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Analytical Methods

A comparison of three amperometric phenoloxidase–Sonogel–Carbon based biosensors for determination of polyphenols in beers

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ABSTRACT

Three phenoloxidases based biosensors were successfully developed using as electrochemical transducer a new type of electrode recently developed by our group: the "Sonogel–Carbon electrode". The employed enzymes were *Trametes versicolor* laccase (*Lac*), *Mushroom* tyrosinase (*Tyr*), and *Horseradish* peroxidase (*HRP*). Immobilization step was accomplished by doping the electrode surface with a mixture of the individual enzyme and Nafion ion exchanger as additive-protective. The biosensor responses, optimized in beer real samples, were evaluated for five individual polyphenols. It was found that the developed biosensors were sensitive to nanomolar concentrations of the tested polyphenols. As example, the limit of detection, sensitivity, and response linear range for caffeic acid for Nafion-*Lac*/Sonogel–Carbon biosensor were 0.06 μmol L^{-1}, 99.6 nA μmol^{-1} L, and 0.04–2 μmol L^{-1}, respectively. The stability and reproducibility of the biosensors were evaluated by applying them directly to beer real samples. It has been demonstrated that the Nafion-*Lac*/Sonogel–Carbon system is the more stable with a relative standard deviation of 3.3% ($n = 10$), maintaining 84% of its stable response for at least three weeks. Estimation of polyphenol index in eight lager beers and a comparison of the results with those obtained by a classical method was carried out.

1. Introduction

It is well known that polyphenols present in beverages play an important role in their quality and stability as well as in the prevention and protection of some pathologies (Shi et al., 1994; Stewart, 2004; Williamson & Manach, 2005). For these reasons, and due to their variety and the complexity of samples, several efforts have been dedicated to quantification of polyphenols in food and beverages (Santos-Buelga & Williamson 2003). Between the available techniques, bioanalytical tools offer interesting advantages over classical analytical techniques such as high selectivity and sensitivity, short assay times, and reduced cost of analysis.

Various electrochemical biosensors, based specially on phenoloxidases enzymes as tyrosinase (Cumming et al., 1998, 2001; Eggins, Hickey, Toft, & Zhou, 1997; Jewel & Ebler, 2001; Kiralp & Toppare, 2006; Sanz, Mena, González-Cortés, Yáñez-Sedeño, & Pingarrón, 2005), laccase (Gamella, Campuzano, Reviejo, & Pingarrón, 2006; Ghindilis, Gabrilova, & Yaropolov, 1992; Gomes, Nogueira, & Rebelo, 2004), and peroxidase (Imabayashi, Kong, & Watanabe, 2001; Kong, Imabayashi, Kano, Ikeda, & Kakiuchi, 2001; Mello, Sotomayor, & Kubota, 2003) have been developed for polyphenols determination in wine, beer, tea, and vegetables extract. All amperometric biosensors based on these enzymes have a similar detection approach: the phenols are enzymatically oxidized to quinones or radicals and then detected at the electrode by their reduction currents; such approach has the advantage to recycle the reaction products at the intimate electrode surface and consequently increases drastically the current response, improving the sensitivity of the method. However, the enzymatic products can partially electropolymerize to polyaromatic compounds damaging the electrode surface and cut the total assays number as well as the life time of the biosensor. A judicious choice of the electrode transducers and the use of additive-protective matrix on the surface of the biosensor can protect the bioprobe from this undesirable phenomenon and improve the signal transducer.

The employment of sol–gel chemistry to produce electrochemical transducers and its biocompatibility with biological sensing has received increasing interest in recent years (Collinson & Howells, 2000; Jin & Bernnan, 2002; Rabinovich & Lev, 2001; Sun, Zhu, & Zhu, 2006; Wang, 1999). Our group proposed a novel sol–gel-based procedure to obtain solid carbon composite electrodes (Hidalgo-Hidalgo de Cisneros, Cordero-Rando, Naranjo-Rodríguez, Blanco, & Esquivias, 2001), called by us Sonogel–Carbon electrodes.

