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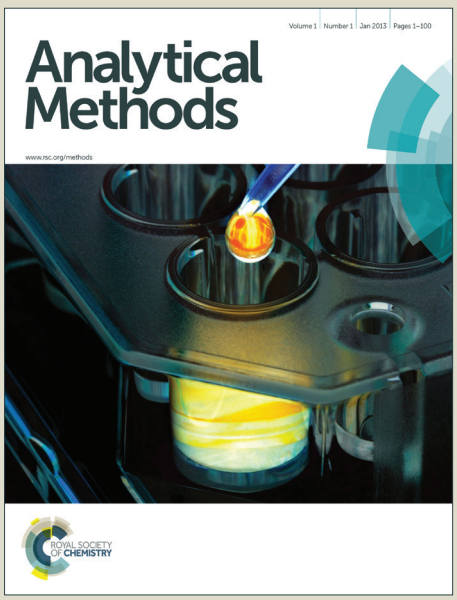

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Modulating the analytical performance of an electrochemical biosensor through varying, at the working electrode, the surface area ratio between that covered by the enzyme and the enzyme-free one

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It is shown that the analytical features namely the sensitivity, the lowest detection (LOD) and quantification (LOQ) limits and the apparent Michaelis-Menten's (K_m') constant as well, of a Laccasa-based electrochemical biosensor for hydroquinone (HQ) quantification, depends on the transductor´s areas ratio, between that covered with the enzyme and the enzyme-free one.

1. Introduction

Signal transduction and general performance of electrochemical biosensors are determined by surface architectures connecting the sensing element to the biological sample. Surface modification techniques, various electrochemical transduction mechanisms, and the choice of recognition receptor molecules all influence the ultimate sensitivity of the sensor [1]. Electrochemical biosensors combine the sensitivity of electroanalytical methods with the inherent bioselectivity of the biological component. The latter in the sensor recognizes its analyte, resulting in a catalytic or binding event, producing hence an electrical signal, monitored by a transducer, that ultimately is proportional to analyte concentration [2]. In general terms, a biosensor can be thought of as comprising a bioactive substance (typically an enzyme, multi-enzyme system, antibody, membrane component, or microorganism) that can specifically recognize species of interest, intimately in contact with a suitable transducing system. Because the biologically sensitive material is responsible for recognizing the analyte, it also regulates, to a large extent, the specificity and sensitivity of the device. The purpose of the transducer is to convert the biochemical signal into an electronic signal that can be suitably processed and output. The transducer can take many forms, but the emphasis to date has been on the following electronic configurations: optoelectronic detectors, field-effect transistors, potentiometric or amperometric electrodes, and thermistors [3]. Most of the electrochemical biosensors reported [4, 5] involve a design where the whole surface area of the electrode (transductor) is covered by the recognition element, that must be attached to the electrode surface by means of different immobilization methods (i.e. entrapment and cross linking). In most cases such a design diminishes the charge transfer rate on the electrode surface, making it necessary to incorporate some catalyst or electron mediator materials, namely: modified core–shell magnetic nanoparticles [6], cytochrome c [7], copper [8], conducting polymers [8,9], Au nanoparticles [10], multiwall carbon nanotubes [11]. In this work it is shown that the analytical performance of an electrochemical biosensor can be modulated by simply controlling the ratio between the recognition element covered and the bare transductor surface area. It is important to mention that a tyrosinase based electrochemical biosensor, having the same architecture as the studies in this work, has been reported to be useful for determining the antioxidant capacity of medicinal plants real samples [12] and phenolic compounds monitoring in tea infusions [13].

2. Experimental

2.1. Chemicals and Solutions.

Laccasa from Trametes versicolor (TvL) > 10 U/mg was from Sigma, the water-soluble polyvinyl alcohol polymer, PVA-AWP, from Toyo Gosei Co., Ltd., the 25% glutaraldehyde (GA). For the buffer solution, 99.36% purity dibasic potassium phosphate (K_2HPO_4) and the 99.6% purity potassium phosphate monobasic (KH_2PO_4) were from Baker Analyzed, the glacial acetic acid (CH_3COOH), and the 99% purity sodium acetate ($NaCH_3COO$), ethanol and hydroquinone (HQ) 99% were from Sigma-Aldrich. All solutions were prepared with deionized water type I.

