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Disposable biosensor based on platinum nanoparticles-reduced graphene oxide-laccase biocomposite for the determination of total polyphenolic content

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ABSTRACT

A disposable amperometric biosensor was developed for the detection of total polyphenolic compounds from tea infusions. The biosensor was designed by modifying the surface of a carbon screen-printed electrode with platinum nanoparticles and reduced graphene oxide, followed by the laccase drop-casting and stabilization in neutralised 1% Nafion solution. The obtained biosensor was investigated by scanning electron microscopy and electrochemical techniques. It was observed that platinum nanoparticles-reduced graphene oxide composite had synergistic effects on the electron transfer and increased the electroactive surface area of the carbon screen-printed electrode. The constructed analytical tool showed a good linearity in the range 0.2–2 μM for caffeic acid and a limit of detection of 0.09 μM. The value of Michaelis–Menten apparent constant was calculated from the electrochemical version of Lineweaver–Burk equation to be 2.75 μM. This disposable laccase biosensor could be a valuable tool for the estimation of total polyphenolic content from tea infusions.

1. Introduction

Polyphenols are natural compounds widely found in vegetables [1,2], cereals, tea leaves [3,4], fruit juices [5] and red wines [6]. Tea contains a variety of polyphenolic compounds (phenolic acids, flavonoids, tannins, catechins, etc.) having a wide range of biological activities that contribute in cancer prevention [7], anti-aging, exhibits protective effect against cardiovascular diseases [8] and brain protection against oxidative stress [9]. Fruit tea infusions have a high content of caffeic acid and therefore can show strong antioxidant properties [10].

There are many analytical methods currently involved in the determination of polyphenols from different sources: UV–vis spectrophotometry [11], chromatography [12–14], capillary electrophoresis [15], electrochemistry [16]. Two standard methods frequently used for the determination of total polyphenolic content from tea are Folin–Ciocalteu colorimetric method [17,18] and high performance liquid chromatography with appropriate detection (HPLC) [19]. Spectrophotometric methods sometimes lack in sensitivity and specificity (e.g. Folin–Ciocalteu), while chromatographic methods are laborious, require skilled personnel and high amounts of solvents, reagents and resources. Considering tea extracts, the major drawback of Folin–Ciocalteu method is the detection of other interfering compounds (i.e. ascorbic acid, several amino acids, carbohydrates, etc.) besides polyphenolic compounds. To overcome the drawbacks of the previously mentioned methods, biosensors represent a valuable alternative for the determination of polyphenolic content. There are several amperometric biosensors reported in the literature for the analysis of polyphenols from tea. The first enzyme-based biosensor used for the determination of polyphenols was developed by Ghindilis et al. [20], the biological recognition element being laccase. Laccase [21–23] is rather used than tyrosinase [24,25] in developing biosensors for polyphenolic content determination, due to its wider catalytic effect against polyphenols (it catalyses both *ortho* and *para* di- and polyphenols). To provide resolution and a better quantification of each compound from a phenolic or polyphenolic mixture, two groups of researchers developed independently two analytical tools, one based on a dual amperometric approach (laccase- and tyrosinase-based biosensors) for rapid evaluation of mixtures of phenolic compounds [26] and the second system was based on an array of enzymatic biosensors (tyrosinase and laccase) for the determination of major polyphenols found in wine [27].

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According to the International Union of Pure and Applied Chemistry (IUPAC), a chemically modified electrode is an electrode made of a conducting or semiconducting material that is coated with a selected monomolecular, multimolecular, ionic or polymeric film of a chemical modifier and that by means of faradaic (charge-transfer) reactions or interfacial potential differences exhibits better chemical, electrochemical and/or optical properties of the film [28]. The electrode modifier must have specific solubility characteristics, meaning no, or low solubility in the reaction mixture and no electroactivity in the used potential window [29]. New electrode modifiers, such as graphene, graphene oxide, reduced graphene oxide, etc., have been used in biosensors construction mainly due to the fact that they are easy to be obtained with a lower cost than carbon nanotubes. Graphenes have unique properties that make them extremely useful in the biosensors field [30,31]: high surface area, high electrical conductivity and good mechanical and thermal properties [32]. The modification of the working electrode with graphene increases the active surface area of the electrode thus increasing the number of active sites and facilitates the electron transfer between the electrode surface (due to graphene small band gap) and biomolecules [33]. Graphene has been used in developing biosensors for glucose [34], detection of pesticides [35] or detection of hydrogen peroxide [36]. Reduced graphene oxide is a better candidate for enzyme immobilization as it lacks in surface functional groups less prone to aggressively interact with the enzyme, and has more structural defects than graphene and graphene oxides thus improving the electron transfer rate and the enzyme loading [37].