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The fabrication procedure, based on the use of sonocatalysis, that avoids the materials shrinkage and permits a control of the pore size, achieves an excellent electrochemical sensor when comparing with other graphite electrodes (Cordero-Rando, Hidalgo-Hidalgo de Cisneros, Blanco, & Naranjo-Rodríguez, 2002, 2005) and a very competitive bioprobe if it is used as electrochemical transducer in enzymatic biosensors (ElKaoutit, Naranjo-Rodriguez, Temsamani, & Hidalgo-Hidalgo de Cisneros, 2007).

In this work, we report the biocompatibility of this material with various enzymes, such as *Trametes versicolor* laccase (*Lac*), *Mushroom* tyrosinase (*Tyr*), and *Horseradish* peroxidase (*HRP*). The use of the resultant biosensors to measure polyphenols in several beer types constitutes the aim of this paper. As far as we know, a comparison in the same paper of three enzyme-based biosensors for determination of a bioelectrochemical index for polyphenols in beer was never reported. These points together with other developed in this paper constitute the originality of this work.

2. Experimental

2.1. Reagents

Methyltrimethoxysilane (MTMOS) was from Merck (Darmstad, Germany) and HCl was from Panreac (Barcelona, Spain). Graphite powder (spectroscopic grade RBW) was from SGL Carbon (Ringsdorff, Germany). *Mushroom* tyrosinase (E.C. 1.14.18.1, 3000 U mg^{-1}), and *Horseradish* peroxidase (E.C. 1.11.1.7, 269 U mg^{-1}) were from Sigma (Steinheim, Germany). *Trametes versicolor* laccase (E.C. 1.10.3.2, 23.3 U mg^{-1}) was from Fluka (Steinheim, Germany), KH_2PO_4/K_2HPO_4 and acetic acid/ sodium acetate for phosphate or acetate buffer were from Fluka (Buchs, Switzerland) and Merck (Darmstad, Germany), respectively. Nafion-perfluorinated ion-exchange resin (Cat. No. 27, 470-4) 5% (w/v) in a mixture of lower aliphatic alcohols and water, and glutaricdialdehyde 25% wt solution in water were from Aldrich (Steinheim, Germany). Nanopure water was obtained by passing twice-distilled water through a Milli-Q system (18 M cm, Millipore, Bedford, MA). All phenolic compounds tested in this work (caffeic acid (CA); ferulic acid (FA); gallic acid (GA); (+)-catechin ((+)-cat); and (−)-epicatechin ((−)-epi)), were of analytical grade, used as received, and purchased from Merck, Fluka or Panreac. Folin–Ciocalteu reagent was from Panreac and used as received.

Stock solutions of the phenolic compounds (0.01 mol L^{-1}) were prepared daily by dissolving the appropriate amount either in 0.05 mol L^{-1} buffer solution or in ethanol, depending on the phenolic compounds solubility. More dilute standards were prepared by suitable dilution with 0.05 mol L^{-1} phosphate or acetate solution at working pH, which was also used as the supporting electrolyte.

Glass capillary tubes, i.d. 1.15 mm, were used as the bodies for the composite electrodes.

2.2. Samples

The samples analyzed were commercial beers purchased in a local market. Eight lager beers were investigated (five with alcohol contents around 5% vol. and three non-alcoholic). The samples were previously degasified by means of centrifugation in order to take an exact beer volume and to dilute it in the working solution at a 1:5 ratio when necessary.

2.3. Apparatus

Chronoamperometric measurements were performed with an Autolab PGSTAT20 (Ecochemie, Ultrecht, The Netherlands) potentiostat/galvanostat interfaced with a personal computer, using the AutoLab software GPES for waveform generation and data acquisition and elaboration.

Colorimetric essays were performed with UV/VIS Spectrophotometer *Jasco* V-550 (Japan), using the Jasco 32 software.

