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Ultrasensitive Amperometric Biosensing of Polyphenols Using Horseradish Peroxidase Immobilized in a Laponite/Au/DNA-Bioinspired Polycation Nanocomposite

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An ultrasensitive electrochemical biosensor for the determination of hydroquinone (HQ) and chlorogenic acid (CGA) has been prepared by horseradish peroxidase (HRP) immobilization onto nanohydrogels made of laponite, gold nanoparticles (AuNP) and a vinylbenzyltriethylammonium polycation copolymerized with vinylbenzylthymine groups. The structure and active site of the enzyme were not modified upon immobilization, as determined by UV-Vis and FTIR spectroscopies. The biosensor showed remarkable electroanalytical properties for detection of HQ and CGA, e.g. linear stationary current up to 120 μM and 4.2 μM, limit of detection (LOD) of 1.6 ± 0.2 nM and 2.7 ± 0.1 nM for CGA, and sensitivities of 218 ± 4 $\mu A.mM^{-1}$ and 132 ± 4 $\mu A.mM^{-1}$, respectively. The electroanalytical capabilities of the biosensor was successfully tested in the quantification of the total polyphenol content in green coffee and yerba mate beverages, yielding equivalent results than those obtained with the classical Folin-Ciocalteu method. Nonetheless, our biosensor showed remarkable advantages due to its ultra-sensitivity, together with smaller sample volumes and shorter detection times required, improving its analytical application.
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Edible polyphenols are widely spread in the vegetal kingdom, including fresh foods and beverages,[1] and their intake is highly beneficial for health due to their antioxidant activity as free radical scavengers.[2] Hence, the development of analytical methods that allow a precise and highly sensitive detection and quantification of polyphenols in food matrices and/or samples with minimal preparation is crucial.[3] With that in mind, amperometric biosensors containing suitable enzymes seem to be an excellent alternative for polyphenol detection since their as high stability, low noise, fast response, low detection limit, reproducibility and wide linear range of detection.[4–8]

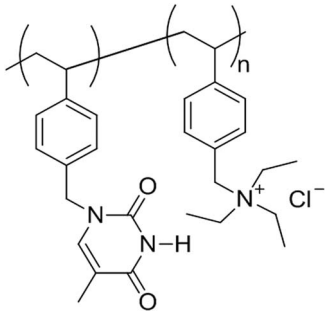
On the other hand, the good performance of bioelectrodes also depends on the physicochemical properties of the immobilization matrix where the enzyme is located; searching for the highest mechanical stability, substrate permeability, and electrical conductivity, respectively. Regarding these points, in previous studies we have shown the success of lactate oxidase (LOx)[9] and glucose oxidase (GOx)[10] immobilization using hydrogel films made of the synthetic nanoclay laponite and DNA-bioinspired polycations, which were obtained by copolymerization of vinylbenzylthymine (VBT) and vinylbenzyltriethylammonium chloride (VBA), e.g. $\{[(VBT)(VBA)_n]^{n+}\}_{25}$ (P^{n+}), with n = 4, 8, or 16 (Scheme 1). Besides of the strong positive charge electrostatic effect of the DNA-bioinspired polycations, the thymine pendant group also allows the modulation of weak electrostatic and non-electrostatic interactions, e.g. hydrogen bonding and π-stacking respectively, yielding extra interaction forces whose largely improve the interfacial properties of the bioelectrodes.[10] Thus, both the sensitivity and stability of the bioelectrodes were improved by the presence of these polycations, and it was also obtained a successful quantification of the respective substrate in real food samples, without interference effect of other compounds.[9,10]

In the present work we extend our studies to enzyme immobilization on these hydrogels, but now incorporating AuNP capped with citrate in order to improve the electrocatalytic properties of the bioelectrodes. Bearing in mind the influence of the hydrophilic and hydrophobic balance on the electroanalytical properties exerted by the DNA-inspired polycations, the hydrogel composition was optimized relative to the analytical performance of a horseradish peroxidase (HRP) based biosensor toward hydroquinone detection and the developed biosensor was applied to the detection of total polyphenol content in yerba mate and green coffee beverages samples. The resulting biosensor exhibited ultra-sensitivity behavior and one of the lowest detection limits compared to several similar devices previously reported.

