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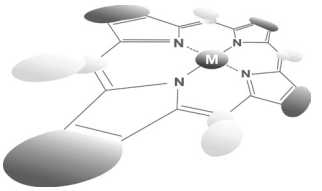

Electronic tongue formed by sensors and biosensors containing phthalocyanines as electron mediators. Application to the analysis of red grapes

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Dedicated to Professor Aslan Tsivadze on the occasion of his 70th birthday

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ABSTRACT: An electronic tongue formed by voltammetric sensors and biosensors containing phthalocyanines has been developed and used to analyze grapes of different varieties. The sensors are prepared using the carbon paste technique and have been chemically modified with different metallophthalocyanines. In turn, biosensors consist of carbon paste electrodes modified with phthalocyanines combined with tyrosinase or glucose oxidase. The response of the individual sensors towards model solutions of glucose and catechol have demonstrated that the voltammetric responses depend on the nature of the phthalocyanine, evidencing the important role of the electron mediator in the performance of the sensors. The capability of the system to discriminate grapes according to their sugar and their polyphenolic content has been evidenced using Principal Component Analysis. It has been demonstrated that the proposed array of sensors combines the advantages of classical phthalocyanine based sensors — that provide global information about the sample —, with the specificity of the enzyme substrate reaction typical of biosensors. For this reason, the selectivity of the multisensor system and its capability of discrimination is clearly improved when biosensors containing glucose oxidase or tyrosinase are included in the array.

KEYWORDS: electronic tongue, phthalocyanine, voltammetric sensor, biosensor, grape, must.

INTRODUCTION

During the last few years efforts have been made to develop a new type of instrument for the analysis of liquids, the electronic tongue (ET) [1–4]. According to the IUPAC, an ET is a multisensor system formed by an array of low-selective sensors combined with advanced mathematical procedures for signal processing based on Pattern Recognition and/or Multivariate data analysis [5]. These instruments provide global information about the sample instead of giving information of particular constituents. Due to this holistic approach, ET are particularly useful for the analysis of complex liquids such as wines [6–9]. Most of the works in the

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field of electronic tongues involve signal generation from electrochemical sensors including potentiometric, impedimetric or voltammetric sensors [9–12].

In the last few years, our group has developed an ET based on voltammetric sensors chemically modified with phthalocyanines (MPc) [7, 13–15]. Sensors based on phthalocyanines show interesting sensing properties due to their rich electrochemical and electrocatalytic behavior, and because their properties can be tuned by chemical modification [16–19]. In addition, sensors can be prepared using a variety of approaches that include simple methods such as the carbon paste technique or more sophisticated methods that produce nanostructured devices [11, 13, 20].

The voltammetric signals obtained from electrodes modified with phthalocyanines show redox peaks associated with the studied solution (as in classical metallic or carbonaceous electrodes), but in the modified electrodes, the position and intensity of such redox process are modulated by the interaction between the solution and the phthalocyanine (*i.e.* by the electrocatalytic behavior of the phthalocyanine) [18]. Phthalocyanines have also demonstrated their ability to act as electron mediators in voltammetric biosensors. In such devices, phthalocyanines are used to facilitate the electron transfer between the enzyme (*i.e.* tyrosinase) and the electrode surface [21, 22].

Arrays of sensors prepared from different phthalocyanine molecules have shown a high degree of cross-selectivity and have been successfully used to analyze model solutions of basic tastes and other complex liquids of industrial interest such as wines [4, 7, 14, 15].

The cross-selectivity of a multisensor system could be improved by constructing hybrid arrays combining low-specific sensors, with biosensors containing enzymes selective to specific compounds of interest. This can be of particular importance in the field of viticulture and enology where the content of certain compounds is directly related with the quality of wines and also with the quality of grapes from which wines are prepared [23, 24]. For instance, the quality of grapes is usually established by measuring their sugar content and acidity. In addition, it is becoming evident that the quality of grapes is also closely related with other parameters such as their phenolic composition [25, 26].

The aim of this work is to develop a hybrid multisensor system combining voltammetric sensors and biosensors and to evaluate its capability to discriminate musts prepared from different varieties of grapes according to their polyphenol and glucose content. For this purpose, an array of sensors based on phthalocyanines (MPc-CPE) has been combined with two arrays of biosensors based on enzymes (tyrosinase or glucose oxidase: MPC-Tyr-CPE and MPc-GOx-CPE respectively) where phthalocyanines have been used as electron mediators. The sensors have been immersed in model solutions of phenols (catechol) and sugar (glucose) and the responses have been evaluated. Then, the array has been used to analyze musts prepared from different varieties of grapes. The capability of discrimination of the sensor array has been investigated using Principal Component Analysis (PCA).

EXPERIMENTAL

Grape samples

Grapes of five different varieties were harvested in 2012 in the D.O. Ribera de Duero region (Spain). The varieties included were *Tempranillo*, *Garnacha*, *Cabernet-Sauvignon*, *Prieto Picudo and Mencía.* Samples were selected and harvested by the ITACYL and Bodega Cooperativa de Cigales (Spain). The Enological Centre of Castilla y León carried out the chemical analysis of the grapes and prepared the corresponding musts. Grapes were analyzed following international regulations [27]. **T**he results are compiled in Table 1.

Reagents and solutions

All reagents were of high purity and used without further purification (Sigma-Aldrich). All solutions were prepared with deionized water (18.3 $M\Omega.cm^{-1}$ resistivity, Milli-Q, Millipore). A 70 $\mu g.mL^{-1}$ solution of enzymes (tyrosinase and glucose oxidase) in buffer phosphate 0.01 M (pH 7.0) was used for the enzyme immobilization. 10^{-3} M solutions of catechol and glucose were prepared in phsophate buffer (pH 7.0) and citrate buffer (pH 3.1).