A 600-W model, 20 kHz ultrasonic processor (Misonix Inc., Farmingdale, NY) equipped with a 13 mm titanium tip was used. The ultrasonic processor was enclosed inside a sound-proof chamber during operation.

2.4. Methods

2.4.1. Electrochemical transducer preparation

Electrochemical Sonogel–Carbon transducer was prepared as described previously (Cordero-Rando et al., 2002). Before biological modification, the electrodes were electrochemically pre-treated by dipping them in 0.05 mol L^{-1} sulphuric acid and polarized by voltage cycling from −0.5 to 1.5 V for 5 cycles; electrodes with similar current backgrounds were selected, washed carefully with Milli-Q water and let to dry at room temperature.

2.4.2. Biosensors fabrication

In this work Sonogel–Carbon bioelectrodes based on laccase (*Lac*/SNGC), tyrosinase (*Tyr*/SNGC) and peroxidase (*HRP*/SNGC) were developed as follow: An adequate quantity of enzyme was dissolved in 30 μL of different buffer solutions, such as phosphate buffer, 0.2 mol L^{-1} pH 7, for tyrosinase and peroxidase, and acetate buffer, 0.2 mol L^{-1} pH 5, for laccase biosensors. At this enzymatic solution, 1.25 μL of glutaricdialdehyde was added, set to polymerize in ultrasonic bath for 3 min, and modified by adding 3.5 μL of Nafion. From the resulting solution, adequate quantities were deposited on the top of the Sonogel–Carbon electrodes with a μ-syringe and allowed to dry under room conditions. Finally, the three resulting biosensors have 100, 54, and 23 Units/Electrode of *Tyr*, *HRP*, and *Lac*, respectively, 0.9% of glutaricdialdehyde and 0.5% of Nafion. Before using, the enzymes electrodes were dipped in stirred buffer solution for 15 min, to eliminate the excess of enzymes not adsorbed, rinsed with the same buffered solution and stored immersed in the buffer at 4 °C when they were not in use.

2.5. Electrochemical measurement and bioelectrochemical polyphenols index determination

Electrochemical experiments were carried out in a cell containing 25 mL of an aerated adequate buffer, depending on pH values, at 22 ± 2 °C. The three-electrode system consisted of an enzyme-modified Sonogel–Carbon electrode as working electrode, a Ag/AgCl (3 M KCl) and a platinum wire as reference and auxiliary electrodes, respectively. To perform the measurements, a selected potential was applied to the working electrode and the background current was registered until reaching the steady state. The respective polyphenolic compounds standard solutions were added to the cell and the corresponding current–time curves were recorded. The biosensor response was measured as the difference between the total and the background current. A magnetic stirrer and a stirring bar were used to provide continuous convective transport.

Bioelectrochemical polyphenol index determination in beers was performed as follows: Polarization of the biosensor at its optimum potential and registration of the background current under stirring; addition of 500 μL of 1:5 diluted beer sample to 25 mL of 0.05 mol L^{-1} acetate buffer solution of pH 5; application of the standard addition method (addition of three successive aliquots of a gallic acid stock solution with a concentration about 0.40 mg L^{-1}). A linear curve with four bi-replicated points was constructed and a polyphenols index was determined. Its standard

deviation was estimated as statistically assumed (Miller & Miller, 1993).

2.6. Folin–Ciocalteu polyphenol index determination

500 μL of beer and the same volume of the corresponding gallic acid standard solutions were placed in four 50 mL volumetric flasks; 30 mL of Milli-Q water, 2.5 mL of Folin–Ciocalteu reagent, 7.5 mL of 20% anhydrous sodium carbonate solution, and distilled water to make up the total volume were added, according to a described procedure (Singleton & Rossi, 1965). After 2 h the absorbance at 760 nm was read, using a blank prepared with distilled water, and the polyphenol index and its error were obtained from the standard addition curve, employing the same mathematical approach above mentioned.