The use of metallic nanoparticles in the biosensing field aims the improvement of analytical characteristics of the biosensors, namely the sensitivity and the response time [38]. Graphene nanocomposites with metallic nanoparticles (i.e. platinum, gold and silver) present synergetic effects and are usually obtained by the deposition of metallic nanoparticles onto graphenes [35,39]. These newly formed structures of graphene, beside the already mentioned properties, can reduce the over potential of the electrochemical reactions.

In this work we report the construction of a rapid, simple and highly sensitive laccase-based amperometric biosensor for the determination of total polyphenolic content (TPC) from tea infusions. The surface of a carbon based ink screen printed electrode was modified with Pt-nanoparticles, followed by a second layer of reduced graphene oxide (RGO) and finally the enzyme was immobilised by adsorption on the surface of the nanocomposites and stabilised in a 1% (v/v) Nafion membrane. Each step involved in the development of the biosensor was characterised both by scanning electron microscopy (SEM) and electrochemical methods. The developed biosensor proved unique and enhanced properties that make it an ideal analytical tool for TPC determination. In order to confirm the device feasibility, the results obtained, using the laccase based biosensor, were compared with the results obtained by the classical Folin–Ciocalteu method.

2. Experimental details

2.1. Reagents

Laccase from *Trametes versicolor* (EC 1.10.3.2, activity 1.34 U/mg according to the supplier) was provided by Sigma-Aldrich, Steinheim, Germany, and its activity was checked spectrometrically, before each batch of biosensors was fabricated, using the unspecific substrate, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), also supplied by Sigma-Aldrich. Caffeic acid, Nafion perfluorinated ion-exchange resin, 10 wt% dispersion in water, sodium acetate, acetic acid and potassium chloride, sodium carbonate, Folin–Ciocalteu reagent were purchased from Sigma-Aldrich. 0.1 M acetate buffer (pH 5.50) in 0.1 M KCl was chosen as buffer solution and electrolyte. Graphite flakes (SP1) were from Bay Carbon, Inc., USA. All reagents were of analytical grade and all the aqueous solutions were prepared with Milli Q ultra-pure water.

2.2. Tea infusions preparation

The tea infusions were prepared by weighing 2 g of tea and adding 100 mL of hot water (80 °C) allowing the extraction of active principles for 20 min. Then the infusion was diluted as further described and immediately used to assess the total polyphenolic content.

2.3. Apparatus

For electrochemical determinations a PG580 Uniscan potentiostat (Uniscan Instruments Ltd., Buxton, UK) was used, the electrochemical cell being DropSens 110 C-working electrode carbon screen-printed (WE: C-SPE) (DropSens, Spain). The UV–vis spectrophotometric measurements were carried out on a Thermo Evolution 260 Bio (Thermo Fischer Scientific). The micrographs were obtained using a Nova NanoSEM 630 (FEI Company, USA) Scanning Electron Microscope (SEM).

2.4. Preparation of modified electrode

Platinum nanoparticles (Pt-NPs) were obtained accordingly to Brăgaru et al. [40]. Briefly, to 10 mL of H_2PtCl_6 solution (10 mM) – prepared in distilled water – were added 10 mL of freshly prepared $NaBH_4$ (25 mM) solution. The reaction was allowed to proceed under stirring, at room temperature, for 3 h.

Graphene oxide (GO) was prepared accordingly to Hummers method [41]. Reduced graphene oxide (RGO) was obtained by chemically reducing with hydrazine the graphene oxide powder accordingly to Park et al. [42]. Graphene oxide (30 mg) was dispersed by ultrasounds in 45 mL of distilled water during 4 h, and hydrazine monohydrate (10 μL) was subsequently added to the dispersion. The chemical reaction was allowed to proceed under stirring and heating (80 °C), overnight. The reaction product was washed with distilled water on a sintered glass filter (medium pore size), and dried under vacuum, at 80 °C, during 48 h.