Materials and Methods

Materials.—Laponite RD, a synthetic hectorite (monovalent cation exchange capacity, *c.e.c.* = 0.74 meq.g^{-1}) was obtained from Laportes Industries (Detroit, USA). Horseradish peroxidase (HRP) lyophilized powder (EC 1.11.1.7, 193 U.mg^{-1}), citrate buffer-stabilized spherical AuNP (diameter ≈5 nm), hydroquinone, caffeine, chlorogenic, ascorbic, citric, glutamic and tartaric acids were purchased from Sigma-Aldrich S.A. Reagent grade buffer phosphate sodium salts NaH_2PO_4 and Na_2HPO_4, hydrogen peroxide (30 vol.) and glucose were obtained from J. T. Baker. Water-soluble polystyrene copolymers of vinylbenzylthymine (VBT) and vinylbenzyl triethylammonium chloride (VBA) were synthetized as described before.[11]

Biosensor preparation.—Prior to each experiment, glassy carbon surfaces were treated as described previously.[10] Bioelectrodes were prepared depositing a mixture of 30 μg of laponite, 15 μg of the different thymine-based polycations, 7.5, 15 or 30 μg of HRP and different amounts of AuNP, expressed as micrograms of gold per milliliter of solution or mixture (Au μg/ml). The modified bioelectrodes were air-dried at 25°C, and prior to use immersed in phosphate buffer (0.1 M, pH 7.0) during 45 minutes for swelling step.



Scheme 1. Chemical structure of the DNA-bioinspired polycation.

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Electrochemical measurements.—All electrochemical measurements were carried out with a potenciostat/galvanostat (Teq4, Buenos Aires, Argentina), equipped with an impedance analyzer module. A three-electrode electrochemical cell with a platinum wire as counter electrode and Ag|AgCl|Cl^- (3 M) as reference electrode was used. Working electrodes were prepared on glassy carbon disks of 0.07 cm^2. 0.1 M phosphate buffer, at the indicated pH values, was used as supporting electrolyte; while the working temperature was 25°C.

Cyclic voltammograms (CV) were registered between −200 and 600 mV, at a scan rate of 50 mV s^{-1}. Chronoamperometry (CA) studies were performed under magnetic stirring. The current response was registered as a function of time in presence of H_2O_2 after sequential addition of HQ or CGA aliquots.

Electrochemical impedance spectroscopy (EIS) measurements were carried out in 5 mM $K_3[Fe(CN)_6]$ + 5 mM $K_4[Fe(CN)_6]$ prepared in 0.1 M KCl. Amplitude of the alternate voltage was 10 mV at a bias potential of 200 mV and frequency range 0.05 Hz–10 kHz.

Optimal experimental conditions for biosensor response were accomplished by studying the electrode sensitivity with HQ as enzymatic substrate. The analytical parameters of the biosensor were also obtained using CGA, which was the standard for the detection of polyphenols in yerba mate and green coffee samples.

Spectroscopic measurements.—UV-vis absorption spectra over a wavelength range of 300–800 nm of the hydrogel preparations were carried out using the NanoDrop 2000c spectrophotometer (Thermo Scientific) by depositing 1 μL of each solution on the pedestal.

Attenuated total reflection Fourier Transform Infrared (ATR-FTIR) spectra were obtained with a Jasco FT/IR-4600 apparatus equipped with a single-reflection ATR accessory. Sample films were prepared by depositing 2 μL of each solution onto glass slides and they were left to dry until each measurements. Transmittance spectra were recorded over a wavelength range of 3500–650 cm^{-1} at room temperature and under N_2-purged atmosphere (Parker Balston 75–45). Spectra handling were performed using the Spectra Manager CFR, Jasco.

Polyphenol determination in real samples.—Commercial green coffee and yerba mate were purchased from a local supermarket. 0.1 g of each sample were mixed with 10 mL of water at 80°C, shaken for 10 min and then filtered (Whatman Grade 589/2). In all cases, the polyphenol detection was carried out on the supernatant, and concentrations were determined by the standard addition method. In order to check possible matrix effects, the relative recovery of the current signal produced by an aliquot of 0.85 μM CGA before and after the addition of 10, 20 and 30 μL of the supernatant was measured.[12]

The colorimetric Folin-Ciocalteau method[13] was used to validate the amperometric determinations (see Section S1 of Supplementary Material). UV-vis absorption spectra were registered using an Agilent 8453 spectrophotometer (Palo Alto, CA, USA).

Results and Discussion

Optimization of hydrogel film composition for the HRP immobilization and bioelectrode properties.—The modification of electroactive surfaces by materials used for enzyme immobilization and/or improvement of the interfacial electrical properties of the modified electrode can be appropriately monitored by electrochemical impedance spectroscopy (EIS). Figure 1 shows the frequency-dependent impedance in the complex plane (Nyquist diagram) for the redox response of the couple model $[Fe(CN)_6]^{3-/4-}$ upon successive modifications of the surface of the glassy carbon electrode, as described as follows: (a) bare GCE; (b) 30 μg laponite; (c) 30 μg laponite + 15 μg P^{4+}; (d) 30 μg laponite + 15 μg P^{4+} + 4.50 Au μg/ml; and (e) 30 μg laponite + 15 μg P^{4+} + 4.50 Au μg/ml + 15 μg HRP. Results indicate that regardless the composition of the nanofilm deposited onto the electrode surface the impedance response of the redox couple was controlled by combined kinetic and diffusion

