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Amperometric biosensor based on a high resolution photopolymer deposited onto a screen-printed electrode for phenolic compounds monitoring in tea infusions

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ABSTRACT

An amperometric biosensor based on laccase, from Trametes versicolor (LTV), was developed and optimized for monitoring the phenolic compounds content in tea infusions. The fungal enzyme was immobilized by entrapment within polyvinyl alcohol photopolymer PVA-AWP (azide-unit pendant water-soluble photopolymer) onto disposable graphite screen-printed electrodes (SPE). Sensitivity optimization in terms of pH, temperature and applied potential was carried out. The linear range, detection limit, operational and storage stabilities were also determined.

The laccase biosensor (LTV-SPE) was calibrated for o-, m- and p-diphenol as well as caffeic acid. The highest response was found at 0.1 M acetate buffer pH 4.7, though it must be added the good reproducibility and operational stability were also obtained. The useful lifetime of the biosensor is estimated to be greater than 6 months.

LTV-SPE was used for the determination of the equivalent phenol content (EPC) in tea infusions by the direct addition into the electrochemical cell: the results were compared with those from the Folin–Ciocalteu spectrophotometric method. The amperometric detection exhibits some interesting advantages such as high simplicity, minimal sample preparation and shorter response time. A stable and sensitive amperometric response was obtained toward standard diphenolic compounds and herbal infusions. These biosensors are useful for easy and fast monitoring of EPC that can be related to the antioxidant capacity of natural extracts.

1. Introduction

In the last three decades, numerous studies on free radicals are largely found in the clinical and nutritional literature. Free radicals are highly unstable molecules, generated in vivo during metabolic processes that can be neutralized by certain compounds known as antioxidants, naturally produced by the human body. However, some factors like pollution, sunlight exposure, cigarette smoking or simply a malfunction of the antioxidant production may lead to a free radical excess which induces oxidative damage in cell constituents and promote numerous degenerative diseases and aging. That is why cellular antioxidants play an important role in preventing the formation and opposing the cellular oxidants action [1–4].

In this way, countless dietary compounds have been purportedly and intensely marketed as being important supplementary antioxidants, among the most frequently mentioned ones are: vitamins C and E, carotenoids, tocopherols, etc. [5–7].

The polyphenols, known to have antioxidant properties, form an extensive group of compounds commonly found in legumes, cereals, fruits and medicinal plants [8]. Therefore, it is of great interest for health and food science researchers to evaluate the antioxidant capacity of natural foodstuff related to its polyphenolic constituents [9]. To determine the relative effectiveness of specific compounds acting as antioxidants is not an easy task due to the complexity of food composition [10]. Separating each antioxidant or families of antioxidants and studying them individually is not suitable as well, thus, it would be advantageous to have a simple enough method for quick and reliable evaluation of antioxidant effectiveness. How-

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ever, the antioxidant capacity assessed using only one method seems to be inadequate due to the significant evidence revealing different reaction mechanisms [11].

There are many papers reviewing the large number of assays developed to measure antioxidant capacity of a huge variety of natural samples [12–24]. These assays are useful to rank antioxidant activity of substances and foodstuffs that contain antioxidant compounds and can be an indicator of the antioxidant potential prior to their consumption [25]. Beverages such as herbal infusions and teas do not have any particular nutritional value, but constitute attractive antioxidant's supplementary sources to the human diet. The antioxidant capacity of herbal infusions and teas has been studied by different methodologies [26–39], namely: chemiluminescence, oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant potential (FRAP).

Due to its high specificity, enzyme based-amperometric biosensors, useful for polyphenols quantification, have been developed [9,40–63]. For this purpose, the so called phenoloxidases, namely tyrosinase and laccase have been used, however most biosensors for the detection of polyphenols antioxidants are based on the enzyme tyrosinase [44–47,49–51,57,58]. Laccase, although as effective, has been less used for this purpose [48,52]. While tyrosinase catalyzes the oxidation of monophenols, o-diphenols to o-quinones [64–66], laccase can catalyze the oxidation of o-, m-, and p-benzenediols and phenol to o-, m- and p-quinones or radical species and does not require hydrogen peroxide as a co-substrate or any cofactors for the catalytic reaction [67]. The general performance of this sort of biosensor depends on several aspects; one of the most important is to conserve the activity of the enzyme when it is confined into the polymeric matrix that secures the enzyme onto the electrode surfaces. Since the enzyme should be reached by the substrate molecules and the oxidized phenols should be in direct contact with the working electrode surface, the matrix should provide stability to the enzyme and, at the same time, should allow the diffusion of the substrate molecules and its oxidized forms.