3. Results and discussion

3.1. Parameters optimization

The biosensor optimal response depends on the physical–chemical parameters, as pH and applied potential, as well as on the selected standard substrate (Gomes et al., 2004; Imabayashi et al., 2001). In the majority of the published studies, polyphenols biosensors are optimized by using randomly selected individual substrates. This approach can lead to erroneous results when the bioprobe is used in real samples or for the detection of mixtures of polyphenols. To avoid these problems, and to attain the real optimum response, in this paper the optimization of the response dependence of all biosensors on pH and potential was investigated directly in lager beer as a real sample of a mixture of polyphenols.

The influence of the operating potential on the response of the biosensors is presented in Fig. 1. An adequate amount of lager beer, depending of the biosensor sensitivity, was added in the cell (in the case of *HRP* biosensor, H_2O_2 was previously added until a 10 μmol L^{-1} concentration), the applied potential was varied in the range from −300 to +50 mV, and the corresponding response was registered. As can be expected, for all biosensors the reduction responses of the liberated quinones or free radicals rise sharply at the beginning, reach a plateau around the optimum values and then decreases for negative potentials. At extreme negative potentials (−250 mV), the biosensors give respectively 80%, 75%, and 30% of their maximum responses. The maximum responses were attained at potentials of −200 mV, −150 mV, and −100 mV for *Tyr*, *Lac* and *HRP* based biosensors, respectively. Consequently these potential were adopted in all subsequent experiments with the respective biosensors.

The sensitivities dependence for lager beer over the pH range from 3.5 to 8.2 is shown in Fig. 2 (the buffers used were acetate or phosphate). *HRP*/SNGC displays a larger plateau response, from pH 5.8 to 8.2, with a response variation of only 15%, and shows the maximum value at pH 7. *Lac*/SNGC and *Tyr*/SNGC show the maximum response at pH 5 and 6.5, respectively. Therefore, to obtain the best performance for all biosensors, the pH values of 7, 6.5 and 5 were, respectively, selected in all subsequent experiments.

3.2. Sensitivity and selectivity of biosensors

To comprehend the interaction of the immobilized enzymes in their new environment with phenolic compounds present in beers, we carried out a comparative kinetic and characteristic study of the response of enzyme modified Sonogel–Carbon biosensors in presence of polyphenols. From the current–time curves recorded as described in Section 2.5, calibration curves of the three biosensors for CA, FA, GA, (+)-cat and (−)-epi are constructed, and parameters as the detection limit (LOD, calculated as follow: LOD = 3 SB/b, where SB is the statistic standard deviation of the blank and *b* is the slope of the calibration curve), direct linear range (DLR), and sensitivity as well as its deviation (calculated as statically assumed in Miller & Miller, 1993), are resumed in Table 1. It can be seen that there are great differences in sensitivity for the different phenolic substrates tested by the same biosensor. *Tyr*/SNGC responded to nanomolar concentrations of the two flavan-3-ol compounds, to micro molar concentrations of GA and CA, and did not respond to FA as a consequence of the occupation of the ortho-position.

With regard to the *HRP*/SNGC, a similar response was obtained for the two hydroxycinnamic acids and flavan-3-ols, and a low sen-