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† Electronic supplementary information (ESI) available: HQ Cyclic voltammograms obtained in the bare SPE, EIS plots recorded in the systems: SPE, SPW/GA and SPE/PVA, chronoamperograms (CA) and respective enzymatic kinetics for hydroquinone using the SPE/LTv/PVA and CA for recorded with SPE/TvL/GA-30% and SPE/TvL/GA-100% biosensors See DOI: 10.1039/c2ay25454a

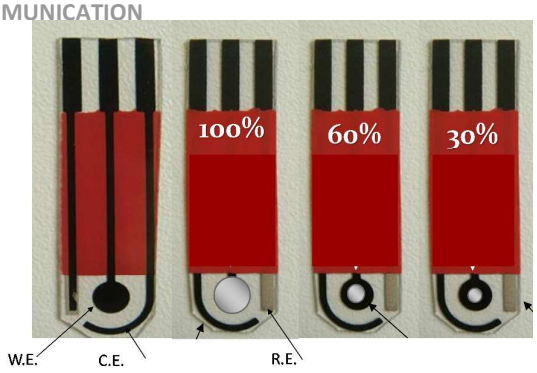

Figure 1. Screen-printed electrodes used, indicating in gray the zones of the respective working electrode that were covered during the enzyme immobilization process.

2.2 Construction of the Biosensors.

The enzyme immobilization onto the electrode surface was carried out by means of the cross-linking at 40 °C method [12]. A 50% v/v mix of the enzymatic solution (5 mg Laccase mg/mL) and 2.5 % glutaraldehyde was prepared. 5 µL of this solution was added over the screen-printed working electrode covering different quantities of its surface areas namely 30, 60 and 100% (see Figure 1) and subsequently the system was thermocured at (40.0 ± 0.5) °C for 1 h. These biosensors were labeled as SPE/TvL/GA-30%, SPE/TvL/GA-60% and SPE/TvL/GA-100%.

3. Results and discussion

3.1 Amperometric response of the SPE/TvL/GA biosensors.

With the different SPE/TvL/GA biosensors, immersed into a thermostated cell containing acetates' buffer 0.1 M at pH 4.7 ± 0.01 at (30.0 ± 0.5) °C under constant stirring, a potential of -0.30 V vs. Ag/AgCl pseudoreference electrode [13] was applied[‡] and the current response was monitored, see Figure 2a, the chronoamperograms recorded with SPE/TvL/GA-30% and SPE/TvL/GA-100% biosensors can be found as Figure S3 in ESI. Once the steady state was reached, aliquots of a standard HQ solution were added and the current variations were recorded, when stable, as a function of the HQ concentration, see Figure 2b.

In all cases the amperometric evaluation of the biosensors displayed a Michaelian-type kinetic behavior; therefore, the Hills model [12] was used to calculate the apparent Michaelis-Menten's (K_m') constant. It was evaluated following the Quinone, Q, potentiostatic reduction, that was enzymatically formed by mushroom TvL for different HQ concentrations. The results are reported in Table 1. It is possible to note that the lowest value of K_m' was found using the SPE/TvL/GA-60% biosensor. Furthermore, from the linear zone of the I vs HQ concentration plot, see Figure 3, it is possible to evaluate the analytic features of these biosensors towards HQ quantification, namely the sensitivity, the lowest detection (LOD) and quantification (LOQ) limits. These values are also reported in Table 1, from which it is possible to note that the best analytical performance corresponds to the biosensor of which the working electrode surface was solely covered up to 60% with the enzyme.