In this work, preparation and performance of a disposable screen-printed laccase biosensor (with all the electrodes included, namely: working, reference and counterelectrode) is reported, where the enzyme was immobilized into the working electrode surface by entrapment within a novel polyvinyl alcohol photopolymer. This biosensor showed a fast, stable, and sensitive amperometric response to several standard phenolic compounds and was used for the estimation of the Equivalent Phenolic Content (EPC) in different teas real samples, by direct addition of an appropriate diluted sample aliquot to the electrochemical cell.

2. Experimental

2.1. Reagents and solutions

All reagents were analytical grade, supplied by Sigma–Aldrich, except for the ethanol 96.2° purchased from Carlo Erba reagents. The polymer PVA-AWP was provided by Toyo Gosei Co., Ltd., Japan. The laccase from Trametes versicolor (EC 1.10.3.2) 18,000 IU/mg was purchased from Sigma. KH_2PO_4 and Na_2HPO_4 were used to prepare 0.1 M phosphate buffer at pH 6.5 and pH 7.0. Also, 0.1 M acetate buffer at pH 4.7 and pH 5.5 was prepared with sodium acetate and acetic acid; Folin–Ciocalteu's phenol reagent (FCR) 2N was supplied by Sigma; 0.1 M sodium carbonate, caffeic acid, resorcinol (m-diphenol), hydroquinone (p-diphenol) and catechol (o-diphenol) stock solutions were prepared in ethanol. Subsequently, 10^{-2} M, 10^{-3} M and 10^{-4} M stock solutions were freshly prepared by suitable dilutions the day they were used, kept in a dark flask at 4 °C half immersing them on an ice tray during measurements.

2.2. Sample preparation

Herbs and teas bags samples were obtained from on-the-shelf commercial products. The following herbs were studied: Arnica (*Arnica chamissonis*), Basil (*Ocimum basilicum*), Orange leaves (*Citrus sinensis*), Fennel (*Foeniculum vulgare*), Mint (*Mentha sativa*), Palo Azul (*Cycloleptys genistoides*) and Hibiscus (*Hibiscus sabdariffa* L.). Infusions were prepared by adding 5 mL of distilled water (room temperature) to 30 mg of herbal material previously dried during 8 h at 40 °C. The infusions were brewed for 10 min at 70 °C. 1.0 mL, aliquots were collected from the resulting solutions and immediately used to assess their EPC using amperometric and spectrophotometric methods

2.3. Electrodes and biosensors manufacture

The SPE used are three-electrode configuration comprising working, counter and reference electrodes, that were manufactured at IMAGES EA 4218 laboratory, Université de Perpignan, France, using a DEK 248 printing machine. The working electrode surface was 12 mm^2 and Ag/AgCl was used as pseudoreference electrode. For the sake of comparison in some figures, the potential values are also quoted to the commercial $Ag/AgCl/Cl^-_{(aq)}$ 3 M electrode. A BAS electrode MF-2079 was used for this purpose.

For LTV-SPE preparation, the enzyme was immobilized on the working electrode surface on a polymeric film that was prepared by mixing 100 μL of LTV aqueous solution 10 mg mL^{-1} and 100 μL of PVA-AWP polymer. When perfectly homogeneous, 5 μL of the mixture were placed onto the working electrode surface and dried under white light at 5 °C during 3 h. Once the electrode surface had dried out, the LTV-SPE was stored under vacuum in a refrigerator at 5 °C. Before use no previous conditioning was necessary.

2.4. Apparatus and measurements

The dependence of the biosensor performance on the applied potential is one of the most important aspects to focus on since quinoid species, product of the enzyme reaction at the working electrode surface, can be electrochemically reduced back to diphenol species at an appropriate potential.

2.4.1. Cyclic voltammetry, CV, was mainly used to optimize the potential range during the amperometric quantification of polyphenolic compounds under selected experimental conditions

Cyclic voltammograms were recorded using a BAS-Epsilon potentiostat with unmodified SPE in a stirred batch system with a thermostated cell. An initial voltammogram was recorded with the SPE immersed into pure buffer solution to verify the background signal. Subsequently, a diphenol solution aliquot was added into the stirred cell and a CV was performed straight after, in order to minimize adsorption of the diphenol compound onto the working electrode surface. Because the hydroquinone is considered the natural substrate of laccase, the analysis of its redox behavior was carried out. The redox processes associated to hydroquinone were studied using SPE whose working electrode surface has been modified with the photopolymer film excluding the enzyme. The initial scan was recorded within the −0.6 V to 0.6 V potential range at 0.10 V s^{-1} scan rate.