Cobalt(II), copper(II) and zinc(II) phthalocyaninates (CoPc, CuPc, ZnPc) were purchased from Sigma Aldrich. The lutetium(III) bisphthalocyaninate ($LuPc_2$) was synthesized and purified in its neutral radical state following previously published procedures [28].

Preparation of the electrodes

Array of sensors formed by Carbon Paste Electrodes (CPE) modified with phthalocyanines (MPc-CPE). An array formed by five CPE electrodes was prepared. It was formed by three electrodes modified with metalo-phthalocyanines including CoPc (CoPc-CPE), CuPc (CuPc-CPE), ZnPc (ZnPc-CPE), one electrode modified with $LuPc_2$ ($LuPc_2$-CPE) and one unmodified carbon paste electrode (C-CPE). CPEs were prepared as previously reported by mixing graphite powder and the corresponding phthalocyanine (15%, w/w). Nujol was used as the binder of the composite mixture. Pastes were packed into the body of a 1 mL plastic syringe and compressed. A metallic copper wire was used as a contact [29].

Array of biosensors formed by CPE electrodes modified with phthalocyanines and tyrosinase (MPc-Tyr-CPE). Five CPE sensors modified with phthalocyanines were prepared as described in the previous paragraph. The enzyme, tyrosinase (Tyr), was then immobilized on the surface of those electrodes using the drop casting

Table 1. Results of chemical analysis of the grapes

Number of sample	Variety of grape	Brix	Density	Sugar, g/l	Degree (16.8)	Degree (17.5)	pH	Total acidity, g/l	Malic acid, g/l	Tartaric acid, g/l	Potassium, mg/l	Total polyphenol index
1	Tempranillo	24	1.0998	238.2	14.15	13.6	4.18	4.14	2.03	5.23	1770	20
2	Prieto Picudo	23.3	1.0965	229.9	13.66	13.15	3.59	4.94	0.83	7.62	1830	15
3	Mencía	22	1.0906	214.8	12.76	12.3	4.17	4.26	1.53	5.03	1820	12
4	Cabernet	19.43	1.0791	185.1	11	10.6	3.71	3.48	1.68	3.88	1450	15
5	Garnacha	23.12	1.0956	227.6	13.52	13	3.42	4.37	0.53	6.87	1340	15

technique followed by cross-linking. For this purpose 10 μL of 0.01 M phosphate buffer (pH 7.0) containing 70 $\mu g.mL^{-1}$ of enzyme, were deposited onto the electrode surface. After drying at room temperature for 45 min, the biosensors were placed in a saturated glutaraldehyde vapor for 30 min and dried in air for 15 min at room temperature. The biosensors were then rinsed with phosphate buffer to remove any unbound enzyme and stored at 4 °C. Using this method, five biosensors were prepared (C-Tyr-CPE; CoPc-Tyr-CPE, CuPc-Tyr-CPE, ZnPc-Tyr-CPE and $LuPc_2$-Tyr-CPE) [30].

Array of biosensors formed by CPE electrodes modified with phthalocyanines and glucose oxidase (MPc-GOx-CPE). Five CPE sensors modified with phthalocyanines were prepared as described in previous paragraphs but in this case, 10 μL of 0.01 M phosphate buffer (pH 7.0) containing 70 $\mu g.mL^{-1}$ of glucose oxidase (GOx), was immobilized on the surface of those electrodes using the drop casting technique followed by the cross-linking above described. Using this method, five biosensors were prepared (C-GOx-CPE; CoPc-GOx-CPE, CuPc-GOx-CPE, ZnPc-GOx-CPE and $LuPc_2$-GOx-CPE).

Electrochemical measurements and data analysis

Electrochemical experiments were performed using a three electrode system with a Ag/AgCl reference electrode, a 1 cm^2 platinum as the counter electrode and a sensor or a biosensor as working electrode (the sensors forming the array were polarized sequentially). The potentiostat used was an EG&G PARC, Model 273 potentiostat/galvanostat (Princeton Applied Research Corp). Voltammograms were carried out at a scan rate of 0.1 $V.s^{-1}$ and the working range was adapted to the nature of each class of electrodes. Seven repetitions per sample were measured. In order to reduce the high dimensionality of the recorded signals (samples x sensors x potentials), a preprocessing stage was required. For this, a feature extraction tool based on "bell-shaped-windowing" curves called "*kernels*" was used to compress the information from the original signals and to extract meaningful data from the readings [31, 32]. Then, the obtained coefficients (10 coefficients per curve) were used as the input variables for Principal Component Analysis (PCA), used for recognition of sample patterns and dis(similarities) between varieties of grapes.

RESULTS AND DISCUSSION

As stated in the introduction section, the quality of grapes is mainly related with their sugar concentration and their polyphenolic content. For this reason, an array of CPE sensors combined with two arrays of CPE biosensors purposely dedicated to detect the sugar and polyphenolic content of grapes has been developed.

In a first step of this work, the response of the three arrays (MPc-CPE sensors, MPc-Tyr-CPE biosensors modified with tyrosinase and MPc-GOx-CPE biosensors modified with glucose oxidase) were tested separately using two model solutions: a 10^{-3} M glucose solution to evaluate the capability of the sensors to detect sugars and a 10^{-3} M catechol solution to evaluate the capability to detect phenols. The responses of the three arrays *vs.* catechol and glucose were also used to evaluate the electrocatalytic properties of the different phthalocyanines and their capabilities as electron mediators. Once characterized, the three arrays were exposed to musts prepared from grapes of different varieties. Finally, the three arrays were combined and the capability of the hybrid array formed by sensors and biosensors was evaluated.