The surface of the DropSens carbon working electrode (C-SPE) was modified by deposition in a first step of 5 μL platinum nanoparticles (Pt-NPs) and then of 5 μL of RGO (2 mg/mL), the electrode being named C-SPE/Pt-NPs/RGO. Between each deposition step, the electrodes were allowed to dry at 20 °C. As control Pt-NPs modified electrodes (C-SPE/Pt-NPs) and RGO (2 mg/mL) (C-SPE/RGO) modified electrodes were prepared.

2.5. Biosensor construction

The modified electrodes were coated with 5 μL of laccase solution, ensuring an enzyme activity onto the working electrode of 300 mIU, and dried at 20 °C. The biosensor stabilisation was finally achieved by casting 5 μL of 1% neutralised Nafion. The disposable biosensors were stored at 4 °C, on silica gel layer. The obtained bio-electrodes were named C-SPE/Pt-NPs/RGO/lacc/Nafion.

2.6. Electrochemical methods

All the cyclic voltammetry and amperometric measurements were performed at room temperature (24 ± 0.5 °C) in drop mode, the electrochemical cell containing 0.1 M acetate buffer (pH 5.50). In order to characterise the modified electrode redox properties cyclic voltammetry measurements were performed in 1 mM $K_3[Fe(CN)_6]$. The amperometric measurements were carried out at optimum applied potential value of −100 mV vs. Ag/AgCl in order to assess the total polyphenolic content (TPC) from tea infusions; the TPC was finally expressed as molar equivalents of caffeic acid.

2.7. Spectrophotometric method

In order to compare the results, the tea infusions were also analysed by Folin–Ciocalteu spectrophotometric method. The Folin–Ciocalteu reagent was diluted 10 times with MilliQ water and 0.5 mL from this solution were taken and added to 0.1 mL sample (tea infusions were diluted 1:5 *v/v* with MilliQ water). After 8 min 0.4 mL sodium carbonate 7.8% were added to the reaction mixture and the solutions were allowed to react for 1 h in dark, at room temperature. The absorbance was then read at 760 nm. The same protocol was followed for the calibration curve with standard solutions of caffeic acid. The total polyphenolic content was evaluated from the absorbance value interpolated into the calibration curve obtained for caffeic acid and the results were expressed as molar equivalents of caffeic acid.

3. Results and discussion

3.1. Electrode characterisation

3.1.1. SEM characterization

Scanning electron microscopy (SEM) indicated that Pt-NPs size was around 100 nm (Fig. 1 (B)), while the RGO layer was formed from planar sheets of C (Fig. 1 (C)). When the biocomposite was present over the surface of the electrode a porous three-dimensional surface appeared (Fig. 1 (D)).

3.1.2. Electrochemical characterization

In order to investigate the redox electrochemical properties of the modified electrodes, cyclic voltammetry experiments were performed in 1 mM $[Fe(CN)_6]^{3-/4-}$ solution. In the case of C-SPE/RGO and C-SPE/Pt-NPs modified electrodes it was noticed that the peak current intensities corresponding to the solubilised redox probe reduction, respectively oxidation, increased, meaning that a fast electron transfer occurred between bulk solution and electrode surface. In the same time it was noticed that in the case of C-SPE/Pt-NPs modified electrode the peak potentials values have also changed, the separation between reduction and oxidation peak potential values being improved ($\Delta E = 25$ mV for modified electrode with respect to $\Delta E = 95$ mV), demonstrating that the use of Pt-NPs enhanced the redox reversible character (see Fig. 2). Moreover, the reduction in peak potential separation indicates a faster electron transfer between the electrode surface and solution mainly due to the increase in the surface to volume ratio (high surface area provided by the Pt-NPs). Another observed aspect related to the use of Pt-NPs was the large shift of the both waves towards more negative potentials, suggesting that the surface modified with Pt-NPs efficiently catalyse the redox reaction. This behaviour is in accordance to Kumar and Zou findings about nanoelectrodes ensembles where (i) increasing in the particle coverage on the electrode surface and (ii) high catalytic activity of the nanoparticles may shift both anodic and cathodic waves to less positive potentials [43].