2.4.2. Amperometric measurements

Electroreduction of enzymatically generated quinoid products was achieved using the standard injection method under constant potential in a LC-4C BAS amperometric detector interfaced with a personal computer using DAISY LAB 6.0 software for experimental control and data acquisition. The LTV-SPE performance was studied by applying a constant potential, selected after the voltammetric

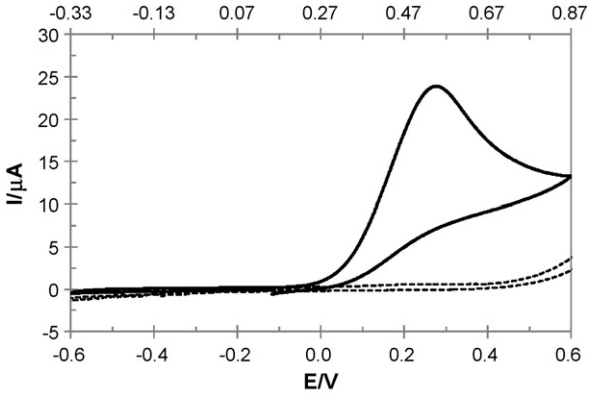

Fig. 1. Experimental CVs recorded in the system SPE/0.1 M acetate buffer (pH 4.7) with 500 μM (solid line) and without (broken line) hydroquinone, at 0.1 V s^{-1} potential sweep rate. The potential scan was started at null current conditions $E_{i=0}$ = −0.12 V, toward the negative values. In the second *X*-axis it is reported de potential values vs. a commercial Ag/AgCl/$Cl^-_{(aq)}$ 3 M electrode.

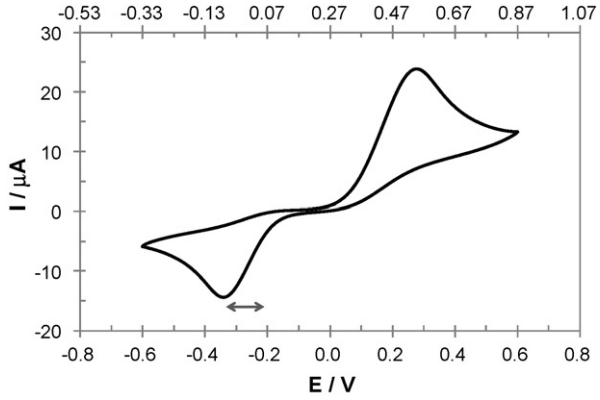

Fig. 2. Experimental CVs recorded in the system SPE/0.1 M acetate buffer (pH 4.7) with 500 μM at 0.1 V s^{-1} potential sweep rate. The potential scan was started at null current conditions, toward the positive values. In the second *X*-axis it is reported de potential values vs. a commercial Ag/AgCl/$Cl^-_{(aq)}$ 3 M electrode.

study, in a stirred batch system using a thermostated cell under specific pH and temperature conditions. To produce the calibration plots, the LTV-SPE was immersed in 10.0 mL of the buffer solution and the selected potential was applied; once the steady state had reached a plateau, aliquots of the standard diphenolic compound solution were injected and the ensuing current variations were recorded. The calibration plot shows the overall current as a function of the substrate concentration, such that when the substrate concentration increases, the amperometric response of the LTV-SPE biosensor also increases. Hydroquinone, catechol, resorcinol and caffeic acid were thus evaluated.

2.5. Determination of EPC

Tea infusion analysis was performed after assessment of the calibration plot for the selected standard compound. 50 μL of tea infusion 6 mg mL^{-1} was added into the cell under optimal experimental conditions. The corresponding current response was interpolated in the standard compound calibration plot and the EPC was calculated and expressed in equivalent mg of caffeic acid per liter of tea sample. After each tea measurement, the cell and the LTV-SPE were rinsed and the biosensor was evaluated again with caffeic acid stock solution in order to monitor the response which should be constant during the analysis. Every tea infusion was tested by triplicate using the same biosensor. In order to establish a comparison between methods, the EPC was also determined by the Folin–Ciocalteu spectrophotometric method, which in the traditional version of the method [68–70] was applied using a PerkinElmer UV–vis spectrophotometer. 500 μL of 0.2 N FCR aqueous solution were added to 100 μL of the tea infusion 6.0 mg mL^{-1} sixfold. After stirring, the mixture was kept in darkness during 8 min and 400 μL of the 75 mg mL^{-1} sodium carbonate aqueous solution were added and thoroughly mixed. The resulting mixture was also kept in darkness during 1 h: then, the absorbance was read at 766 nm. Several caffeic acid concentrations were used instead of infusions to construct a calibration plot.