Response of the array of CPE sensors modified with phthalocyanines (MPc-CPE)

The response of the bare C-CPE electrode towards 10^{-3} M catechol in buffer phosphate (pH 7.0) showed the expected anodic (0.5 V) and cathodic (0.05 V) waves associated with the oxidation of the o-dihydroquinone to benzoquinone ($\Delta E_{1/2}$ = 0.45 V) (Fig. 1). CoPc-CPE, CuPc-CPE and ZnPc-CPE showed a similar response than C-CPE, however, the intensity of the redox peaks related with the phenol increased their intensity (from 4 μA in C-CPE to *ca.* 15–13 μA in CoPc, CuPc and ZnPc). Note that in the case of the CoPc-CPE electrode, a peak due to the oxidation of Co was also clearly observed at 0.2 V. The most important electrocatalytic effect was observed when using the $LuPc_2$-CPE sensor: the anodic peak produced by the oxidation of the phenol appears shifted to lower potentials (observed at 0.4 V instead of 0.5 V) and the cathodic wave were at 0.15 V (ΔE = 0.25 V). According to these results it can be confirmed that in the $LuPc_2$-CPE sensor the overpotential was reduced and the reversibility was clearly improved. It is also important to remark that the redox activity of the lutetium bisphthalocyanine associated to the $LuPc_2/LuPc_2^-$ was observed at -0.2 V (anodic) and -0.4 V (cathodic).

CPE sensors modified with phthalocyanines were also used to detect glucose. It is well-known that glucose it is difficult to detect using non-catalytic electrodes, because the oxidation of glucose to gluconic acid requires large overpotentials [33]. This is the reason why the C-CPE electrode could not detect the presence of glucose and the voltammograms were characterized by a complete absence of peaks. When using CPE chemically modified with ZnPc-CPE or CuPc-CPE the response towards glucose was weak and only one small cathodic wave was observed at *ca.* -0.5 V (Fig. 2a). In contrast, when electrodes chemically modified with CoPc-CPE and $LuPc_2$-CPE were tested, a drastic increase in the cathodic response at *ca.* -0.8 V was observed (from -10 μA in C-CPE and ZnPc-CPE, to -50 -55 μA in CoPc-CPE and $LuPc_2$-CPE) (Fig. 2b).

According to these results, it can be concluded that the chemical structure of the phthalocyanine plays a

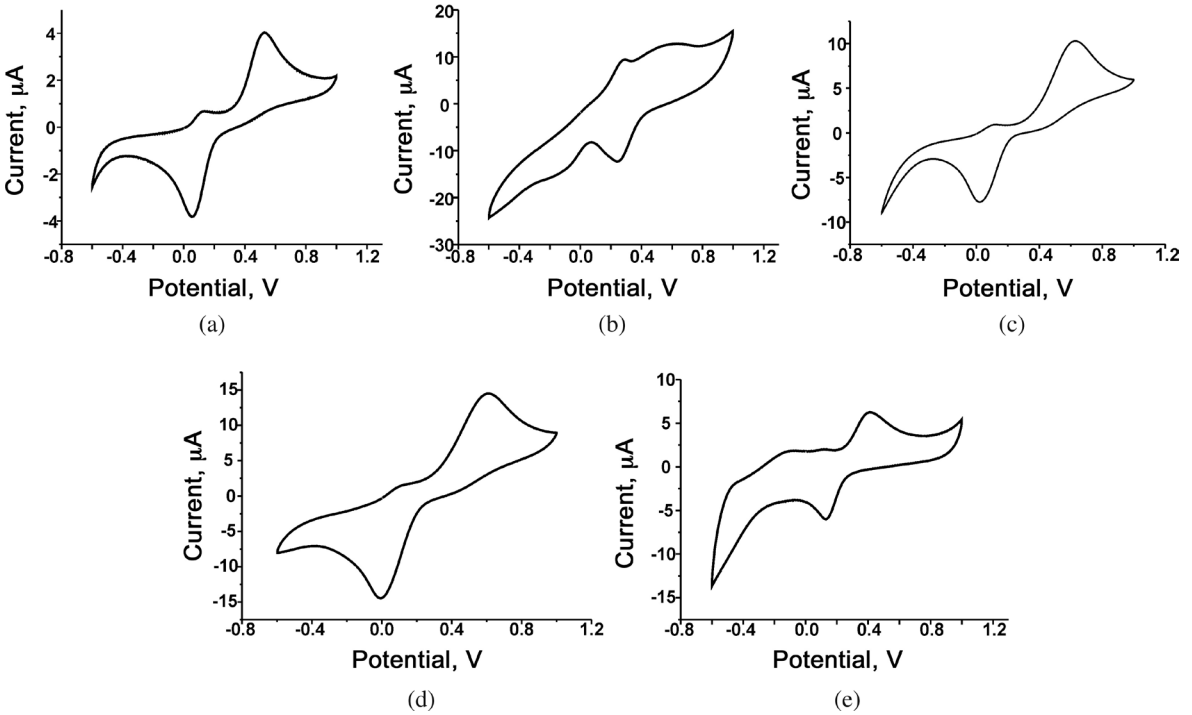

Fig. 1. Response of CPE electrodes modified with phthalocyanines to 10^{-3} M catechol in buffer phosphate (pH 7.0) (a) bare C-CPE, (b) CoPc-CPE, (c) CuPc-CPE, (d) Zn-CPE and (e) $LuPc_2$-CPE. Scan rate 0.1 $V.s^{-1}$