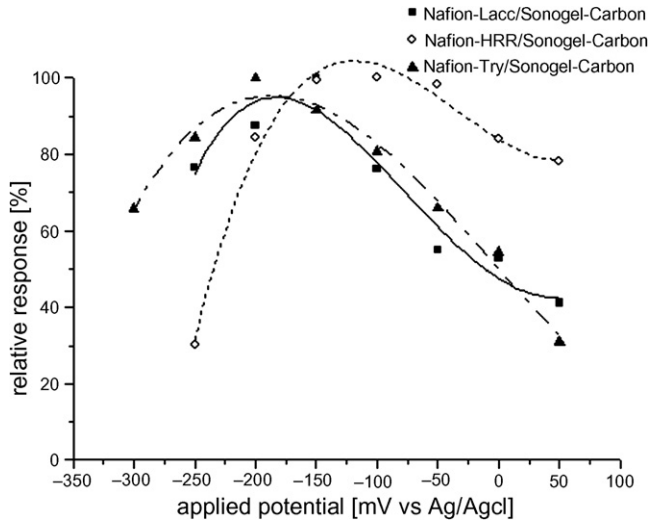

Fig. 1. Influence of the applied potential on amperometric response for the three biosensors in beer real samples. Medium and pH: 0.05 mol L^{-1} acetate buffer pH 5 for *Lac*/SNGC, and 0.05 mol L^{-1} phosphate buffer pH 7 for *Tyr*/SNGC and *HRP*/SNGC. Beer presence: injection of 100, 500, and 100 μL in 25 mL of solution, for each respective biosensor and after adding 10 μmol L^{-1} of H_2O_2 in the case of *HRP* based biosensor. Absolute optimal responses were: 28.5 ± 1.5, 11.9 ± 1.2 and 9.0 ± 1.1 nA, respectively.

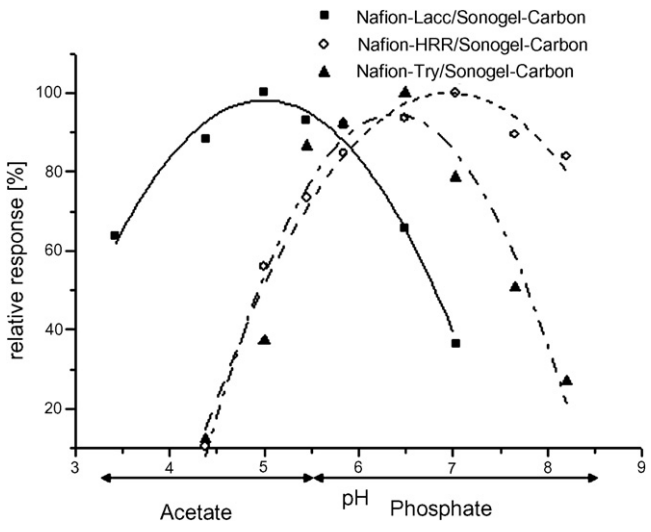

Fig. 2. Influence of pH on amperometric response for the three biosensors in beer real samples. Applied potential: −150, −200, and −100 mV for *Lac*/SNGC, *Tyr*/SNGC and *HRP*/SNGC, respectively. Beer presence: injection of 500 (1:5 diluted beer), 500, and 200 μL in 25 mL of solution for each respective biosensor and after adding 10 μmol $L^{-1}$of H_2O_2 in the case of *HPR* based biosensor. Absolute optimal responses were: 27.02 ± 0.1, 15.3 ± 2.1 and 15.1 ± 0.4 nA, respectively.

Table 1
Analytical performances

Biosensor	DLR (μmol L^{-1})	Sensitivity (nA/μmol L^{-1})	Sensitivity error (nA/μmol L^{-1})	LOD (μmol L^{-1})
Nafion-Tyr/Sonogel–Carbon				
Caffeic acid	0.6–24.5	0.729	0.016	1.43
Ferulic acid	–	–	–	–
Gallic acid	3.6–58	0.081	0.002	3.38
(+)-Catechin	0.6–10.3	1.57	0.012	0.33
(−)-Epicatechin	0.6–20	1.23	0.012	0.42
Nafion-HRP/Sonogel–Carbon				
Caffeic acid	0.2–5.15	8.55	0.140	0.20
Ferulic acid	0.2–4	4.32	0.141	0.32
Gallic acid	20–836	0.024	0.001	94.9
(+)-Catechin	0.2–2	15.925	0.344	0.14
(−)-Epicatechin	0.2–3.2	11.192	0.299	0.18
Nafion-Lac/Sonogel–Carbon				
Caffeic acid	0.04–2	99.454	1.090	0.06
Ferulic acid	0.04–2	12.752	0.412	0.16
Gallic acid	0.1–22	11.009	0.122	0.41
(+)-Catechin	0.04–3	89.066	0.570	0.10
(−)-Epicatechin	0.04–8	28.139	0.194	0.16

