM catechol was obtained using 2 μL of PEI-AuNP-LAC solution for the film formation on the GCE surface. This volume was therefore used for the subsequent biosensor construction.

After the optimization of the biosensor construction, the effects of supporting electrolytes of different natures and at various pH values, including acetate buffer solution (pH 3.0–5.0) and phosphate buffer solution (pH 6.0–7.0), in all of the solutions in a concentration of 0.1 M, were investigated by SWV. The best electroanalytical response to catechol was obtained in acetate buffer solution at pH 5.0.

Subsequently, the SWV parameters were optimized seeking to obtain the best performance of the biosensor under the conditions cited above. The frequency (10–100 Hz), pulse amplitude (10–100 mV) and scan increment (1–10 mV) were investigated in the determination of catechol and the optimized parameters can be summarized as follows: frequency 60 Hz; amplitude 100 mV and scan increment 5 mV. Thus, these instrumental conditions were selected for all subsequent determinations of catechol and other phenolic compounds.

3.6. Analytical performance of the biosensor

Catechol, guaiacol, pyrogallol and hydroquinone were the phenolic compounds selected for this study, particularly in view of their high toxicity. These compounds are important contaminants in groundwater and surface water and they are commonly present in industrial wastewater. Thus, the monitoring of these pollutants is an issue of considerable importance.

Under the conditions previously optimized using SWV, the proposed biosensor showed a linear response in the ranges of 0.36–11.00 μM for catechol, 0.79–17.42 μM for guaiacol, 1.74–19.60 μM for pyrogallol, and 2.90–22.00 μM for hydroquinone. The calibration curves for the phenolic compounds were constructed from plots of the resultant current peak (μA) *vs.* phenolic compound concentration (μM). The respective regression equations, correlation coefficients (r^2), and limits of detection (LOD = three times the standard deviation of the intercept/slope) are shown in Table 1. The responses in terms

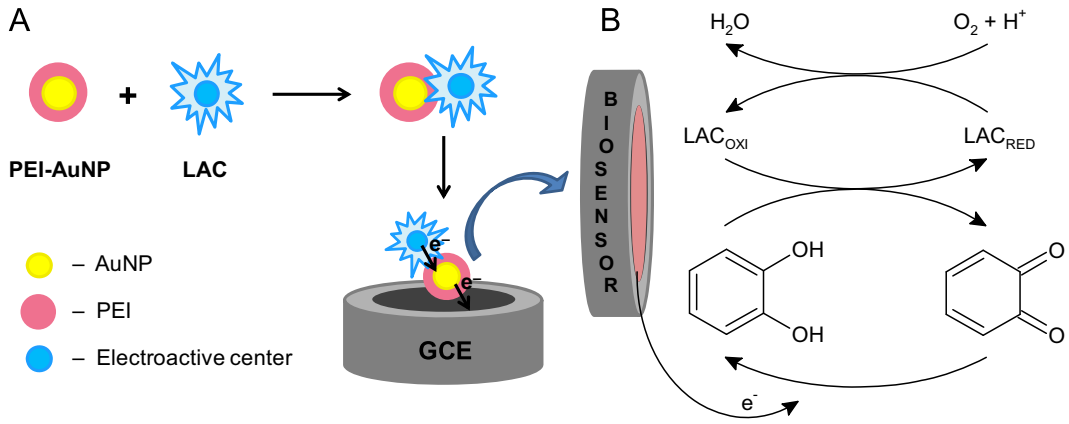

Fig. 3. Schematic representation of (A) components used for construction of the GCE coated with bio-nanostructured film and (B) enzyme-catalyzed oxidation of catechol with subsequent electrochemical reduction of the quinone formed on the biosensor surface.

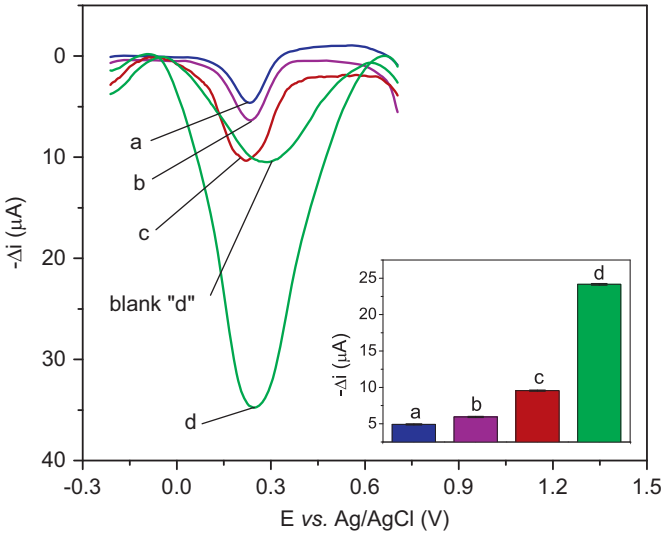

Fig. 4. Square-wave voltammograms obtained using different electrodes: (a) bare GCE, (b) GCE-PEI, (c) GCE-PEI-AuNP and (d) GCE-PEI-AuNP-LAC (biosensor) in 0.1 M acetate buffer solution (pH 5.0) with 5.0 μM of catechol at frequency 60 Hz, pulse amplitude 100 mV and scan increment 5 mV. Blank "d" was obtained in 0.1 M acetate buffer solution (pH 5.0) without catechol. Inset: current values for catechol obtained using each of the electrodes studied.