3. Results and discussion

3.1. Determination of the working potential

Fig. 1 shows a CV recorded in the system SPE/500 μM hydroquinone, 0.1 M acetate buffer (pH 4.7), at 0.1 V s^{-1} potential sweep rate. The potential scan was started at null current conditions, $E_{i=0}$ = −0.12 V, toward the negative values, the potential sweep was reversed at −0.6 V and proceeded up to 0.6 V. It can be clearly noted that when the electrode potential was varied in the negative direction, from −0.12 to −0.6 V, there was not a reduction wave registered, thus indicating that neither hydroquinone nor other component of the system exhibited reduction processes. When the potential sweep was reverted to positive values, an oxidation peak was plainly observed at 0.27 V solely when hydroquinone was present in the solution, otherwise no oxidation was noted, see broken line in Fig. 1.

Fig. 2 shows the CV recorded in the system SPE/500 μM hydroquinone, 0.1 M acetate buffer (pH 4.7), at 0.1 V s^{-1} potential sweep rate. The potential scan was started at null current conditions toward the positive values, the potential sweep was reversed at 0.6 V and decreased until −0.6 V. An oxidation peak was observed during the anodic scan at 0.27 V due hydroquinone oxidation to p-quinone. During the cathodic branch a reduction wave, due to reduction of p-quinone to hydroquinone, was noted with a peak potential at −0.34 V. From these results the potential range: −0.25 to −0.35 V was chosen to be used during the amperometric determinations since it provides both, a good intensity of the signal recorded and minimal possible interference from some other redox processes, like oxygen electroreduction.

3.2. Amperometric response of the LTV-SPE biosensor

With the LTV-SPE immersed into the thermostated cell containing 10 mL of the 0.1 M acetate buffer at pH 4.7 and 30 °C under constant stirring, a potential of −0.30 V was applied and the current response was monitored. Once the steady state was reached, an aliquot of a standard hydroquinone solution was added and the current variations were recorded when stable. Polyphenols are oxidized by LTV to quinoid products whose electrochemical reduction provides a good intensity signal, useful for monitoring the enzyme action. LTV-SPE exhibits a stable signal that directly depends on the substrate concentration into the cell. The biosensor response is clearly instantaneous as indicated by the notable current increase that takes place just as when the injection is carried out, see Fig. 3. Thereafter, the step-wise response reaches a plateau within less than 30 s. Subsequently, the current value becomes quite stable, although it changes increasing slightly at 1 nA min^{-1} rate.

Fig. 4 shows the applied potential dependence of the LTV-SPE to hydroquinone 100 μM in 0.1 M acetate buffer at pH 4.7 and 30 °C. As expected the reduction current increases with the more negative values of potential.

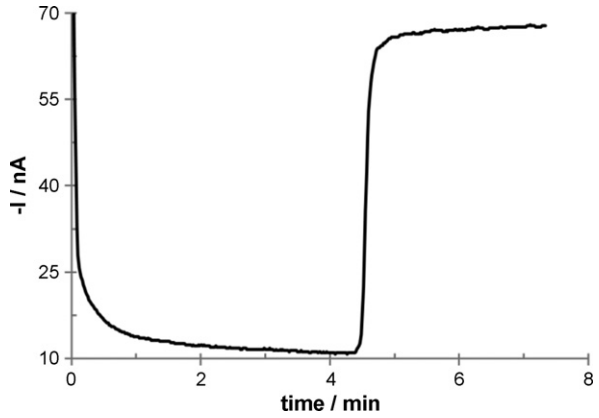

Fig. 3. Current–time curve recorded on LTV-SPE corresponding to the addition of one aliquot of hydroquinone solution.

3.3. Optimization of experimental conditions

Because the LTV-SPE biosensor performance depends not only on the applied potential, the effect of some other experimental variables as temperature and pH were also considered. The aim was to ensure that the amperometric response is as higher as possible avoiding interferences. A plot of the total current vs. the molar concentration of the substrate in the cell is named calibration plot, with a slope known as the sensitivity of the biosensor. Fig. 5 shows a LTV-SPE calibration curve using hydroquinone as a substrate in phosphate buffer 0.1 M pH 7.0, −0.30 V and 30 °C. The plot shows the interval where the current increases linearly as the substrate concentration does so: this analytical characteristic of the biosensor is known as linear range. The linear dependence was found to be approximately from 25 μM until 200 μM of hydroquinone with the slope in this range equal to 9.12 ± 0.05 nA μM^{-1}. A decrease in sensitivity was observed at a concentration greater than 200 μM, which may be due to the limited amount of LTV in the polymer matrix.