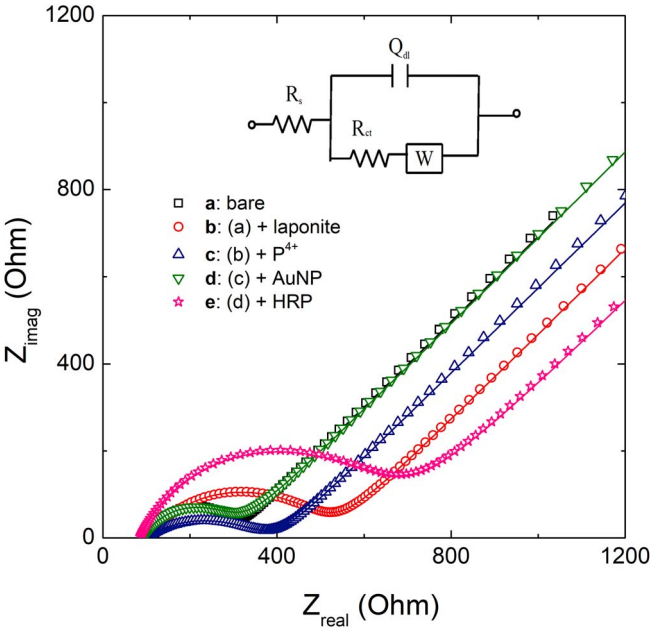


Figure 1. Nyquist plots for (a) bare and different modified GCE from (b) to (e).

process as a function of the frequency regime (semicircle shape at high-frequency and linear response at low-frequency ranges, respectively), and the impedance curves were satisfactory fitted by using the classical Randles circuit model (inset of Figure 1), in which R_s is the electrolyte resistance between working and reference electrodes; Q_{dl}, the constant phase element representing the double-layer capacitance; R_{ct} is the charge-transfer resistance (R_{ct}); and W, the Warburg impedance.

Table S1 of Supplementary Material collects the Randles's circuit parameters obtained by fitting of the impedance curves, and it was observed that only the charge transfer resistance R_{CT} was sensitive to the interface composition of the electrode. For GCE only modified with laponite a R_{CT} = 433 Ω was observed, since the negatively charged surface of the nanoclay impedes the diffusion of the redox probe $[Fe(CN)_6]^{3-/4-}$. Nevertheless, the successive incorporation of both P^{4+} and AuNPs, decreased the R_{CT} value to 333 Ω and 205 Ω, respectively. In the presence of P^{4+}, the reduction of R_{CT} can be explained in terms of both electrostatic neutralization and/or formation of a hydrogel structure with higher porosity than in the case of the neat laponite film.[14] The subsequent reduction of the R_{CT} by inclusion of the AuNPs confirms the conductivity improvement effect exerted by the metal nanoparticles.[15]

Finally, the subsequent incorporation of HRP into the hydrogel film increased again the R_{CT} value to 579 Ω. This effect has also been observed in laponite hydrogels containing lactate oxidase[14] and polyphenol oxidase,[16] and it is attributed to the biomolecule hindrance toward the electron transfer from the redox couple, confirming the effective enzyme immobilization at GCE.

The operative functionality of the modified GCE loading the nanocomposited hydrogel with different amount of AuNPs was tested by measuring the stationary currents at −100 mV of the electrochemical cell containing 600 μM H_2O_2 in 0.1 M phosphate buffer pH 7 after addition of successive aliquots of hydroquinone (HQ), Figure 2A. The bioelectrode sensitivity was calculated from the linear slopes of the corresponding calibration curve (Figure 2B). In order to probe that the reduction current observed by the addition of polyphenol corresponded to the enzymatic process at the bioelectrode, control experiments adding aliquots of HQ or CGA in the absence of H_2O_2 and vice versa were performed (Figure S2 of Supplementary Material). Under such experimental conditions, no amperometric signal was observed in any case. Hence, the monitoring current is associated with