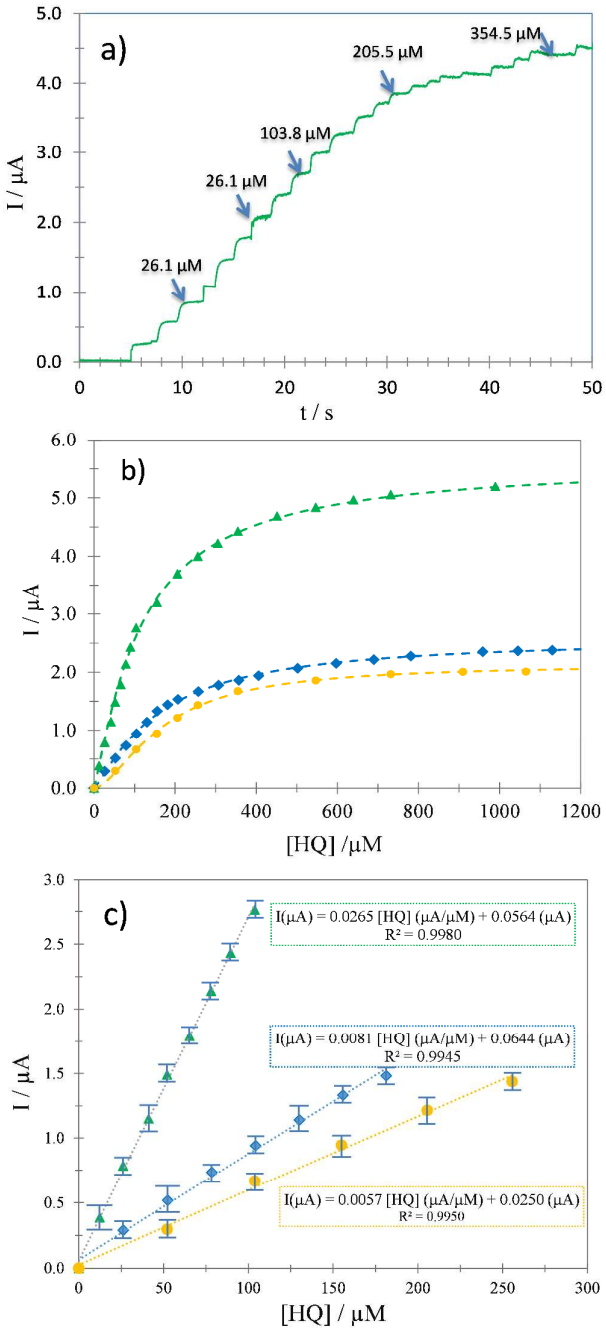

Figure 2. a) Chronoamperogram recorded with the SPE/TvL/GA-60% biosensor b) comparison of the respective enzymatic kinetics for hydroquinone recorded using the different laccasa biosensors: (◆) SPE/TvL/GA-30%, (▲) SPE/TvL/GA-60% and (●) SPE/TvL/GA-100% in acetates' buffer 0.1 M at pH 4.7 ± 0.01 and (30.0 ± 0.5) °C. Following the measured current, at –300 mV imposed potential, as a function of hydroquinone's concentration. The broken lines correspond to the fitting of the Hill's model [12], the best fit values for Km' are shown in Table 1. c) Linear zone of the I vs. HQ plots, see Figure 2b, alone with the respective linear fit. Each current value has been calculated on the basis of three different experiments; the error bars shown indicate the associated standard deviation.

Notwithstanding, even when the biosensor, constructed covering 30 % of its working electrode surface area with the enzymatic recognition agent displayed a lower analytical performance than that of the SPE/TvL/GA-60 % biosensor, its K_m' and sensitivity are comparable with those of the SPE/TvL/GA-100 % biosensor, although its LOD and LOQ are better than that of the fully covered working electrode biosensor.

Furthermore, this same study was conducted using biosensors where the enzyme was immobilized to the working electrode of the SPE by means of the entrapment method [12], see Table 2 and Figure S3 as ESI, and the same conclusions were reached regarding the influence of the ratio between the recognition element covered and the bare transductor surface area on the analytical performance of the electrochemical biosensor.

It is important to mention that even when TvL is responsible for the selective oxidation of HQ to Q, see Reaction R1, the electrochemical quantification of HQ requires the reduction of the Q enzymatically formed onto the surface of the transductor see Reaction R2. If this surface is blocked with the immobilization agent, namely namely SPE/GA or SPE/PVA, the charge transfer resistance increases notoriously as compared with the bare SPE surface, which can be inferred from the Electrochemical Impedance Spectroscopy, EIS, plots included in the ESI section, and provoking the observed changes in the biosensors performance.

In order to evaluate the stability of the different biosensors studied in this work and their reliability to carry out with each of them long-term quantification of the HQ concentration, the evaluation of their sensitivity as a function of time is depicted in Figure 3. From Figure 3a it is possible to note that the sensitivity of all the SPE/TvL/PVA biosensors varies linearly with time. Even when the sensitivity diminishes with time it is possible to use these electrodes during one year at least (The biosensors were stored at room temperature, under dry conditions kept away from the light and prior to using they were activated for ten minutes by immersion in an acetates' buffer 0.1 M at pH 4.7 ± 0.01 and (30.0 ± 0.5) °C. On the contrary, see Figure 3b, the SPE/TvL/GA biosensors stability is of about 4 months, after which their sensitivity starts to decrease almost to nil. Therefore, reliable quantification of HQ using the same SPE/TvL/GA biosensor is limited to 4 months.

Conclusions

In this work, a screen printed electrode (SPE) was used. Different quantities of the SPE working electrode surface areas (30, 60 and 100 %) were covered with Laccasa by means of two different sorts of immobilization, namely, cross-linking (using glutaraldehyde) and entrapment (PVA). The biosensor that displayed the lowest K_m', LOD and LQD values, having the highest sensitivity corresponded to that which working electrode surface was solely covered up to 60 % with the enzyme. Furthermore, the biosensor less covered with the enzyme (30 %) showed similar analytical performance than that completely covered (100 %). The same results were obtained regardless of the enzyme immobilization method. The surface areas ratio between that covered of the recognition element to that of the bare transductor plays a key role on the analytical performance of an electrochemical biosensor.