sitivity was observed for the GA. In the case of the *Lac*/SNGC the higher sensitivities and lower detection limits were achieved for the five compounds tested. The sensitivity for this biosensor follows the trend: CA > (+)-cat > (−)-epi > FA > GA. The most common enzymatic product of this enzyme is the phenoxy radical and arguments about the stability of this product can explain the selectivity of this enzyme.

3.3. Kinetics factor

Table 2 shows the kinetics parameters of the enzymatic reactions for the different polyphenolic compounds. Maximum rate (I_{max}), Michaelis–Menten constant. (K_m^{App}), and Hill coefficient (h) were evaluated from the corresponding Eadie–Hofstee plots. As can be seen in all the cases the value of h is close to one, so the Michaelis behaviour of all enzymes, in their new immobilization matrix, was demonstrated. In addition, when we compare K_m^{App} and sensitivity, we observe that the lower K_m^{App} values were found for the phenolic compounds exhibiting the higher sensitivities, as a consequence of the substrate recycling phenomenon confirmed for amperometric biosensors. However, the K_m^{App} parameter is independent of the enzyme concentration and usually reflects the enzyme mobility as well as the biocompatibility of the immobilization matrix; therefore, it is appropriate to perform a comparative study of the values of this parameter obtained in this paper with those reported in literature. We found that the value of K_m^{App} obtained for *Tyr*/SNGC is lower than that reported for tyrosinase entrapped in a copolymer conducting, using GA as substrate (Kiralp & Toppare, 2006), or immobilized on a gold nanoparticles modified glassy carbon electrode in the case of CA and GA as substrates (Sanz et al., 2005) and the values obtained for *Lac*/SNGC are in the range of the values obtained using laccase immobilized on glassy carbon surface (Gamella et al., 2006). This indicates a high biocompatibility of our bioelectrode configuration, which allows the achievement of a high sensitivity.

Table 2
Kinetic factors for the proposed biosensors

Biosensor	I_{max} (nA)	Error (nA)	K_m^{App} (μM)	Error (μM)	h
Nafion/Tyr/Sonogel–Carbon					
Caffeic acid	184.3	4.87	241.1	24.20	1.04
Ferulic acid	–	–	–	–	–
Gallic acid	22.77	0.98	266.9	0.44	1.07
(+)-Catechin	122.0	4.21	72.55	1.93	0.97
(-)-Epicatechin	104.9	7.00	92.59	9.67	0.99
Nafion/HRP/Sonogel–Carbon					
Caffeic acid	80.02	1.81	5.88	0.31	1.07
Ferulic acid	41.52	1.70	6.75	0.52	1.02
Gallic acid	53.48	0.40	1521	116	1.10
(+)-Catechin	82.22	8.47	4.21	0.13	0.93
(−)-Epicatechin	79.38	1.78	5.74	0.83	1.02
Nafion/Lac/Sonogel–Carbon					
Caffeic acid	3238	276.1	32.99	3.18	1.02
Ferulic acid	757.8	16.23	60.95	2.34	1.09
Gallic acid	933.4	46.71	76.47	9.96	0.97
(+)-Catechin	3628	173.0	38.02	2.43	1.07
(−)-Epicatechin	4435	128.4	57.16	5.27	1.12