Optimization was carried out by comparing the sensitivity of the sensor evaluated under different experimental conditions. Using different temperatures, pH and applied potentials which produce eight different experiments, each one corresponding to every possible combination of values, namely, two per variable. This analysis was completed using hydroquinone within the concentration range from 25 μM to 125 μM, according to the calibration plot shown in Fig. 5.

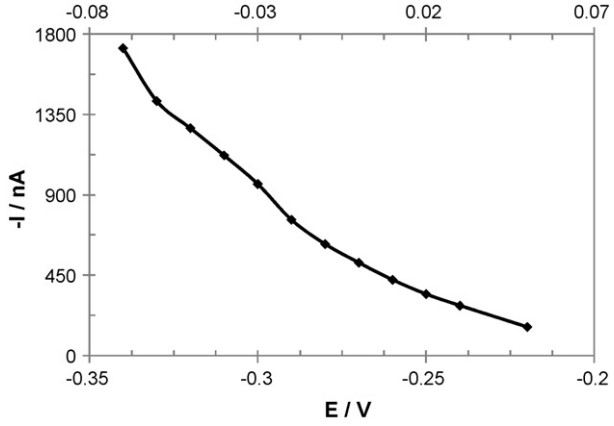

Fig. 4. Effect of applied potential on the LTV-SPE response to hydroquinone 100 μM in 0.1 M acetate buffer at pH 4.7 and 30 °C. In the second *X*-axis it is reported de potential values vs. a commercial Ag/AgCl/Cl$^-_{(aq)}$ 3 M electrode.

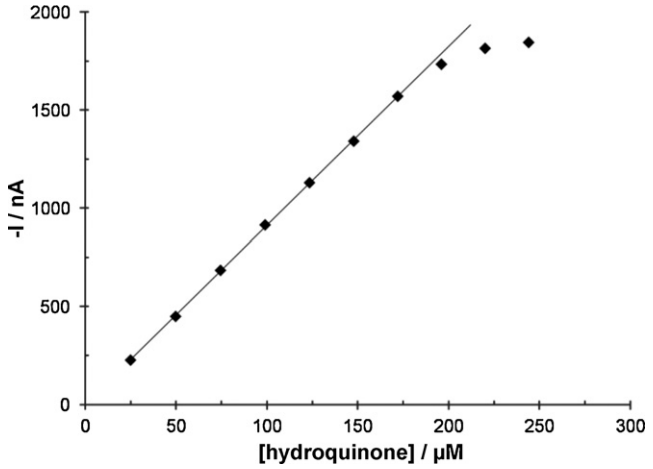

Fig. 5. Calibration plot for the LTV-SPE recorded with hydroquinone in 0.1 M acetate buffer at −0.30 V, pH 4.7 and 30 °C.

Table 1
Sensitivity variation of the LTV-SPE toward hydroquinone in 0.1 M acetate buffer with different factors namely: pH, applied potential and temperature.

Temperature (°C)	−0.27 V		−0.30 V	
	pH 4.7	pH 5.5	pH 4.7	pH 5.5
25	4.15 ± 0.03	2.21 ± 0.04	6.97 ± 0.02	4.52 ± 0.05
30	5.55 ± 0.02	3.28 ± 0.11	9.44 ± 0.07	6.61 ± 0.14

Sensitivity in nA μM^{-1}.

As can be seen in Table 1, the sensitivity of the biosensor varies considerably according to selected experimental conditions. Since an enzyme is involved, the pH value is definitely one of the parameters that provoke a strong effect on the performance of the sensor.

Fig. 6 shows the sensitivity of the LTV-SPE measured at four pH values, at −0.30 V and 30 °C. The highest sensitivity obtained was 9.44 nA μM^{-1} in 0.1 M acetate buffer at pH 4.7. According to the results obtained, these conditions were selected for other standard compounds and herbal infusion analysis.

Three diphenol isomers were analyzed under optimal experimental conditions, namely 0.1 M acetate buffer at pH 4.7, 30 °C and at −0.30 V. Fig. 7 shows the calibration plots at a low concentration range corresponding to hydroquinone, catechol, see Fig. 7a, and resorcinol, see Fig. 7b.