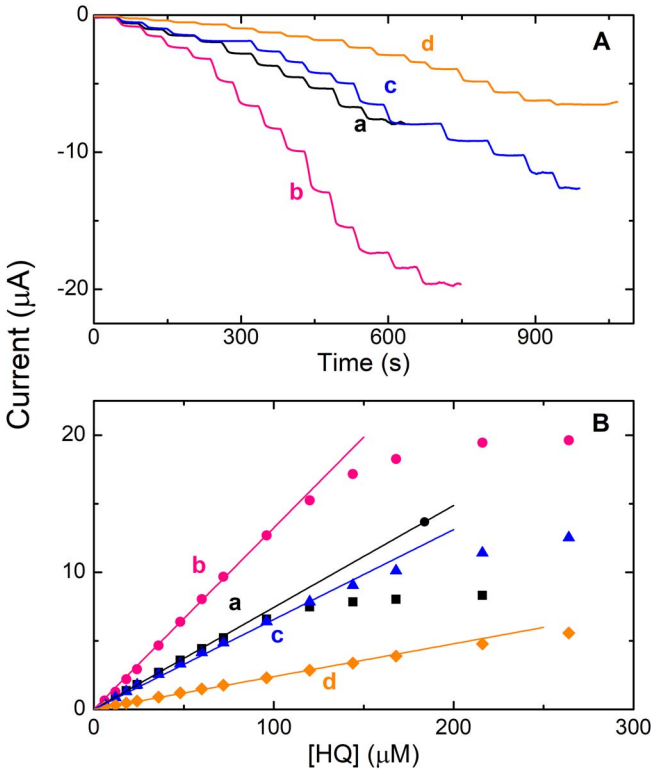
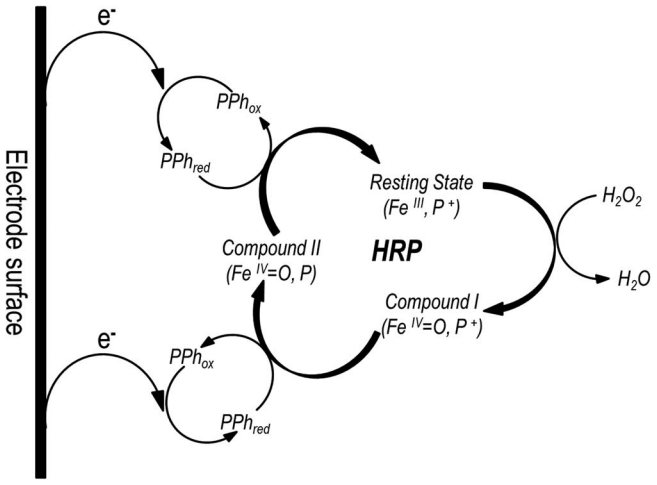


Figure 2. (A) Amperometric responses and (B) calibration curves for HQ on the modified bioelectrode with 30 μg of laponite, 15 μg of P^{4+}, 15 μg of HRP and different amount of AuNP: a) none, b) 4.50 Au μg/ml, c) 9.00 Au μg/ml, and d) 13.5 Au μg/ml.

the reduction of the oxidized polyphenol (PPh_{ox}) products resulting from the previous enzymatic oxidation, Scheme 2. The well-known reaction mechanism of HRP-based biosensors involves a cyclic enzymatic reaction in the presence of H_2O_2 which results in the formation of oxidized HRP, which can be reduced electrochemically by a direct or mediated electronic transfer (ET). In the case of the mediated ET, the oxidized enzyme takes the electrons from the reduced polyphenols (PPh_{red}) in solution and the resulting PPh_{ox} (quinone or free radical forms) are reduced back to their initial forms at the electrode surface yielding a reduction current proportional to the concentration of the



Scheme 2. Enzymatic and electrode reactions scheme involved in the response of HRP electrochemical biosensors to polyphenolics compounds. Adapted from Ref. 20.

phenolic compounds in the solution, where the corresponding enzyme intermediates are compound I (oxidation state +5) comprising a ferryl species ($Fe^{4+} = O$) and compound II (oxidation state +4).[17] This behavior is known as ping-pong or double displacement mechanism, in which two substrates, e.g. the phenolic compound and H_2O_2 are involved.[18] Accordingly with the cyclic enzymatic reaction of HRP shown in Scheme 2, the H_2O_2 content influences the concentration of polyphenol at which the enzyme saturation is reached. Present results indicated that for H_2O_2 concentrations lower than 600 μM the enzymatic mechanism was limited by the latter. This behavior was coincident with former results that indicated that at higher concentrations the enzyme inhibition occurs.[19]

Figure 3 summarizes the behavior of the bioelectrode sensitivity for HQ detection under different experimental conditions. The effect of the loaded amount of AuNPs in the hydrogels was compared for preparations with fixed amounts of HRP, laponite, and polycation, but for the latter modifying the VBA/VBT ratios, e.g. n = 4, 8, and 16 (Figure 3A). It can be observed that the sensitivity enhancement was dependent on both the amount of AuNPs and the positive charge density of the polycation. The best electroanalytical performance was obtained for the bioelectrode with the hydrogel film containing the polycation with four positive charges per copolymeric unit, e.g. the P^{4+} and 4.50 Au μg/ml. These results can be explained in terms of the balance of electrostatic forces between the hydrogel components, e.g. laponite, AuNPs, polycation and enzyme. Since the isoelectric point of HRP is 8.9,[21] it would be expected that the enzyme is positively charged at neutral pH. Thus, reduction of electrostatic repulsion interactions by using the less positive polycation allowed the larger incorporation and stabilization of HRP into the hydrogel film. The opposite effect was observed for bioelectrodes containing GOx ($pI = 4.2$) into the laponite hydrogel, since the sensitivity increased with the increment of positive charge of the polycation P^{n+}.[10] On the other hand, it is well-known that AuNPs are very suitable nanomaterials for bioelectrode preparation due to their excellent biocompatibility, large specific surface area, and electrical conductivity.[15] However, in the present case, incorporation of AuNPs into the hydrogel slightly improved the sensibility up to 4.50 Au μg/ml, independently of the positive charge loaded in the polycation. Considering that citrate-capped AuNPs are negatively charged[22] as well as the laponite nanolayer,[23] it may be expected that both nanomaterials contribute to the neutralization of the positive charges carried by the enzyme and polycation molecules. Thus, any excess of AuNPs can contribute to disrupt the electrostatic neutral balance and structure of the hydrogel film with the consequent loss of sensitivity.

