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Multisensor system based on bisphthalocyanine nanowires for the detection of antioxidants

Mónica Gay Martín [a], José Antonio de Saja [b], Raquel Muñoz [c], María Luz Rodríguez-Méndez [a,*]

[a] Inorganic Chemistry Department, Engineers School, University of Valladolid, Spain
[b] Condensed Matter Physics Department, Faculty of Sciences, University of Valladolid, Spain
[c] Biochemistry Department, Faculty of Sciences, University of Valladolid, Spain

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ABSTRACT

Electrophoretic deposition has been used to prepare thin films based on nanowires of three lanthanoid bisphthalocyaninates (including dysprosium, gadolinium and lutetium). Nanowires of similar structural characteristics have been obtained for the three compounds by tuning the electrophoretic conditions according to the redox properties of each phthalocyanine. The three electrodes have been used to form an array of sensors that has been employed to discriminate phenolic antioxidants of interest in the food industry including caffeic, gallic, vanillic and ferulic acids. The Principal Component Analysis (PCA) and the Partial Least Squares Discriminant Analysis (PLS-DA) of the electrochemical signals has allowed a clear discrimination of the four phenols analyzed according to the number of phenolic groups attached to the structure (monophenol, diphenol or triphenol). The PCA loading plots indicate that the three electrodes bring complementary information facilitating the discrimination of the studied solutions. In addition, good correlations between the intensity of the redox processes observed in the electrodes and the concentration of phenolic compounds have been found with detection limits in the range of 10^{-5}–10^{-6} mol L^{-1} and good reproducibility.

The fast preparation of these nanowires based films and their excellent performance offer a new sensing platform for the detection of antioxidants in a fast, reliable way.

1. Introduction

In food analysis, a wide range of traditional methodologies, including chromatography, spectroscopy and electrochemistry, are used to determine or detect characteristic compounds. During the last years, a new technology for the analysis of foods has been developed, called electronic tongue that consists of an array of electrodes with cross selectivity coupled to a pattern recognition software [1,2]. The most-used methods in e-tongues employ electrochemical techniques, including potentiometry [3–5], amperometry [6], cyclic voltammetry [7,8] or impedance measurements [9,10]. Much attention has been paid to the analysis of foods and beverages, and in particular, to the analysis of antioxidants [11,12], beers [13] and wines [5,14–17].

Our group has developed an innovative type of multisensors based on voltammetric electrodes chemically modified with electroactive materials [18,19]. In such electrodes, the interactions between the sensing material and the studied solution (diffusion of ions inside the electrode, electrocatalytic effect of the sensing material, oxidant or reducing nature of the studied solution, etc.) produce specific electrochemical signals for each analyzed solution. The treatment of the electrochemical signals, using chemometric techniques, has allowed discriminating a variety of foods and beverages, such as wines [17], fishes [20], and oils [21].

Lanthanoid bisphthalocyanines ($LnPc_2$) have shown two important advantages as electrochemical modifiers for voltammetric sensors. The first advantage is related to their remarkable electrochemical and electrocatalytic properties [22–26]. The second benefit is that electrodes can be prepared using different techniques giving rise to electrodes with different structures and hence, with different properties. Electrodes based on $LnPc_2$ have been prepared by classical methods such as the carbon paste technique, or by depositing thin films onto conductive substrates by casting, spin coating or ultrahigh vacuum evaporation [17,18]. Nanostructured films prepared by the Langmuir–Blodgett (LB) [18,26,27] or the layer by layer (LbL) techniques [28,29], have shown enhanced surface to volume ratios, and well controlled structures that facilitate the diffusion of ions inside the film, giving rise to sensors with faster kinetics than non nanostructured films [18].

Electrophoretic deposition (EPD) can be an alternative technique to prepare nanostructured sensors based on bisphthalocyanines [30–32]. Using EPD our group prepared recently sensors

* Corresponding author. Tel.: +34 983 423540; fax: +34 983 423310.
E-mail address: mluz@eis.uva.es (M.L. Rodríguez-Méndez).

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consisting of lutetium bisphthalocyanine ($LuPc_2$) nanowires [33]. The morphology of the films and their electrochemical behavior in simple electrolytes was also reported [33].