3.4. Stability and reproducibility of the biosensors

The stability of the biosensor response in real samples constitutes a primordial stage for select the best bioprobe able to achieve our objective. Many different aspects regarding this step were considered. The repeatability of the three enzymes based biosensors was calculated by ten repetitive measurements in the same day using the same electrode in presence of lager beer. Relative standard deviations (RSD) of 3.3%, 9.8%, and 15% were obtained for laccase, peroxidase, and tyrosinase–Sonogel–Carbon biosensors, respectively. The useful lifetime of the biosensors was checked by performing repetitive measurements every day, using some fresh lager beer as samples and storing the biosensor at 4 °C when it is not in use. Great differences in the life time for the three biosensors were observed: The *Lac*/SNGC exhibits an excellent stability since no change in its response was observed in the first week and only a response loss of 15.4% was found at the end of the third week. However, in the cases of *HRP*/SNGC or *Tyr*/SNGC, a response decrease of 16.5% and 25%, respectively, was observed after the first week. The different behaviour of the three biosensors toward the presence of a complex matrix as beer can be explained by (1) the differences existing in the resistance to the inhibition effect of the enzymes caused by the present compounds or those which are developed in a very alive medium like beer, such as carbonate, ascorbic acid, ethanol, or Maillard reaction products (Roux, Billaud, Maraschin, Brun-Mérimee, & Nicolas, 2003); (2) the difference in the biocompatibility of the immobilization matrixes as well as the Sonogel–Carbon electrode with the different enzymes; (3) the

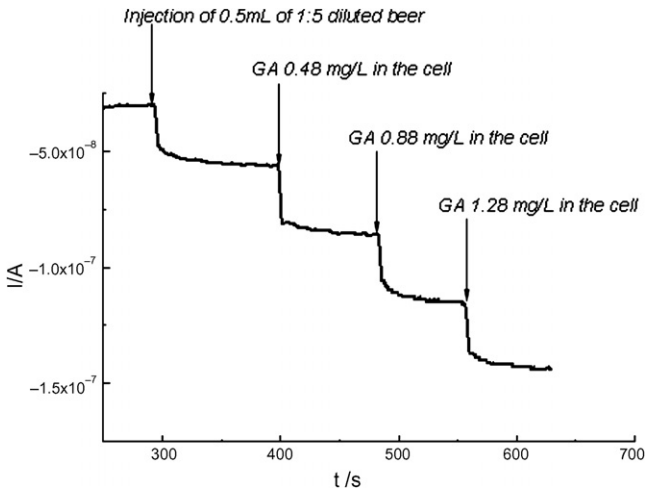

Fig. 3. Example of amperomogram obtained for polyphenols index determination in beer with *Lac*/SNGC: 0.05 mol L^{-1} acetate buffer, pH 5, applied potential is −150 mV. For other details see the figure.

Table 3
Polyphenol index determination with *Lac*/SNGC and Folin–Ciocalteu reagent, and their correlation

Beer	I_{F-Ci} (equiv mg L^{-1} GA)	Error (%)	I_{BEi} (equiv mg L^{-1} GA)	Error (%)	Estimated I_{F-C} (equiv mg L^{-1} GA)	I_{F-C} recovery error (%)
Lager 1	368.53	6.6	155.46	1.5	391.31	+6.2
Lager 2	283.96	3.5	106.80	1.5	268.83	−5.3
Lager 3	311.14	4.2	120.60	2.0	303.56	−2.4
Lager 4	356.44	5.5	144.95	1.8	364.85	+2.4
Lager 5	258.40	5.3	84.66	1.6	269.22	+4.2
Non-alcohol 1	255.45	5.3	83.04	1.5	264.07	+3.4
Non-alcohol 2	215.25	6.8	65.33	1.5	207.75	−3.5
Non-alcohol 3	180.28	6.1	54.66	1.6	173.82	−3.6

Factor 1 = 2.5 (for lager 1, lager 2, lager 3, and lager 4).
Factor 2 = 3.2 (for lager 5, non-alcohol 1, non-alcohol 2, and non-alcohol 3).

difference in the protective effect showed by Nafion toward each enzyme.