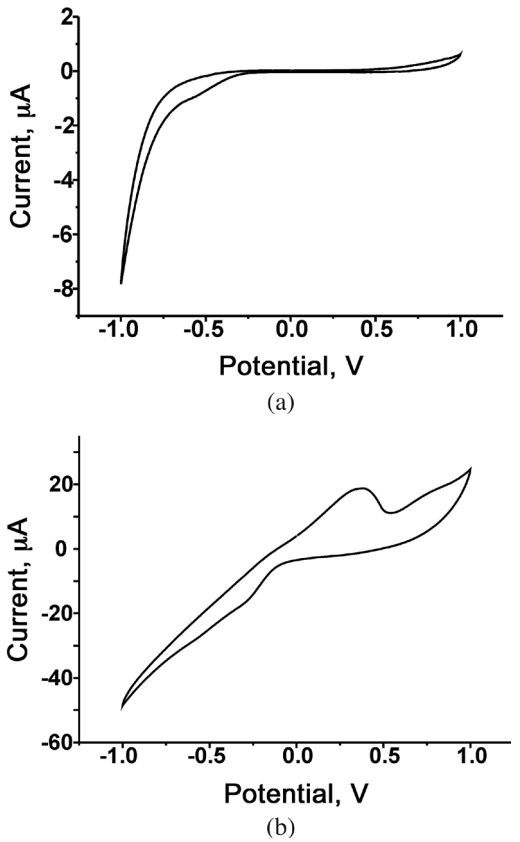

Fig. 2. Response of CPE electrodes modified with phthalocyanines to 10^{-3} M glucose in buffer phosphate (pH 7.0) (a) ZnPc-CPE and (b) CoPc-CPE. Scan rate 0.1 $V.s^{-1}$

critical role in detection of catechol and glucose. It is particularly remarkable that CoPc and $LuPc_2$ (which have redox reactivity in the working range) show enhanced electrocatalytic properties facilitating the electrochemical oxidation/reduction of both catechol and glucose.

Taking into account that grapes and musts obtained from the berries have a pH close to 3.5, the response of the CPE sensors modified with phthalocyanines towards catechol and glucose was also tested at an acidic pH obtained from a buffer citrate (pH 3.1). The effect of the acidic pH is illustrated in Fig. 3 where the responses of the $LuPc_2$-CPE towards catechol and glucose at pH 7.0 and at pH 3.1 are shown.

It is well-known that pH affects the electrochemical response of phenols and when decreasing the pH, the oxidation of phenols occurs at higher potentials [34, 35]. This effect was also observed in our phthalocyanine-based electrodes (Fig. 3a). Moreover, at pH 3.1, the intensity of the voltammograms increased drastically. This increase in the sensitivity could be due to the protonation of the phthalocyanine molecule and can be a good advantage in the detection of phenols in musts.

The effect of pH in the detection of glucose was the opposite and the intensity of the peaks decreased at pH 3.1 (Fig. 3b). This result is in accordance with previously published results that conclude that gluconic acid in its free form reversibly inhibits the oxidation process in acidic media [36]. Nevertheless, the inhibition is only

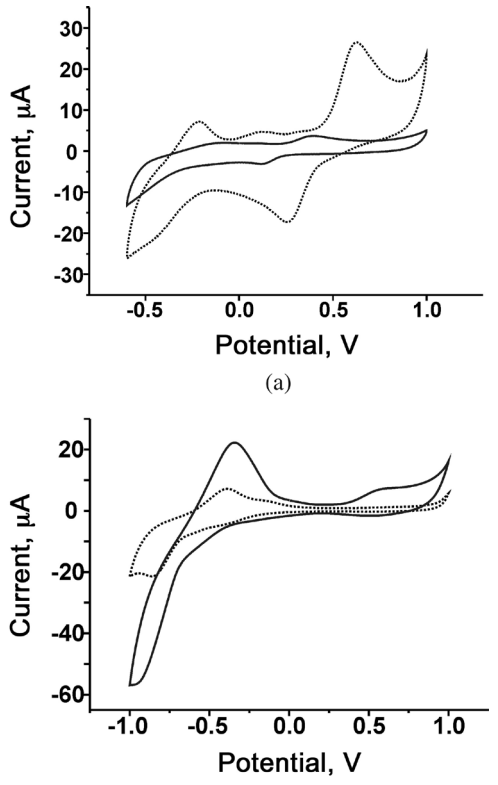

Fig. 3. Cyclic voltammograms of $LuPc_2$-CPE electrodes immersed in (a) 10^{-3} M catechol and (b) 10^{-3} M glucose. Solid lines correspond to measures in buffer phosphate (pH 7.0) and dashed lines correspond to measures registered in buffer citrate (pH 3.1). Scan rate 0.1 $V.s^{-1}$

partial and the intensity of the signals is good enough for applications in the field of wines and musts.

Response of the array of biosensors formed by CPEs modified with phthalocyanines and tyrosinase (MPc-Tyr-CPE)

In order to improve the selectivity of the electronic tongue towards polyphenols, an array formed by biosensors containing tyrosinase was prepared. Tyrosinase is involved in the oxidation of diphenols to the corresponding quinoid form. Then, the formed benzoquinone can be reduced electrochemically at the electrode surface. Phthalocyanines used as electron mediators can facilitate the transfer of electrons from the enzyme to the electrode, improving the performance of the biosensors [21, 22, 37].

The response of the array MPc-Tyr-CPE electrodes towards catechol is illustrated in Fig. 4. The influence of the pH in the response is also illustrated in the figure.