Figure 3B shows the variation of the sensitivity of a selected bioelectrode (e.g. 15 μg of HRP, 4.50 Au μg/ml, 30 μg of laponite, and 15 μg of P^{4+} with the pH of the working buffer solution, and an inverted bell-shaped variation with the highest value at pH 5 was observed, as it is the case of the free enzyme.[19] This behavior strongly suggests that the conformational structure of the active site of the enzyme did not changed significantly after immobilization into the hydrogel. Figure 3C shows that under optimal pH conditions of 5.0, the highest sensitivity values of the bioelectrode were reached around applied potential $E = -(100–200)$ mV. It has been reported that the sensitivity, selectivity and operational stability of the HRP are strongly influenced by the polarization potential.[24] At larger negative potentials the inactivation of the enzyme may occur as a result of a formation of the highly oxidized compound III (Fe^{6+}).[24] Moreover, it is also desirable to work at low potential values to avoid the interference of easily oxidizing substances that could be present in complex samples. The present results confirm the capability of the modified bioelectrode to detect polyphenols and relatively low applied potentials with optimal sensitivity, improving the selectivity of the analytical determination of polyphenols by avoiding spurious current due to the oxidation of interfering substrates in complex samples. Finally, Figure 3D shows that the sensitivity of the bioelectrode did not changed above 15 μg of loaded HRP into the hydrogel, indicating that under the used experimental conditions the enzyme saturation was reached.