of detectability (LOD) followed the order: catechol = guaiacol > pyrogallol > hydroquinone. As can be seen, the levels of sensitivity of the calibrations (slope of calibration curve) obtained for the phenolic compounds analyzed and peak potential values were similar, since their chemical structures are very similar. Thus, for a mixture of these four phenolic compounds, the peak current would represent the total value for all of these compounds and only the peak current of hydroquinone appears at a potential sufficiently different to be determined separately from the other compounds studied.

The lifetime and stability of the biosensors were investigated by performing SWV measurements in 5.0 μM of catechol at different time intervals over a period of 90 days (over 150 measurements for each film formed on the electrode), and were stored at room temperature in a dry place when not in use. A relative response of over 80% was obtained at 90 days of evaluation, in relation to that obtained on the day of construction. These results are considered satisfactory, and indicate that the enzyme was immobilized in a biocompatible environment. Therefore, this method allows the stability and lifetime of the biomolecule, and consequently of the biosensor, to be maintained.

Table 1
Analytical parameters of the biosensor in the determination of phenolic compounds.

Phenolic compound	E[a]	Intercept (μA)	Slope (μA μM^{-1})	r^2	Linear range (μM)	LOD (μM)
Catechol	0.25	1.41 ± 0.040	4.41 ± 0.007	0.9994	0.36–11.00	0.03
Guaiacol	0.25	0.26 ± 0.026	2.23 ± 0.003	0.9998	0.79–17.42	0.03
Pyrogallol	0.18	1.45 ± 0.059	1.22 ± 0.006	0.9996	1.74–19.60	0.14
Hydroquinone	0.12	1.83 ± 0.078	1.12 ± 0.006	0.9990	2.90–22.00	0.21

[a] Peak potential values (*V*) *vs.* Ag/AgCl.

Studies of the repeatability, biosensor-to-biosensor reproducibility, and interfering compounds also were carried out (see supporting information).

3.7. Recovery study and analytical application

The proposed biosensor was applied in a study on the recovery and quantification of catechol in 3 water samples (tap, mineral and urban drainage water) using the standard addition method. The spiked water samples were prepared as described in Section 2.6 with a known amount of catechol (0.80 μM), and the SWV measurements were performed in triplicate for each sample. Recovery measurements were obtained by adding different standard concentrations of catechol to three different types of water samples. The recoveries of 97.4–101.7% obtained for these samples demonstrate the satisfactory accuracy of the proposed method (Table 2). In addition, the catechol contents determined in the samples of tap, mineral and urban drainage water were 0.82 ± 0.01 μM, 0.78 ± 0.02 μM and 0.76 ± 0.04 μM, respectively (as shown in Table 2). The results obtained from these samples showed a maximum relative error of ± 5.0% compared to the known content of the spiked samples. Therefore, the developed method is presented as an adequate alternative for the determination of catechol in water samples with good precision and accuracy.

4. Conclusions

This paper describes the synthesis and characterization of PEI-coated gold nanoparticles (PEI-AuNP). LAC enzymes were immobilized on PEI-AuNP, and applied in the construction of a biosensor based on a GCE coated with a bio-nanostructured film. The presence of AuNP in the film promotes the DET between the electroactive center of LAC and the electrode surface. The optimized biosensor

Table 2
Study on recovery and quantification of catechol in spiked water samples using the proposed biosensor.

Sample[a]	Catechol (μM)		
	Standard concentration added	% Recovery[b]	Quantified[c]
Tap water	1.54	101.3	0.82 ± 0.01
	1.90	101.6	
	2.62	98.8	
Mineral water	1.54	100.7	0.78 ± 0.02
	1.90	100.5	
	2.62	99.2	
Urban drainage water	1.17	101.7	0.76 ± 0.04
	1.90	97.4	
	2.62	100.8	

[a] Spiked water samples = 0.80 μM catechol.
[b] Recovery = (mean value found/added value) × 100%.
[c] Mean ± standard deviation; $n = 3$.

showed good stability, acceptable lifetime, adequate repeatability and reproducibility, and a low detection limit for the determination of catechol and other phenolic compounds. This biosensor was successfully applied to catechol quantification in spiked water samples, and may be appropriate for the determination of other phenolic compounds in environmental samples. In addition, this method proved to be a suitable platform for the immobilization and DET of enzymes, and also showed great potential for application in the development of new devices for biosensing.

Acknowledgments

The financial support from CNPq (Processes 470430/2009-5 and 470505/2011-7), MCT/CNPq/PADCT, and also the scholarship granted by CNPq to DB and BS are gratefully acknowledged. We also thank INCT-Catálise, the LCME/UFSC for technical support in the TEM, and Silvane Morés for metal determination by AAS.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.10.087.

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