As can be seen in Fig. 7, there is a remarkable difference in the sensitivity among compounds. The highest slope corresponds to catechol 18.82 ± 0.76 nA μM^{-1} whereas the lowest one was

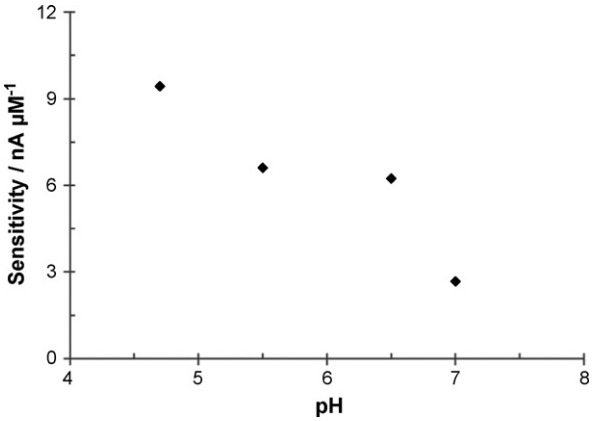

Fig. 6. pH dependence of LTV-SPE response toward hydroquinone at −0.30 V and 30 °C.

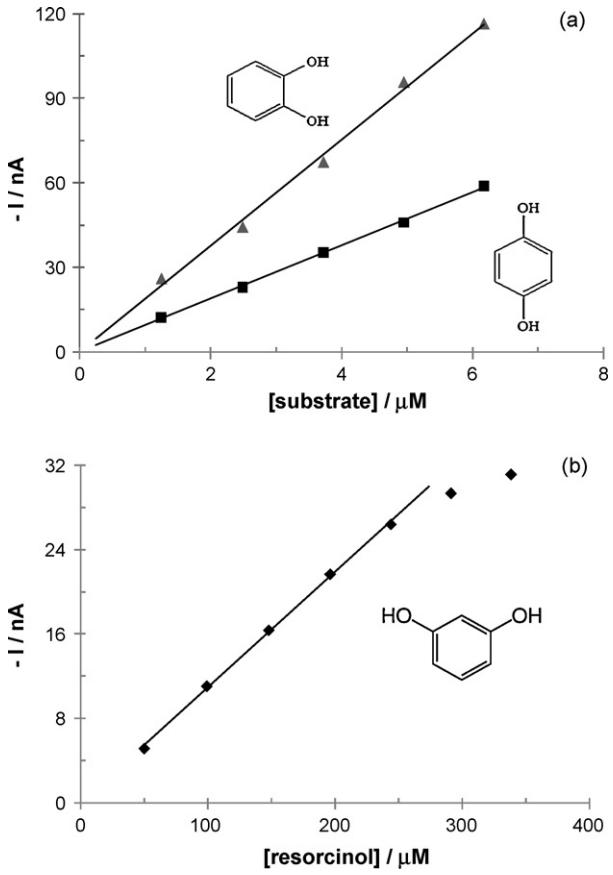

Fig. 7. Calibration plots of LTV-SPE for diphenol isomers in acetate buffer 0.1 M pH 4.7, 30 °C at −0.30 V. (a) catechol (▲) and hydroquinone (■) and (b) resorcinol.

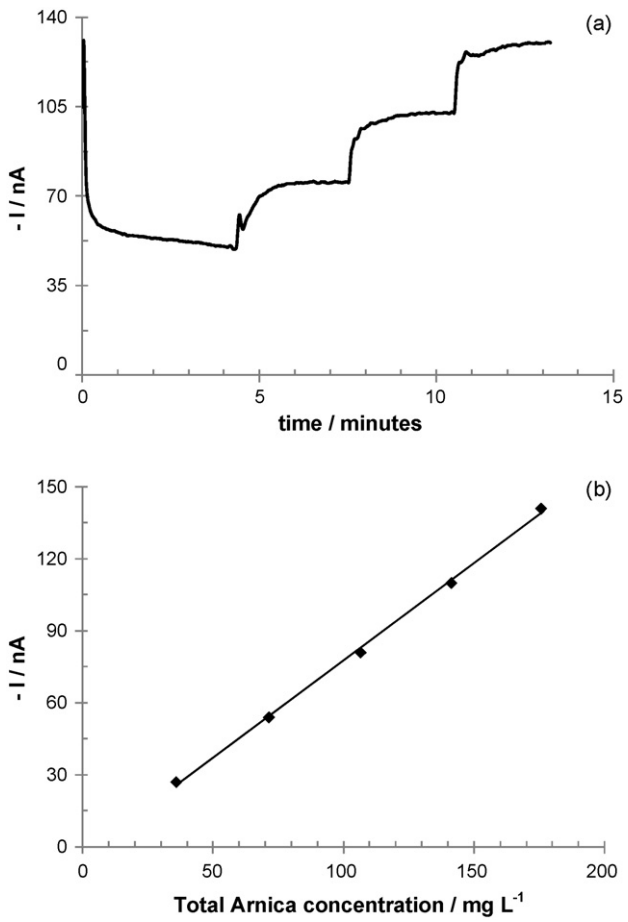

Fig. 8. Current–time plot for successive 50 μL additions of the arnica infusion 6.0 mg mL^{-1} to the electrochemical cell (a) and the overall current plotted as a function of arnica tea concentration in the cell (b).

obtained for resorcinol 0.110 ± 0.002 nA μM^{-1}. Table 2 gives the analytical parameters obtained from the calibration curve shown in Fig. 7 and that corresponding for caffeic acid as well. From these results it becomes clear that the LTV-SPE biosensor responds to different diphenols. In order to analyze possible interferences during the quantification of real samples, an equimolar mixture of catechol and hydroquinone was also tested. For this case a sensitivity of about 11 nA μM^{-1} was obtained, which means that the LTV-SPE can still be used in the presence of diphenol mixtures.