In all MPc-Tyr-CPE electrodes, voltamograms registered at pH 7.0, showed the electrochemical reduction of the previously formed benzoquinone in the -0.4 V region (Fig. 4a). The reduction of the quinone to the phenolic form, shifted to lower potentials in the presence of phthalocyanines (results not shown). It is particularly remarkable the shift observed in CoPc-Tyr-CPE, and $LuPc_2$-Tyr-CPE where this process occurs at *ca*. 0.0 V. The electrochemical behavior of these two electrodes are also different from the rest, because redox processes of the phthalocyanines which occur in the

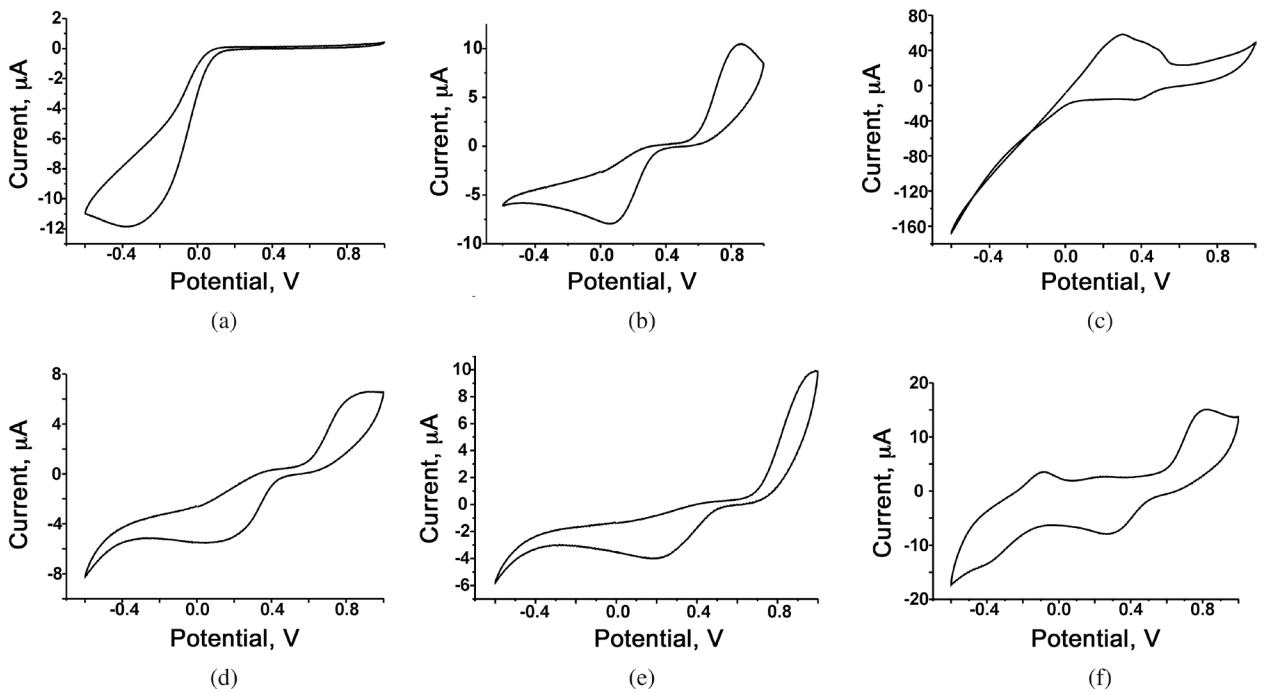

Fig. 4. Response of CPE electrodes modified with phthalocyanines and tyrosinase towards 10^{-3} M catechol (a) bare C-Tyr-CPE in buffer phosphate (pH 7.0), (b) bare C-Tyr-CPE in buffer citrate (pH 3.1), (c) CoPc-Tyr-CPE in buffer citrate (pH 3.1), (d) CuPc-Tyr-CPE in buffer citrate (pH 3.1), (e) ZnPc-Tyr-CPE in buffer citrate (pH 3.1) and (f) $LuPc_2$-Tyr-CPE in buffer citrate (pH 3.1). Scan rate 0.1 $V.s^{-1}$

working range (Co^{I}/Co^{II} and $LuPc_2^{-}/LuPc_2$), can facilitate the electron transfer to the electrode.

The pH of a solution has several effects on the structure and activity of enzymes. The optimal pH for tyrosinase is about 6–7 [37]. The acidic pH of grapes and musts (close to 3.5) could inhibit the enzymatic activity. In Figs 4a and 4b the voltammograms of the C-Tyr-CPE biosensor towards catechol dissolved in buffer phosphate (pH 7.0) and in buffer citrate (pH 3.1) are compared. The enzymatic reduction of the benzoquinone shifted from -0.35 V at pH 7 to +0.17 V at pH 3.1 while the intensity of the signal decreased. The most remarkable difference is that at pH 3.1, a new peak was observed at *ca*. 0.8 V. The acidity dissociation constants (pKa) for catechol are both above pH 7, but the pKa (1) for the catechol radical is close to 4 [38]. Thus, the pH can affect the dissociation state and hence the electroxidation/reduction mechanism of the catechol, facilitating the electrochemical oxidation at 0.8 V.

As shown in Figs 4c–4f, similar trends were observed in all the MPc-Tyr-CPE sensors immersed in catechol solved in citric acid (pH 3.1).

Response of the array of biosensors formed by CPEs modified with phthalocyanines and glucose oxidase (MPc-GOx-CPE)

In order to increase the selectivity towards glucose, a third array formed by biosensors containing glucose oxidase was prepared. In electrochemical biosensors based on the glucose oxidase, the enzymatic oxidation of glucose produces gluconic acid and hydrogen peroxide with consumption of oxygen [39]. The reduced glucose oxidase is not able to transfer electrons to conventional electrodes because the distance between its redox centers and the electrode surface exceeds the distance across which electrons are transferred at sufficient rates. Therefore, electrical communication between the redox centers of this enzyme and electrodes require the presence of electron mediators. A suitable mediator for the GOx enzyme must have a more positive redox potential than the redox potential of the coenzyme FAD (*ca*. -0.4 V *vs.* Ag/AgCl) [40]. It has been reported that some phthalocyanines such as the CoPc can act as electron mediators of the GOx enzyme [41]. In this work, the electron mediator properties of CoPc, CuPc, ZnPc and $LuPc_2$ have been analyzed and compared.