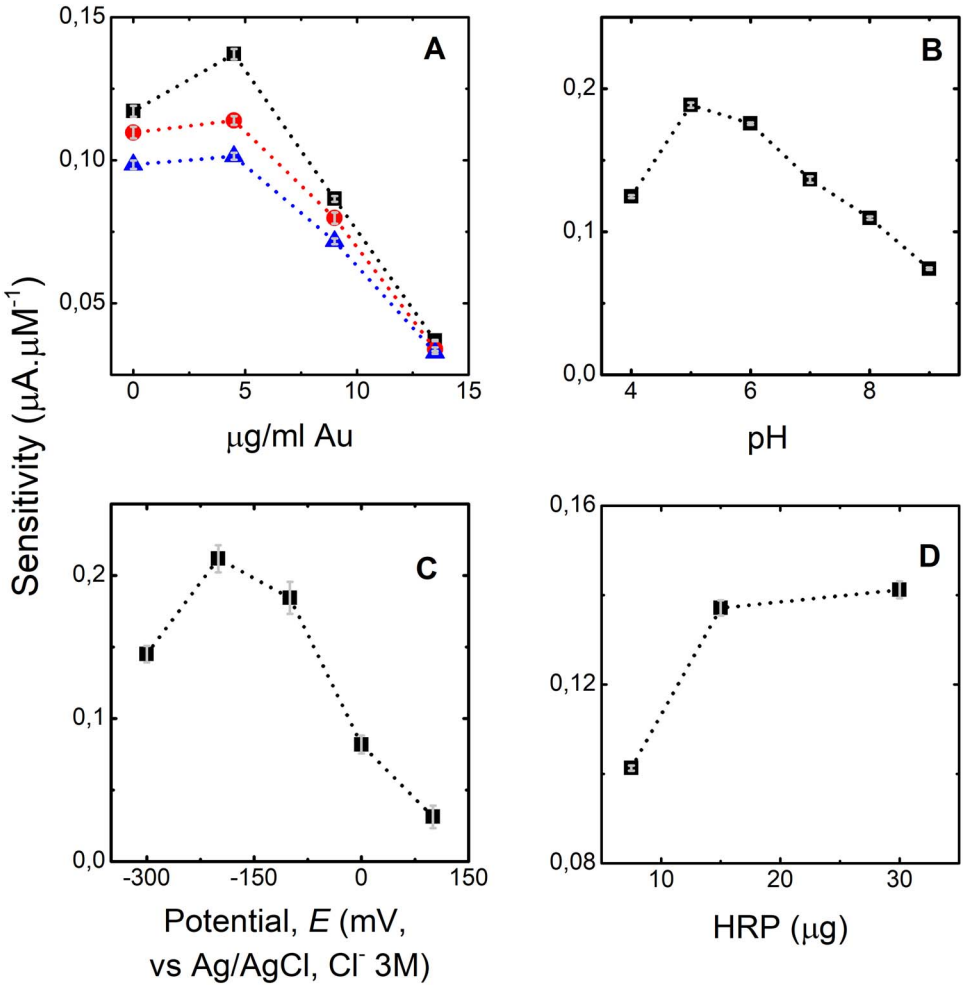


Figure 3. Sensitivity of bioelectrodes exerted by: (A) Au content (μg/ml) in hydrogels containing 30 μg of laponite, 15 μg of HRP and 15 μg of P^{n+} with n = 4 (■), 8 (●) and 16 (▲); (B) buffer pH (C) applied potential at pH 5 and (D) amount of HRP at pH 7 for the bioelectrode used in (B). Working buffer was 0.1 M phosphate with 600 μM H_2O_2. In A, B, and D the applied potential was −100 mV.

As well as the sensitivity behavior of the modified bioelectrode confirmed that the loaded enzyme retained its activity, the structural stability of HRP into the hydrogel films can be easily monitored by UV-vis absorption spectroscopy by the strong Soret band absorption around 400 nm ($\varepsilon \approx 1 \times 10^5$ $M^{-1}cm^{-1}$) of the heme prosthetic group, which is sensitive to conformational enzymatic changes.[18,25] Figure 4A shows the spectrum of the hydrogel film without HRP (a), and only the UV absorption of the vinylbenzyl moiety of the polycation and the visible absorption band around 520 nm due to the surface resonance plasmon of AuNPs were observed.[26] Thus, in the presence of HRP in the hydrogel film, the strong absorption band at 403 nm is entirely associated to the Soret band transition of the heme prosthetic group (b). In fact, the HRP spectrum obtained after subtraction of the hydrogel absorbance contribution was coincident with the spectrum of the enzyme in buffer solution (inset of Figure 4A), confirming that that the secondary structure of enzyme was not modified by the immobilization and therefore its biological activity conserved. An absorbance –concentration calibration linear plot (Figure S3 of Supplementary Material) was performed in order to estimate the retention yield of the enzyme into the hydrogel film by measuring the Soret absorbance of HRP. The amount of HRP loaded into the hydrogel calculated from the UV-Vis calibration curve was coincident (e.g. 14.7 ±0.3 μg) with the mass mixed in the hydrogel preparation (15 μg), confirming the total retention of the enzyme into the hydrogel film.

Attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) measurements of the HRP in buffer and in hydrogel films also demonstrated that no conformational changes occurred by interaction of the enzyme with the nanocomposited film (Figure 4B), since no modifications were observed of both typical amide I (1700–1600 cm^{-1}) attributed to the C=O stretching vibration modes of the peptide linkage in the background of protein and amide II (1625–1500 cm^{-1}) due to the combination of N-H bending and C-N stretching.[27] On the other hand, the Si-O stretching band vibration at 1070 cm^{-1} of the laponite backbone[28] was not shifted by adding the others components of the nanocomposite, but the transition was slightly increased after the addition of the polycation suggesting a subtle rearrangement of the laponite structure due to both electrostatic and intermolecular hydrogen bonds interactions (Figure 4C).

In summary, considering the above results and the highest sensitivity obtained, the modified bioelectrode with 30 μg of laponite, 15 μg of P^{4+}, 4.50 Au μg/ml and 15 μg of HRP was chosen for further analytical experiments.

Analytical parameters toward hydroquinone and chlorogenic acid detection.—Figure 5A shows the current-time profile and the respective calibration curve for the quantification of hydroquinone (HQ) obtained by using the selected bioelectrode under optimized experimental conditions, e.g. pH 5.0, $E = -200$ mV and 600 μM H_2O_2. For a set of three identically prepared bioelectrodes the relative standard deviation between sensitivities values was $\sigma = 2\%$, indicating the high reproducibility of the preparation method. Table I collects the average values of the electroanalytical parameters obtained with