The same experiments were performed for C-SPE/Pt-NPs/RGO modified electrode, C-SPE/RGO modified electrode and C-SPE/Pt-NPs (see Fig. 3). It was noticed that the current intensity of the peaks obtained when C-SPE/Pt-NPs/RGO electrode was used was higher than that registered for the other two modified electrodes, while the peak potential values corresponding to $[Fe(CN)_6]^{3-/4-}$ reduction, respectively oxidation are closer to those of C-SPE/Pt-NPs. The higher value of peak current intensity for C-SPE/Pt-NPs/

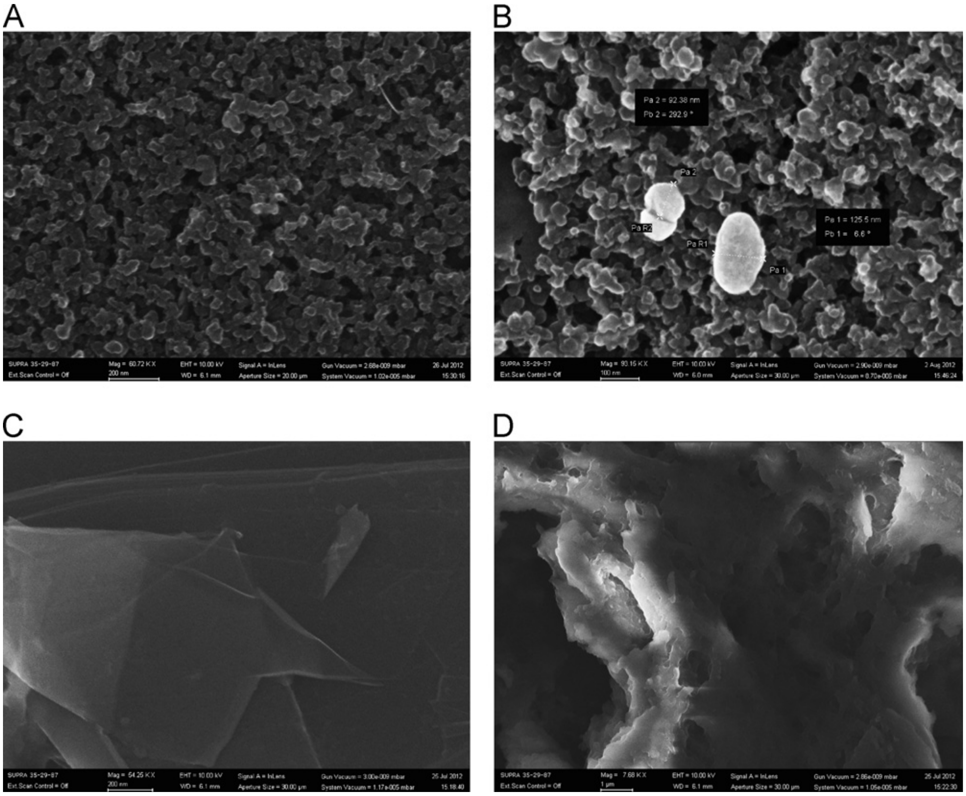

Fig. 1. SEM images of: (A) bare C-SPE, (B) C-SPE/Pt-NPs, (C) C-SPE/RGO and (D) C-SPE/Pt-NPs/RGO/lacc/Nafion.

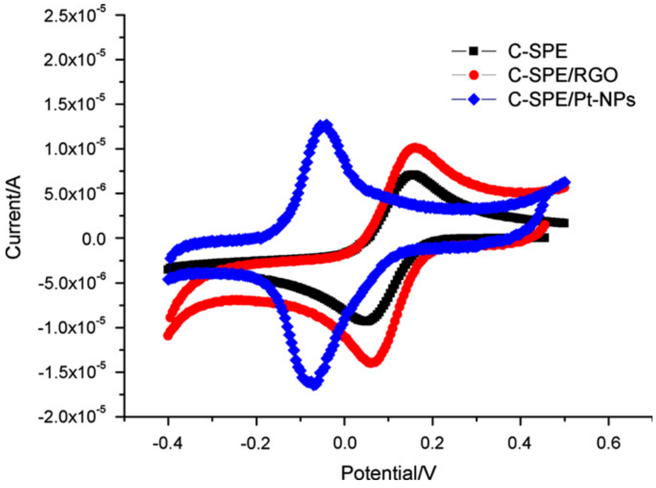

Fig. 2. Cyclic voltammograms of 1 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl on C-SPE-bare, C-SPE/RGO and C-SPE/Pt-NPs; scan rate of 50 mV/s.