3.4. Real samples analysis

For practical purposes, the LTV-SPE was used for assessing the phenolic content in tea infusions. The analysis of different herbs was carried out by direct addition of small aliquots to the electrochemical cell containing 10 mL of acetate buffer solution under optimized experimental conditions of pH, temperature and applied potential. Fig. 8a shows the current–time response curve for successive additions of arnica infusion 6.0 mg mL^{-1}. As can be seen, the time response is considerably short, in the curve every step corresponds to a 50 μL aliquot injection. The overall current is plotted as a function of tea concentration in the cell to obtain the sensitivity of the biosensor toward the herb infusion see Fig. 8b which corresponds to 0.81 ± 0.02 nA/mgL^{-1}.

The overall phenolic content in infusions was determined according to the Folin–Ciocalteau spectrophotometric method, using caffeic acid as a standard. Briefly, appropriate aliquots of the samples (100 μL) were added to 500 μL of 0.2 N Folin–Ciocalteau reagent (2 N, diluted tenfold). After 5 min, 400 μL of sodium carbonate 75 mg/mL was added. The mixtures were incubated for 2 h and the absorbance of the resulting blue color was measured at 766 nm using an UV–vis spectrophotometer. Quantification was carried out on the basis of the caffeic acid standard plot, and the results were expressed as mg of caffeic acid per liter of tea sample.

The results obtained using both methods for six common herbs are summarized in Table 3, with triplicate measurements. These results are the evaluation of the practical usefulness of our LTV-

Table 2
Analytical parameters corresponding to phenolic compounds calibration curves.

	Sensitivity (nA μM^{-1})	R^2	Detection limit (μM)	Linear range (μM)
Caffeic acid	24.91 ± 0.42	0.9991	0.524	0.5–130
Catechol	18.83 ± 0.76	0.9951	0.558	0.5–175
Hydroquinone	9.44 ± 0.19	0.9988	1.071	1.1–130
Resorcinol	0.110 ± 0.002	0.9989	35.432	50–250

Table 3
EPC of tea infusions determined by the amperometric method proposed in this work ($EPC_{LTV\text{-}SPE}$) and spectrophotometric methods (EPC_{FCR}).

Herb	$EPC_{LTV\text{-}SPE}$	EPC_{FCR}
Arnica	47.0	120.1
Basil	5.8	205.7
Orange leaves	4.0	142.5
Fennel	25.3	162.8
Mint	37.6	171.0
Hibiscus	35.4	188.4
Palo azul	109.2	297.5

Results expressed in mg of caffeic acid per liter of tea sample.

Table 4
Some analytical characteristic of polyphenol oxidases base-biosensor.

Enzyme	Electrode	Immobilization method	Substrate	Sensitivity (nA μM^{-1})	LOD (μM)	Reference
Tyr	Sonogel	Nafion	Hydroquinone	2.6	3.5	[73]
Lac	Carbon-fiber electrodes	Crosslinking with Carbodiimide-glutaraldehide 10%	Catechol	16.1	–	[41]
Lac	Graphite screen-printed electrodes modified with ferrocene	Sol–gel matrix of diglycerysilane (DGS)	Catechol	3.0	10	[74]
			Caffeic acid	6.2	6	
Lac/Tyr			Caffeic acid	1.2	24	
Tyr			Caffeic acid	0.8	78	
Lac	Ferrocene-modified screen-printed graphite	Crosslinking with glutaraldehide (2.5%)	Caffeic acid	21.6	1.6	[42]
Tyr	Glassy carbon modified with electrodeposited gold nanoparticles	Crosslinking with glutaraldehide (25%)	Catechol	107	1.5	[65]
			Caffeic acid	14	6.6	
Lac	Screen-printed graphite electrodes	Entrapment in polyvinyl alcohol film	Catechol	18.8	0.5	This work
			Caffeic acid	24.9	0.5	
			Hydroquinone	9.4	1.1	

SPE for application on the determination of phenols contents in real tea samples. Similar results have been obtained by Carralero Sanz et al. [65], for the determination of the "pool" of polyphenols in wines using a tyrosinase based biosensor, by Chang et al. [71], during the estimation of total phenols in real water samples using a horseradish peroxidase modified screen-printed carbon electrode and by ElKaoutit et al. [72], during determination of polyphenols in real beer samples using a phenoloxidase (laccase, tyrosinase and Horseradish peroxidase) – Sonogel – Carbon based biosensors.