At pH 7.0 (buffer phosphate), the presence of phthalocyanines increased the intensity of the response towards glucose with respect to the signal obtained using an electron mediator free electrode. This is illustrated in Fig. 5a where the responses of the phthalocyanine-free C-GOx-CPE electrode and the CuPc-GOx-CPE and the ZnPc-GOx-CPE electrodes are compared. However, the difference between the response of an electrode modified with both phthalocyanine and GOx and one electrode modified only with a phthalocyanine is not so dramatic.

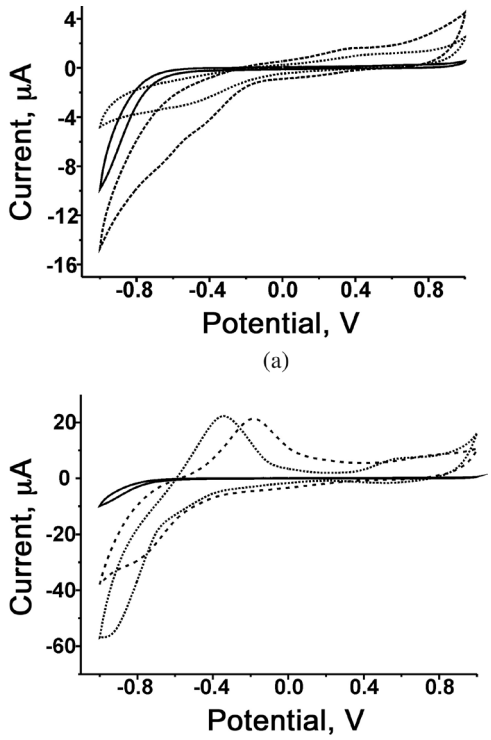

Fig. 5. (a) Voltammetric response of CPE electrodes modified with phthalocyanines and glucose oxidase to glucose 10^{-3} M in buffer phosphate (pH 7.0): (solid line) C-GOx-CPE, (dotted line) CuPc-GOx-CPE, (dashed line) ZnPc-GOx-CPE. (b) Cyclic voltammograms of (solid line) C-GOx-CPE, (dotted line) $LuPc_2$-CPE, (dashed line) $LuPc_2$-GOx-CPE immersed in glucose 10^{-3} M in buffer phosphate (pH 7.0). Scan rate 0.1 $V.s^{-1}$

Except in the case of electrodes containing $LuPc_2$ (Fig. 5b) because $LuPc_2$ has a stronger effect than the other phthalocyanines, showing a higher reduction current. Moreover, in the presence of GOx, the reduction peak is shifted by *ca*. 0.2 V towards more positive potentials.

On the other hand, the optimal pH for glucose oxidase is 5.5 (a broad range of 4–7 is acceptable). Surprisingly, the voltammograms registered in buffer citrate at pH 3.1 (closest to the pH found in wines and musts) showed a clear increase in the intensity of the responses, that was particularly noticeable for the $LuPc_2$-GOx-CPE (Fig. 6). This enhancement can be the result of the protonation of the phthalocyanine ring that modifies the electrochemical properties of the phthalocyanines [13, 29] improving their electron mediator activity.

Response towards musts

The sensors and biosensors described in the previous sections were exposed to musts prepared from grapes of different varieties. In order to decrease the complexity of the samples, musts were diluted 50% in water (final pH of *ca*. 4.2) (Fig. 7). Voltammograms were dominated by the redox response of the phenolic groups present in

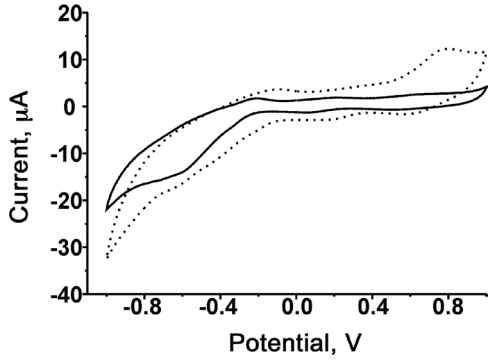

Fig. 6. Response of (solid line) $LuPc_2$-CPE, (dotted line) $LuPc_2$-GOx-CPE to 10^{-3} M glucose in buffer citrate (pH 3.1). Scan rate 0.1 $V.s^{-1}$

region was also observed that could be associated with the glucose but also with the presence of protons.

The intensity and position of the peaks depend on the nature of the phthalocyanine used as modifier. As expected, in the case of sensors modified with CoPc and $LuPc_2$ the peaks increased their intensity and shifted to lower potentials.

Obviously, the presence of tyrosinase or glucose oxidase also introduced important modifications in the electrochemical responses. The intensities and positions of the peaks were related with the total polyphenols index and the content of glucose measured by chemical methods, that in turn depend on the grape variety (Table 1).

In summary, each electrode provided a different response towards the same must sample and an important degree of cross-selectivity was attained.