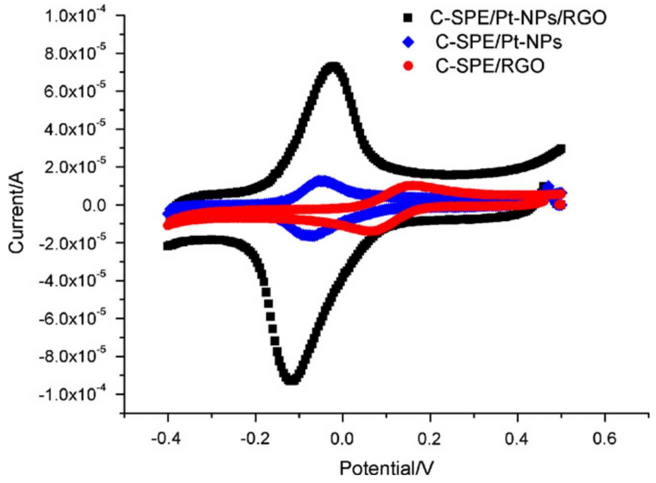

Fig. 3. Cyclic voltammograms of 1 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl on C-SPE/Pt-NPs/RGO, C-SPE/Pt-NPs and C-SPE/RGO; scan rate of 50 mV/s.

RGO observed in Fig. 3 could be ascribed to the synergistic effects of RGO and Pt-NPs, which play an important role in facilitating the electron transfer.

3.2. Optimization of the experimental parameters of the biosensor

The detection principle of caffeic acid using a laccase biosensor is depicted in Fig. 4.

Considering the operational parameters of an amperometric biosensor, the following parameters: applied potential, pH and enzyme loading were optimised. The results are presented in Fig. 5.

Considering our group previous experience on polyphenol oxidases based biosensors development [44,45], in order to optimise all the afore mentioned analytical parameters, the amperometric measurements were carried out for a concentration of 0.6 μM caffeic acid. The applied potential was investigated from −200 mV up to +50 mV and the results are presented in Fig. 5 (A). As it can be seen the highest sensitivity towards caffeic acid was obtained for an applied potential of −100 mV vs. Ag/AgCl. Consequently this potential value was used for further amperometric experiments.

The effect of pH on the amperometric response was also tested in the pH range 3.76–5.50 (the acetate pH range considering its pKa of 4.75), for the same concentration of caffeic acid, the applied potential being −100 mV vs. Ag/AgCl. It was noticed that the current intensity increases with the increase of pH value up to 5.00 and then it decreases as the pH is further increased (Fig. 5 (B)). Therefore a value of pH of 5.00 was chosen for further experiments, the value being in agreement with the optimum pH of laccase from *T. versicolor* [46].

Another factor that influences the amperometric response is the enzyme loading. The current intensity increased with the increase in the laccase activity and reached a maximum for an enzyme activity of 300 mUI (see Fig. 5 (C)). Therefore, 300 mUI were chosen as the optimal enzyme loading.

Consequently, the optimal conditions for further experiments were 5.00 for pH, −100 mV vs. Ag/AgCl for applied potential and 300 mUI for laccase loading.

3.3. Electrode response characteristics

The response characteristics of the developed disposable biosensor were assessed using the optimal operational parameters settled previously. The analytical performance characteristics of the amperometric biosensor with respect to caffeic acid as substrate for enzymatic reaction are given in the Table 1.

As it can be observed from Table 1, the sensitivity of the biosensor calculated as the slope of the linear calibration curve was approximately 2147 nA/μM, value extremely higher with respect to other laccase-based biosensors (see Table 2), proving that the constructed biosensor has enhanced sensitivity. Another improved characteristic is the affinity of the enzyme toward substrate, since the apparent Michaelis–Menten constant calculated from the electrochemical version of Lineweaver–Burk equation, 2.75 μM, is better than other reported values.