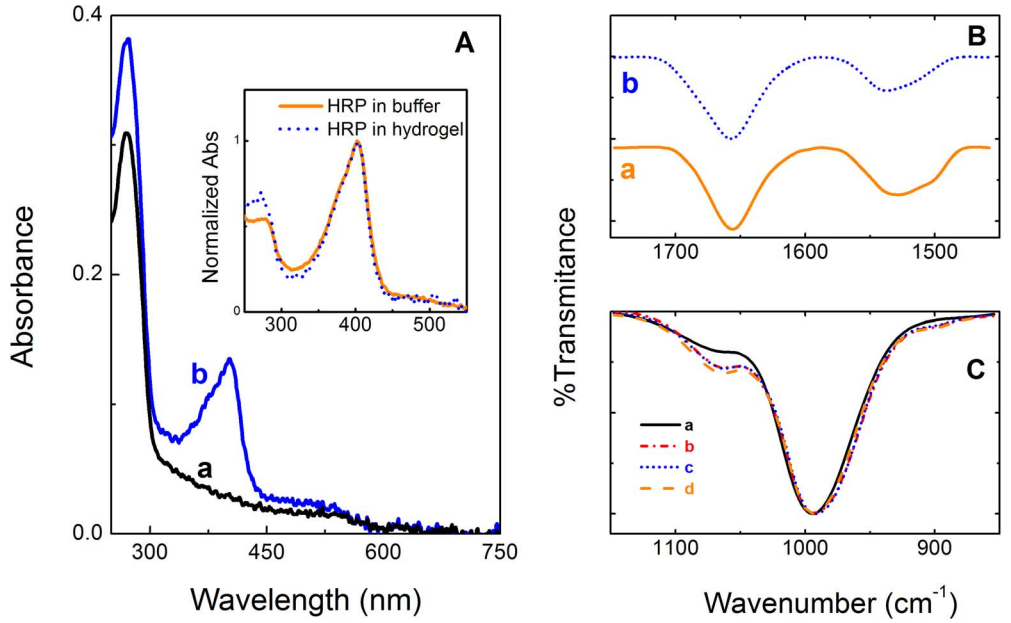


Figure 4. A) UV-Vis absorption spectra of the nanocomposited hygrogel: a) mixture of 30 μg of laponite, 15 μg of and 4.50 Au μg/ml; b) the same than before, but without HRP. Inset: Normalized absorption spectra of HRP in buffer (solid line) and in the hydrogel preparation (dotted line) after subtraction of the background absorbance of the hydrogel. B) ATR-FTIR spectra of HRP in buffer (a) and in the hydrogel preparation obtained after subtraction of the background absorbance of the hydrogel (b). C) ATR-FTIR spectra of (a) laponita Si-O stretching band vibration after the successive incorporation of: (b) 15 μg of P^{4+}, (c) 4.50 Au μg/ml and (d) 15 μg of HRP.

a set of three bioelectrodes prepared in same fashion, and compared with those reported for others bioelectrodes containing immobilized HRP. The present bioelectrode showed remarkable analytical properties, such as a linear range up to 120 μM, high sensitivity (e.g. s = 218 ± 4 μA.mM^{-1}), and ultra-low detection limit of 1.6 ± 0.2 nM (equivalent 0.2 ppbv) calculated as $3.3\sigma_b/s$, where σ_b is the standard deviation of the blank signal and *s* the bioelectrode sensitivity.

Although direct electrochemical detection of hydroquinone and chlorogenic acid on glassy carbon electrodes is also possible (see Figure S4, Supplementary Material), the present biosensor allows the detection of both compounds at much lower potentials.

Furthermore, the evaluation of the biosensor stability indicated that almost 95% and 62% of the initial sensitivity was preserved after 7 and 30 days of storage in buffer solution at 8°C, respectively. Altogether, these electroanalytical hallmarks indicate an ultra-sensitive behavior for the present electrode for the detection of HQ and related polyphenols.

Determination of polyphenol concentration in yerba mate and green coffee.—In order to test the real feasibility of the biosensor, the total polyphenol content of yerba mate and green coffee was analyzed by the standard addition method using CGA as standard. Previously, the biosensor stationary current response under the same experimental conditions used for HQ was analyzed by the successive additions of 0.85 μM CGA (Figure 5B). Once more, ultra-sensitive detection of CGA was found, e.g. a sensitivity of 132 μA.mM^{-1}, linearity range up to 4.2 μM, and detection limit of 2.7 nM (equivalent to 1 ppvb).

The evaluation of possible interferences by other typical substrates present in plant tissues was performed, e.g. glucose, ascorbic acid, tartaric acid and citric acid.[12] The interference effect was evaluated considering the following molar ratios [interferent/CGA] = 2/1, 3/2, 1/1 and 1/2 in the electrochemical cell containing 600 μM H_2O_2 in 0.1 M phosphate buffer pH 5.0 and applying $E = -200$ mV. In any case the tested interferent compounds did modify the electrochemical response of the biosensor (Figure S5 of Supplementary Material).

On the other hand, possible matrix effects were evaluated in terms of apparent recovery (R_A) Eq. 1:[31]

$$R_A = \frac{x(obs,\ O+S) - x(obs,\ O)}{x(S)} \qquad [1]$$

where *x(obs, O+S)* is the observed value for the spiked sample, *x(obs,O)* the observed value for the original unspiked sample, and *x(S)* the value for the spike. Three different sample volumes were examined: 10, 20 and 30 μL. The respective $\%R_A$ values were 92, 84 and 79%; for yerba mate and 91, 74 and 69% for green coffee. According to these results, the smallest volume was used in both samples. Thus, the average polyphenol levels considering of three replicates were 1.29 ± 0.04 and 0.037 ± 0.008 mmol.g^{-1} for yerba mate and

Table I. Electroanalytical properties of bioelectrodes containing HRP for hydroquinone detection.