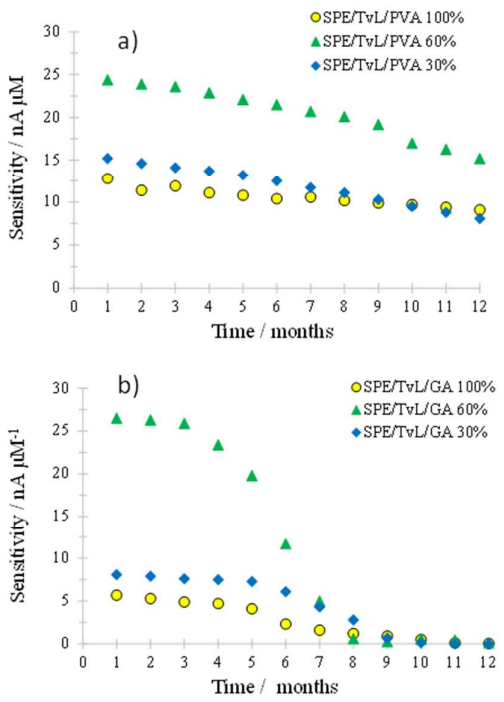

Figure 3. Comparison of the sensitivity time variation of a) SPE/TvL/PVA and b) SPE/TvL/GA biosensors: (◆) towards HQ quantification in acetates' buffer 0.1 M at pH 4.7 ± 0.01 and (30.0 ± 0.5)

Notes and references

‡ This is the peak potential value for Q reduction to HQ on the bare SPE, see the ESI section.

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$$SPE/TvL_{(\text{immobilized})} + HQ_{(aq)} \Leftrightarrow SPE/TvLHQ_{(aq)} \rightarrow SPE/TvL_{(\text{immobilized})} + Q_{(aq)} \quad \text{(R1)}$$

$$Q_{(aq)} + 2e^-_{SPE} \Leftrightarrow HQ_{(aq)} \quad \text{(R2)}$$

Table 1. Analytic parameters of laccase-based biosensors (immobilized onto the electrode surface by means of the cross-linking method) having different ratios between the recognition element covered and the bare transductor surface area, towards hydroquinone concentration in aqueous solution.

Biosensor	K_m' (μM)	Sensitivity (nA μM^{-1})	LOD (μM)	LOQ (μM)	Linear Range (μM)	R^2
SPE/LTv/GA-100%	170 ± 3	5.6 ± 0.4	14 ± 5	48 ± 17	26 ≤ [HQ] ≤ 258	0.99
SPE/LTv/GA-60%	116 ± 2	26 ± 2	0.63 ± 0.02	2.1 ± 0.8	0 ≤ [HQ] ≤ 103	0.99
SPE/LTv/GA-30%	169 ± 3	5.7 ± 0.7	2.2 ± 0.9	7.6 ± 0.3	0 ≤ [HQ] ≤ 255	0.99

Table 2. Analytic parameters of laccase-based biosensors (immobilized onto the electrode surface by means of the entrapment method*) having different ratios between the recognition element covered and the bare transductor surface area, towards hydroquinone concentration in aqueous solution.

Biosensor	K_m' (μM)	Sensitivity (nA μM^{-1})	LOD (μM)	LOQ (μM)	Linear Range (μM)	R^2
SPE/LTv/PVA-30%	180 ± 2	15 ± 1	1.8 ± 0.6	6.0 ± 0.2	0 ≤ [HQ] ≤ 268	0.9
SPE/LTv/PVA-60%	127 ± 2	24 ± 1	1.2 ± 0.4	4.1 ± 0.1	0 ≤ [HQ] ≤ 207	0.9
SPE/LTv/PVA-100%	188 ± 1	12 ± 1	2.7 ± 0.3	9.0 ± 0.3	0 ≤ [HQ] ≤ 268	0.9

*Five μL of the mix 50% v/v 5 mg·mL^{-1} TvL in phosphate buffer (0.1M, pH 7.00 ± 0.01) solution and water-soluble polyvinyl alcohol (PVA) were deposited over the screen-printed working electrode covering different quantities of its surface areas namely 30, 60 and 100% (see inset in Figure 1); subsequently, the electrodes were left to photocure for 3 hrs at 4 °C [8]; these biosensors were labeled as SPE/TvL/PVA-30%, SPE/TvL/PVA-60% and SPE/TvL/PVA-100% respectively.

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