To rationalise on the versatility and potential applicability of the developed biosensor, other polyphenolic compounds frequently found in fruit tea infusions were analysed: rosmarinic acid, gallic acid and luteolin. Determinations were done both for standard compounds and for mixture, to figure out on the biosensor capabilities to provide an accurate analytical information, free of interferences. The chronoamperometric measurements were performed under the optimal experimental conditions, namely 0.1 M acetate buffer containing 0.1 M KCl at pH 5.00 and applied potential −100 mV vs. Ag/AgCl. The analytical parameters obtained from the calibration curves of the three compounds are given in Table 2, linearity range of the response fitting with the usually expected polyphenols concentrations in tea products. The limit of detection (LoD) was calculated as 3.3 × intercept standard error/sensitivity.

From Table 2 it is obvious that the developed C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor sensitively responds for different polyphenolic compounds. The highest sensitivity was obtained for caffeic acid, the compound used for setting the experimental conditions. Good results were also obtained for gallic acid and luteolin.

In order to better understand and apply the developed biosensor for real sample analysis (tea infusions), an equimolar mixture containing the four polyphenolic compounds was tested using the C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor. A sensitivity of 848.93 nA/μM was obtained in the case of the equimolar mixture which means that the C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor can be used in the presence of more polyphenols, but it is in some extent decreased in the presence of gallic acid which is reported to act as an inhibitor of laccase from *T. versicolor* [47].

The analytical performances of the developed biosensor were compared with other reported biosensors and the results are

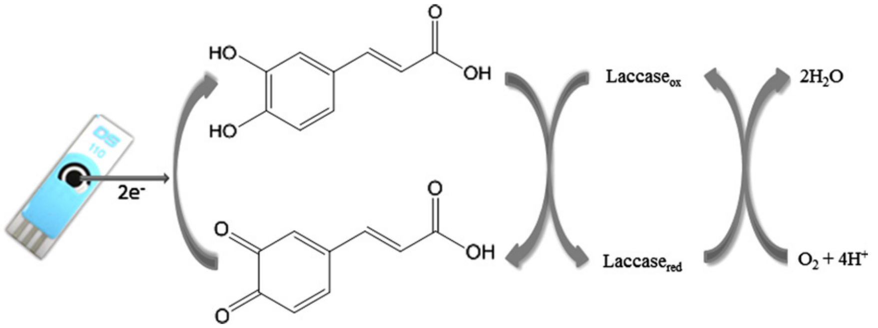

Fig. 4. Schematic representation of the reaction involving caffeic acid and laccase immobilised onto the C-SPE/Pt-NPs/RGO/lacc/Nafion.