Immobilization matrix	Sensitivity (μA mM^{-1})	Linear Range (μM)	Detection limit (nM)	Response time (s)	Ref.
Lap/{[(VBT)(VBA)$_4$]$^{4+}$}$_{\approx 25}$/AuNP/HRP	218 ± 2	up to 120	1.6 ± 0.2	27 ± 3	This work
CNT/PPy/HRP	8	16–240	0.006	2	8
Poly(L-Arg)/AgNP/SiSG-HRP	420	1–150	570	-	6
Au/MMPs/HRP	8.44	0.5–4.5	400	9	29
GC/PVF/PPy-HRP	15.32	1.6–15	600	-	30

Lap: laponite; CNT: carbon nanotube; PPy: poly(pyrrole); SiSG: silica sol-gel; AgNP: silver nanoparticles; Poly(L-Arg); poly(l-arginine); MMPs: magnetic micro particles; GC: glassy carbon; PVF: polyvinylferrocene.

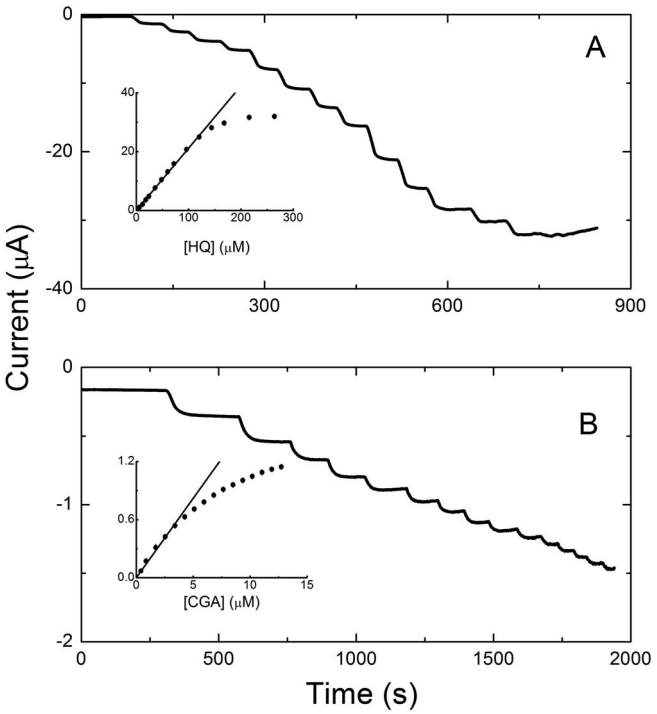


Figure 5. Amperometric response (and corresponding calibrations plots inset) at −200 mV on the selected bioelectrode, for successive additions of (A) HQ and (B) CGA. Supporting electrolyte: 0.1 M phosphate buffer at pH 5.0 with 600 μM H_2O_2.

green coffee, respectively. These results perfectly agreed with the polyphenol content of 1.32 ± 0.04 and 0.042 ± 0.007 mmol.g^{-1}, respectively, as determined with the classical Folin-Ciocalteau colorimetric method. Differences between the two sets of experiments are considered not to be statically significant under a 95% confidence interval.

Conclusions

A simple one-step fabrication of an ultra-sensitive and stable bioelectrode for polyphenol detection was reported. The bioelectrode was obtained by immobilization of HRP with a hydrogel film based on laponite, a DNA-bioinspired polycation and AuNPs. The best amperometric response was obtained for the bioelectrode bearing the polycation P^{4+} and 4.50 Au μg/ml, as result of an optimal balance of electrostatic and non-electrostatic forces, allowing the appropriate nanoenvironment for enzyme activity and electrical conductivity at the electrode surface. The developed biosensor has notable electroanalytical features in comparison to other HRP biosensors for polyphenol detection (Table I). Furthermore, the developed biosensor was able to quantify the total polyphenol content in green coffee and yerba mate beverages without any interference effect of typical substances existing in these beverages, e.g. glucose and ascorbic, tartaric and citric acids. These results were perfectly comparable with those obtained with the Folin-Ciocalteau method, confirming the accuracy of the studied bioelectrode for the direct determination of polyphenols in real food matrixes.

Finally, the present report exerts the biocompatibility of the DNA-bioinspired polycations for the immobilization of redox enzymes onto carbon electrodes as it was previously demonstrated for bioelectrodes with LOx[14] and GOx[10] in the laponite based hydrogel film.

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