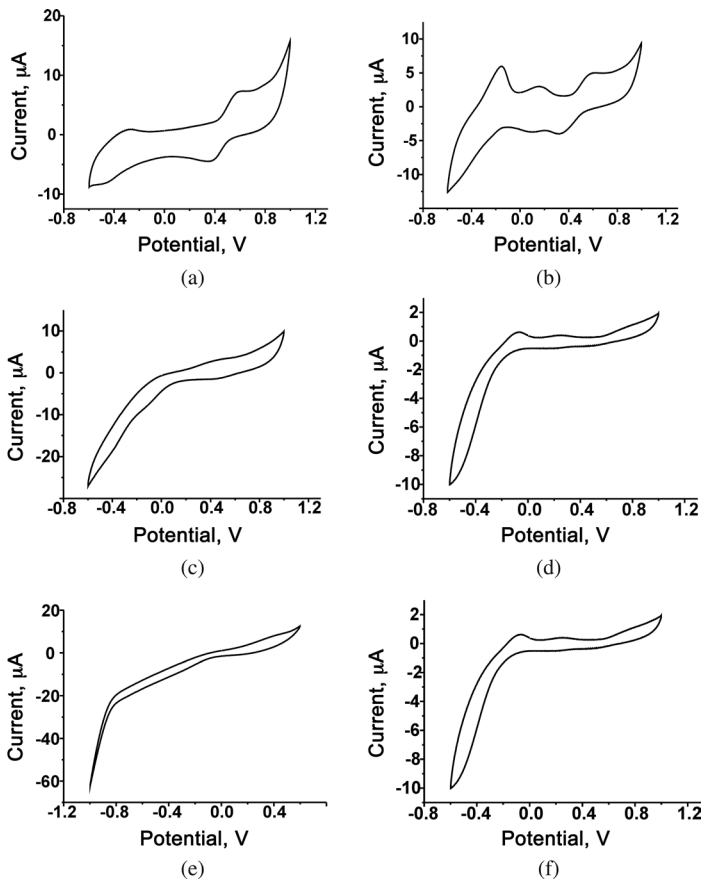

Fig. 7. Response of CPE electrodes modified with phthalocyanines to garnacha must diluted 50% in water (a) CoPc-CPE, (b) $LuPc_2$-CPE, (c) CoPc-Tyr-CPE, (d) $LuPc_2$-Tyr-CPE (e) CoPc-GOx-CPE and (f) $LuPc_2$-GOx-CPE. Scan rate 0.1 $V.s^{-1}$

musts that appear as anodic peaks in the 0.4–0.8 V region, and the corresponding cathodic waves in the 0.35 V region. Obviously in such a complex media, the peaks were broader than in the corresponding model solutions and a variety of other small and not well-defined peaks were detected. In all cases, a cathodic peak in the -0.5 V

Data treatment

As demonstrated in the above sections, the CPE sensors and biosensors show complex voltammograms that contain information about the musts. For this reason, voltammograms can be used to discriminate the must samples, using Principal Component Analysis (PCA) [15, 29, 43].

In order to evaluate the capability of the three arrays to discriminate the variety of grapes, they were immersed in each must and 7 replicas of each sample were registered. Then, voltammograms were pretreated to decrease the number of variables used in the PCA calculations, by means of the kernel method. The 10 values obtained from each voltammogram were scaled between the maximum and minimum values to discard range current effects, then standardized (mean value = 0, standard deviation = 1) to build the matrix used for the pattern recognition techniques.

The three arrays were able to discriminate the five musts. This is illustrated in Fig. 8 where the PCA scores plot and the loadings plot corresponding to the musts analyzed with the array of MPc-Tyr-CPE biosensors containing tyrosinase is shown. As observed in the scores plot (Fig. 8a), the percentage of variance explained using two principal components was 97.28%. That is, almost the total information of the model was contained in two principal components. The array discriminated the musts according to their polyphenol content. For this reason Tempranillo must which has the highest TPI (see Table 1) appears on the right side of the graph, in the positive PC1, far apart from the rest of the musts, whereas, Mencia must that shows the lowest TPI content appears at negative PC1 on the left

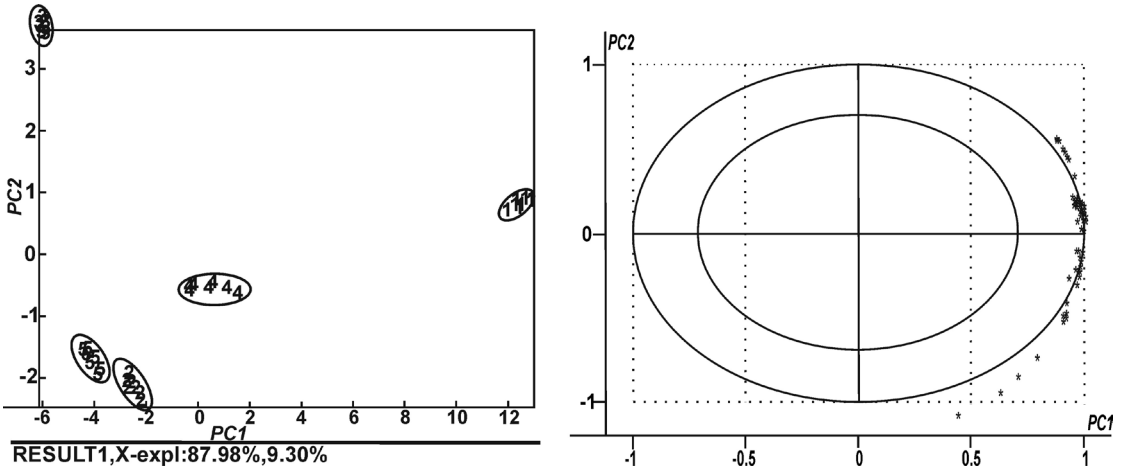

Fig. 8. Left: PCA scores plot corresponding to the classification of the musts. Analysis performed using the CPE modified with phthalocyanines and tyrosinase. 1 — Tempranillo, 2 — Prieto Picudo, 3 — Mencía, 4 — Cabernet and 5 — Garnacha. Right: loading plot of the PCA (ten variables were extracted per sensor using the kernel method)