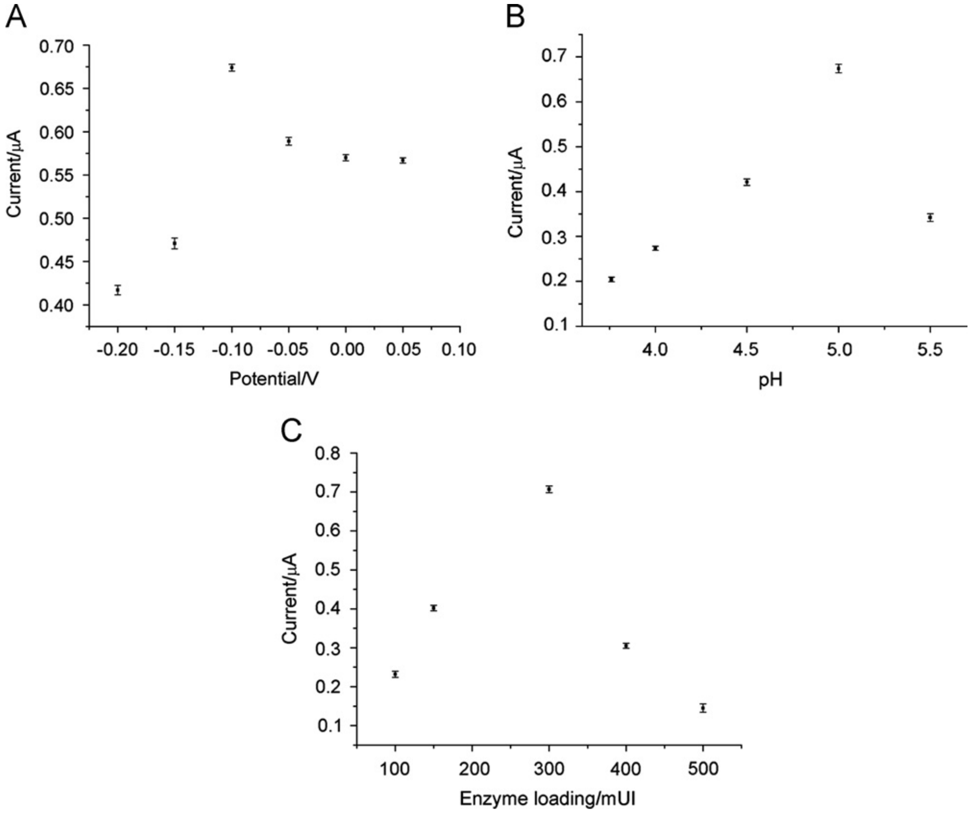

Fig. 5. (A) Effect of the applied working potential on the amperometric response of 0.6 µM caffeic acid at C-SPE/Pt-NPs/RGO/lacc/Nafion in 0.1 M acetate buffer (pH 5.00); (B) effect of pH on the amperometric response of 0.6 µM caffeic acid in 0.1 M acetate buffer, the applied potential: −100 mV vs. Ag/AgCl; (C) effect of enzyme loading on the amperometric response of 0.6 µM caffeic acid at C-SPE/Pt-NPs/RGO/lacc/Nafion in 0.1 M acetate buffer (pH 5.00).

Table 1
The response characteristics of the amperometric C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor.

Equation of calibration curve	Y=(2147.38 ± 65.40)X=(608.89 ± 60.76) Y=nA; X=[caffeic acid] (µM)
R^2	0.9972
Linear range	0.2–2 µM
Response time	60 s (time required to reach 95% of the maximum steady-state current)
K_m^{app}	2.75 µM
LoD	0.09 µM (calculated as 3.3 × intercept standard error/sensitivity)

shown in Table 3. It can be noticed that the performance characteristics of the C-SPE/Pt-NPs/RGO/lacc/Nafion are better than those reported in the literature and therefore it represents an improved analytical tool for the determination of total polyphenolic content from different samples.

3.4. Stability and reproducibility of the biosensor

The storage stability of the developed biosensor was investigated by measuring the current response during storage in the aforementioned conditions. The biosensor maintained the initial response (current intensity) during the first two weeks, while after six weeks a remnant response of 87.4% from its initial value was obtained. The repeatability of the measurements was checked using a concentration of 0.6 μM caffeic acid, on seven different electrodes, the RSD being of 5.7%.

3.5. Real samples analysis

The C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor was used for the estimation of total polyphenolic content from tea infusions as this content may be correlated to the potential antioxidant capacity of these infusions.

Four tea infusions were analysed, the content of polyphenolic compounds being expressed as molar equivalents of caffeic acid. The results obtained with the C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor for four tea infusions provided by local suppliers are summarised in the Table 4 and the results are compared to the results obtained by the Folin–Ciocalteu spectrophotometric method.

It is obvious from Table 4, a significant difference between the TPC obtained by the two methods occurs. This difference is a consequence of the specificity of the two analytical methods used. The higher results obtained by Folin–Ciocalteu method are due to the high content in anthocyanins present in forest fruits and exotic fruits infusions since the reduction of phosphomolybdate ion from Folin–Ciocalteu reagent is a nonspecific reaction for polyphenols and, consequently, the method is affected by chemical interferences from the other compounds present in the matrix [23]. Another apparent controversy in the obtained results might be ascribed to the presence of gallic acid, which interferes in the polyphenolic compounds determination by the amperometric biosensor, gallic acid inhibiting laccase from *T. versicolor* [47]. The big differences in results can be ascertain to the oxidizing agent used in each reaction, laccase or Folin–Ciocalteu reagent, as laccase catalyses the oxidation of *ortho* and *para* polyphenolic compounds, while Folin–Ciocalteu reagent oxidises all polyphenolic compounds as well as many other reduced species found in the sample.

Table 2
Comparison of sensitivities, correlation coefficient (R^2), limit of detection (LoD) and linear range values for different polyphenolic compounds obtained with C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor.