Finally the reproducibility of the biosensor fabrication procedure was also evaluated by comparing the first day response for five *Lac*/SNGC. A RSD of 5.5% for the response of 0.5 mL of lager beer was obtained.

3.5. Estimation of polyphenols index in beer

With the aim to select the best biosensor to determine polyphenols in beers, we perform in this paper a comparative study of the three developed biosensors. Bioelectrochemical polyphenols index (I_{BEi}) of beer, as (+)-cat and GA standards equivalent, was tested by the method of multiple standard additions. A one queue *F* test applied to the variance of I_{BEi} proved that the values for laccase biosensor were significantly lower than those obtained for tyrosinase biosensor. These results, together with other previously exposed, decided us to select the laccase based biosensor to achieve our practical goals.

Five lagers and three non-alcoholic beers were analyzed by *Lac*/SNGC using the standard addition method as described in Section 2. Fig. 3 shows the typical steady state response obtained. As can be seen, comparing the beer signal with that of the GA addition, no difference in the shape of the current–time response curve was observed; no alteration was found for the response time for each current step (response time of around 16 ± 1.8 s). The average slope of the calibration plot calculated by duplicate from the analyzed beers gave 12.9 ± 0.7 nA μmol^{-1} L, for a significance level of 0.05, value moderately superior than that obtained for individual GA calibration curve. These remarks reflect the low interference of the compounds present in beer, such as ascorbate, glucose and alcohol, on the signal response, and also the great stability of the *Lac*/SNGC.

In our opinion, one of the pending challenges in biosensor field is the correlation between the data obtained with the biosensors and those achieved by classical analytical methods. Assuming this challenge, we tried to correlate our I_{BEi} with the Folin–Ciocalteu index which, in spite of several disadvantages and limitations, is one of the most accepted approach for total polyphenol index determination in food chemistry. With this aim, and using both methods, we analyzed eight commercial beers and the results are summarized in Table 3. As can be seen, there are a large difference between theses two polyphenols index that can be attributed to the low selectivity of Folin–Ciocalteu method (Schofield, Mbugua, & Pell, 2001) as well as to the difference on pH values and its effect on the ratio of the free and complexed polyphenols in both systems.

To evaluate the classical index from our I_{BEi} we applied a model of calibration transfer according to the following expression:

$$\text{Estimated } I_{F-C} = \frac{\sum_1^N (I_{BEi}/I_{F-C})}{N} I_{BEi} \quad (1)$$

where the I_{F-C} is the Folin–Ciocalteu index and I_{BEi} is the bioelectrochemical index. Two regions can be observed in the data of Table 3, depending on the type of the beers tested and also on the elaboration process; the first one includes the normal lager beers lager 1, lager 2, lager 3, and lager 4; and the second one includes the non-alcoholic beers, and lager 5 which shows a characteristic light colour. The multiplicative factors calculated as mentioned in Eq. (1) are 2.5 and 3.2 for the two groups, respectively. Table 3 summarizes the new Folin–Ciocalteu polyphenols index calculated from the bioelectrochemical index. It can be seen that the Folin–Ciocalteu index can be estimated directly by means of bioelectrochemical measurement, since when the new calculated polyphenols index was plotted versus the results obtained with the Folin–Ciocalteu method for all samples, a linear correlation with an intercept of −26.9 ± 47.5 and a slope of 1.1 ± 0.2 was obtained.

4. Conclusion

The goal of this work was to compare and characterize various phenoloxidase based Sonogel–Carbon bioelectrodes for polyphenols monitoring in beers. This aim has been possible thank to the biocompatibility of our Sonogel–Carbon electrode with the studied enzymes, as well as to the immobilization matrix used. After a detailed comparison of stability, reproducibility and precision of the three developed bioelectrodes in beer real samples, the laccase based biosensor was selected as the best one to evaluate polyphenols bioelectrochemical index in beers. The proposed index proved to be a useful tool to obtain, in an easy and fast way, a valid estimation of the classical Folin–Ciocalteu index.

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