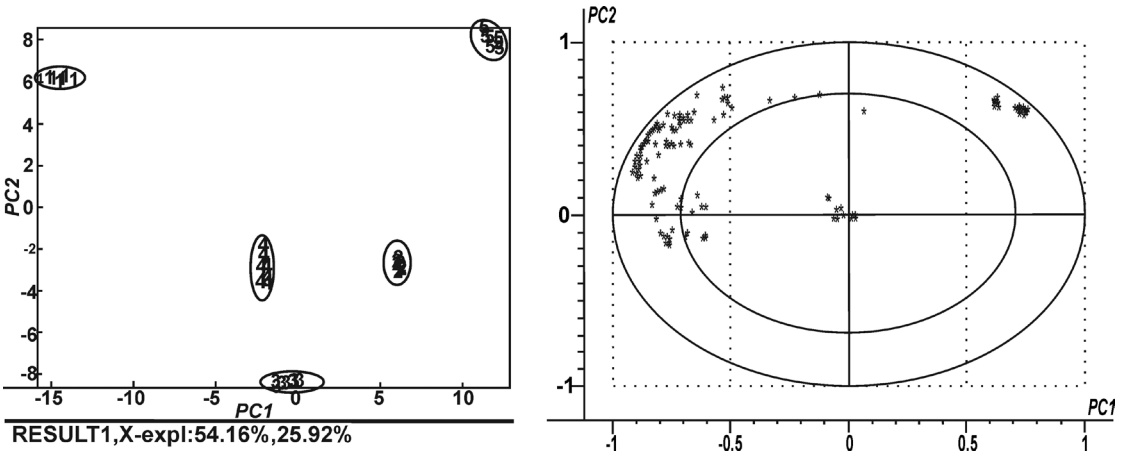

Fig. 9. Left: PCA scores plot and (right) loading plots corresponding to the classification of the musts. Analysis performed using the array formed by the CPE modified with phthalocyanines, CPE modified with phthalocyanines and tyrosinase and CPE modified with phthalocyanines and glucose. 1 — Tempranillo, 2 — Prieto Picudo, 3 — Mencía, 4 — Cabernet and 5 — Garnacha. Right: loading plot of the PCA (ten variables were extracted per sensor using the kernel method)

side of the diagram. Prieto Picudo, Garnacha and Cabernet musts, appear close one to each other because they have a similar polyphenolic content (TPI = 15). As observed in Fig. 8b where the loading plot is presented, the loadings provided by each sensor (ten kernels per sensor) appear in different regions of the diagramme confirming that the central metal ion coordinated to the phthalocyanine ring plays an important role in the selectivity of the sensors. This result demonstrates that sensors based on different phthalocyanines provide complementary information and show an important degree of cross selectivity. Similar results were obtained when using the array of MPc-GOx-CPE biosensors where the array discriminates according to the glucose content.

Finally, the three arrays of MPc-CPE, MPc-Tyr-CPE and MPc-GOx-CPE were combined in a single multisensory system and the PCA was performed. The results are shown in Fig. 9 where the PCA scores plot and loading plot are presented. In this case, due to the increase in the number of sensors (and variables) the three Principal Components bring a noticeable amount of information. The first Principal Component (PC1) explains the 54.16% of the information, the second Principal Component (PC2) the 25.92% and the third one the 9.46%.

As observed in the figure, when using the complete set of sensors a better discrimination is achieved and all the musts appear well-separated. The combined array containing non-specific MPc-CPE sensors with biosensors sensitive to polyphenols (MPc-Tyr-CPE) and glucose (MPc-GOx-CPE) provides a specific signature for each must that allows discriminating the samples according to the variety of grape. It is also remarkable that sensors forming the array have an excellent complementarity as observed in the loading plot (Fig. 9b).

Although the system provides global information about the sample, it is important to notice that some correlations with the composition can be found in the PCA. For instance, the must prepared from grapes of the variety Tempranillo appear on the left and up side of the graph, well apart from the rest of the musts. This result is in good accordance with the results obtained by means of chemical analysis shown in Table 1, where it can be observed that the grapes of the variety Tempranillo have a TPI and a glucose content (and a Brix degree) clearly higher than the levels observed in the other musts. Musts prepared from Mencia, Prieto Picudo and Cabernet appear in the inferior part of the graph, indicating that their TPI and Brix degree is lower. As the quality of the grapes (and of the corresponding musts) depends on the TPI and the sugar content, this result indicates that our bioelectronics tongue could be an interesting instrument to evaluate the quality of grapes.

CONCLUSION

An ET based on voltammetric sensors and biosensors containing phthalocyanines has been developed and used to discriminate musts prepared from different varieties of grapes.

The important degree of cross-selectivity of the MPc-CPE array towards catechol and glucose was related with the electrocatalytic properties of the phthalocyanines used. It has been demonstrated that the electrocatalytic effect of CoPc and $LuPc_2$ towards catechol and glucose is stronger than that observed in ZnPc and CuPc. In addition, the performance of the arrays of biosensors modified with tyrosinase and glucose oxidase (MPc-Tyr-CPE and MPc-GOx-CPE) was also improved in the presence of phthalocyanines as electron mediators.

The electrochemical responses towards musts were related with the content of polyphenols and glucose of the samples. A multivariate data treatment was made to explore the capability of discrimination of the array. PCA showed a good discrimination according to the composition of the musts in terms of polyphenolic content and sugar concentration. The capability of discrimination of the system was improved by combining the MPc-CPE sensors and the MPc-Tyr-CPE and MPc-GOx-CPE biosensors to form an array of sensors with high cross-selectivity. These results confirm the possibility of using this new array of sensors to the study the quality of grapes.

In summary, this multisensor system containing sensors and biosensors could give the classical global information about the characteristics of the samples plus a complementary information about sugars and polyphenolic content that could help to decide important issues such as the retail price (marked by the quality) or the appropriate date for harvesting.

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