Compound	Sensitivity (nA/μM)	R^2	LoD (μM)	Linear range (μM)
Caffeic acid	2147.38	0.9972	0.09	0.2–2.5
Rosmarinic acid	99.78	0.9881	0.15	0.45–2.5
Gallic acid	138.85	0.9964	0.15	0.25–2.5
Luteolin	130.98	0.9964	0.12	0.25–3.0

The results obtained by the laccase based biosensor were plotted against those obtained by Folin–Ciocalteu method and fitted with a $R^2 = 0.9915$, $p < 0.05$ (Fig. 6).

4. Conclusions

A C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor with an improved sensitivity 2147 nA/μM, and a very good detection limit 0.09 μM was developed and applied for the estimation of total polyphenolic content of tea infusions. The combination of reduced graphene oxide and platinum nanoparticles leads to a synergistic effect, resulting in increased electroactive surface area of the

Table 4
Estimation of total polyphenolic content in tea infusions by using the C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor.

Tea infusion	Biosensor (caffeic acid, μM)	Folin–Ciocalteu method (caffeic acid, μM)
Blueberry	57.2	973.6
Lime	49.0	695.7
Forest fruits	83.5	2427.1
Exotic fruits	87.4	2732.2

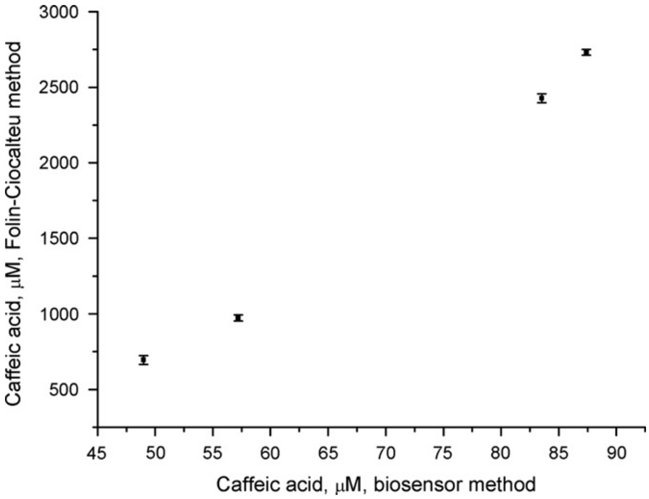

Fig. 6. Correlation between the results obtained for the estimation of the total polyphenolic content in tea infusions by using the C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor and the Folin–Ciocalteau method.

Table 3
Analytical characteristics of several biosensors for the determination of caffeic acid.

Biosensor	E_{appl}(mV)	Sample matrix	Linear range (μM)	Sensitivity (nA/μM)	K_m^{app} (μM)	LoD (μM)	Storage Stability	Ref.
Tyr–nAu-GCE	−100	Wine	2–200	14	220	66	18 days	[48]
Lacc–PVA–AWP	−300	Tea infusions	0.5–130	24.91	–	0.524	4 months	[24]
Lacc-polyethersulphone	−50	Red wine	10–80	24	–	1	–	[49]
Lacc–oxygen electrode	+280	Human plasma	0.1–1	239	–	0.06	–	[50]
Lacc–Fc-C-SPEs	+280	Human plasma	2–30	110	–	1.6	–	
Lacc–graphite	−50	–	1–10	57.92	27.4	0.56	–	[51]
Tyr-AA-LuPc2	−0.5–+0.5	–	10–400	93	62.31	1.98	–	[52]
C-SPE/Pt-NPs/RGO/lacc/Nafion	−100	Tea infusions	0.2–2	2147	2.75	0.09	6 weeks	This work

Tyr: tyrosinase; nAu: gold nanoparticles; GCE: glassy carbon electrode; Lacc: laccase; PVA-AWP: poly(vinyl alcohol) azide-unit pendant water-soluble photopolymer; Fc-C-SPEs: carbon screen-printed electrode modified with ferrocene; Tyr-AA-LuPc2: tyrosinase incorporated in Langmuir films of arachidic acid and bisphtalocyanine.

carbon screen-printed electrode and a better electron transfer towards the electrode. The constructed disposable biosensor showed a Michaelis–Menten constant of 2.75 μM indicating an improved affinity of immobilised laccase toward caffeic acid.

The results obtained for the analysed tea infusions allowed us to conclude that the C-SPE/Pt-NPs/RGO/lacc/Nafion biosensor developed in this paper represent a good analytical tool for the estimation of total polyphenolic content from tea infusions.

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