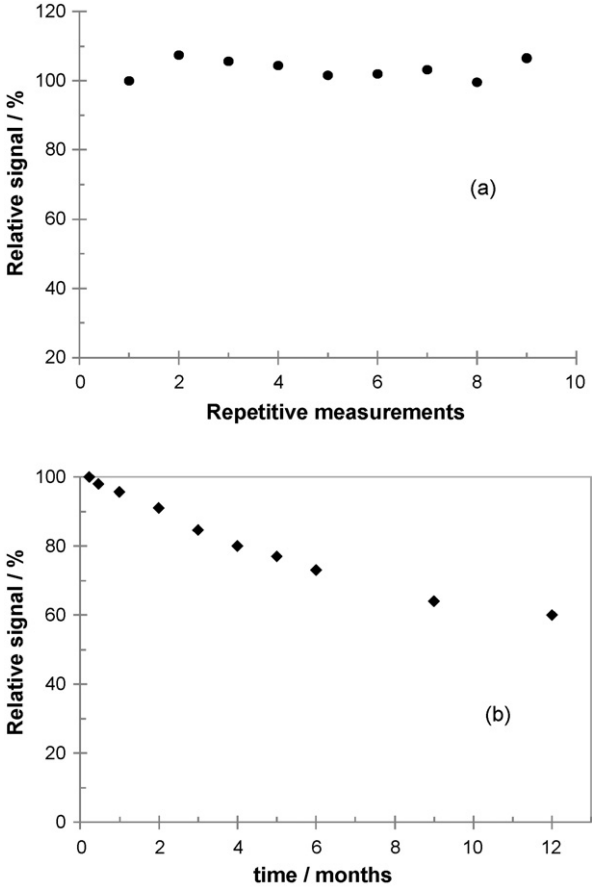

Fig. 9. (a) Amperometric response repeatability of a single LTV-SPE toward catechol quantification, in a aqueous solution containing 50 catechol μM in 0.1 M acetate buffer, pH 4.7 and 30 °C, applying −0.30 V. RSD = 2.73. (b) Stability measured as a plot of the relative signal as a function of elapsed time under dry buffer-less 4 °C storage conditions.

These authors also found that the EPC of the wines samples [65], real water samples [71] and beers samples [72] was much lower using their respective biosensor than when the FCR method was used. Such a difference can be ascribed to the different oxidizing agent, namely, an enzyme (biosensor) or a chemical compound (FC method), used in each case. While the enzyme specifically oxidized diphenolic compounds, in particular laccase oxidize diphenolic compounds in positions ortho, meta and para, the FC reagent can oxidize not only phenolic compounds but any other reduced species in the sample, namely aromatic amines, sulfur dioxide, ascorbic acid, Cu(I) and Fe(II). Therefore, the results obtained by the FC method in real samples cannot be directly associated with the overall phenols contents.

3.5. LTV-SPE stability

Both operational and storage stabilities corresponding to our LTV-SPE are depicted in Fig. 9. Straightforward, these results indicate that a full year passed, under dry buffer-less 4 °C storage, and the electrode still maintained up to 60% of its relative signal.

From Table 4 it is possible to note that our proposed biosensor exhibits superior analytical features for the quantification of most of the typical diphenol compounds as compared with others already published in the literature. Beside this, our sensor has proved to possess a large enough lifetime, it is disposable, does not require an external reference electrode and is suitable for teas real sample analysis.

4. Conclusions

The LTV-SPE constructed by enzymatic immobilization exhibited a good analytical performance for diphenol quantification. A laccase biosensor was developed for the determination of the phenolic content, the results obtained show that LTV immobilization on SPE using PVA-AWP was found to be efficient. The suggested immobilization process provided a useful matrix able to retain the enzyme and also allowed diffusion of the species involved in the enzyme reaction through the polymer film. The experimental conditions have an important influence on the biosensor performance and sensitivity. The biosensor developed showed an excellent stability and exhibited good performance in terms of response time, sensitivity, operational stability, manufacturing process simplicity and can be used for accurate determination of the phenolic content without any pretreatment of the sample.

Simple manufacture procedure and minimal extraction process are combined in these bioelectrodes for a fast and reliable monitoring of the phenolic content in tea infusions.

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