In this work the study has been extended to other $LnPc_2$ derivatives including gadolinium and dysprosium bisphthalocyanines ($GdPc_2$ and $DyPc_2$). The objectives of this work are two. On one hand, the work aims to find the appropriate experimental conditions to prepare for the first time nanowires of $GdPc_2$ and $DyPc_2$ and to study their electrochemical properties, analyzing the influence of the metal in the electrochemical behavior of the nanostructured electrodes. On the other hand the work aims to evaluate the possibility of using such nanostructured sensors as the sensing elements of an electronic tongue with potential applications in the food industry. For this purpose, the sensing properties of the sensors toward hydroalcoholic solutions of antioxidants present in wines including caffeic acid, gallic acid, vanillic acid and ferulic acid will be evaluated in terms of stability and detection limit. These phenols have an important role in the antioxidant and organoleptic characteristics of wines. Finally, an array of sensors will be formed using the three electrodes $GdPc_2$, $DyPc_2$ and $LuPc_2$. The capability of discrimination and of classification of the system toward the antioxidants above mentioned will be analyzed using Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA).

2. Experimental

Three lanthanoid bisphthalocyanines ($LnPc_2$) with different central ions were included in the study. These molecules were gadolinium (III) bisphthalocyanine ($GdPc_2$), dysprosium (III) bisphthalocyanine ($DyPc_2$) and lutetium (III) bisphthalocyanine ($LuPc_2$). They were synthesized following a previously reported method [34,35].

All reactants were purchased from Panreac and were used without further purification. Indium/tin oxide (ITO) coated glass slides (2.5 cm × 1 cm) were used as the cathode and a platinum plate was used as the anode in the EPD experiments. Before deposition, the ITO glass slides were sonicated for 5 min in acetone and finally cleaned with chloroform. The electrophoretic solution was a chloroform solution where the corresponding phthalocyanine and trifluoroacetic acid (TFA) were solved. The electrodes were kept at a constant distance (1 cm) and a voltage of 20 V cm^{-1} was applied between the two electrodes. The concentrations of the bisphthalocyanine as well as the deposition times used depended on the central metal of the molecule. The concentration of the bisphthalocyanines was 5×10^{-5} mol L^{-1} for $GdPc_2$ and $DyPc_2$ and 1×10^{4} mol L^{-1} for the $LuPc_2$. The deposition times were 90 s for $GdPc_2$ and 180 s for $DyPc_2$ and $LuPc_2$. After deposition, the obtained films were annealed at 150 °C for 1 h (at atmospheric pressure) in order to improve their electrochemical stability.

The antioxidants, caffeic acid, gallic acid, vanillic acid and ferulic acid, were solved using a model wine solution prepared from a 12% (v/v) ethanol solution, were 0.033 mol L^{-1} tartaric acid was added. Then NaOH was added to give a pH of 3.6.

Electrochemical measurements were carried out using a Parstat 2273 potentiostat (EG&G) using a conventional three electrode cell. The $LnPc_2$ nanowires deposited on the ITO substrate were used as working electrodes, a platinum sheet was used as the counter electrode and the Ag/AgCl electrode was used as the reference electrode. The three working electrodes were immersed simultaneously the electrolytic solution and connected to the potentiostat using a multiplexor. Cyclic voltammograms were registered sequentially from −0.5 to +1.3 V (vs Ag/AgCl) in the hydroalcoholic solutions and from −1.0 to +1.3 V in the case of aqueous solutions (KCl). The scan rate used was 0.05 V s^{-1}.

A non-supervised multivariate method, the Principal Component Analysis (PCA) was used to analyze the voltammetric curves and to evaluate the capability of the discrimination of the array of nanostructured sensors. The voltammetric curves were mathematically pre-processed and used as a data source for statistical analysis. A windowed slicing method was used to reduce the number of data per sample [17]. In addition, a supervised method, the Partial Least Squares Discriminant Analysis (PLS-DA) was used to evaluate the capability of prediction of the system. All computations and chemometric analysis were carried out using the software Matlab v 6.1 (The Mathworks Inc., Natick, MA, USA) and The Unscrambler v 9.1 (CAMO ASA, Trondheim, Norway).

3. Results and discussion

3.1. Preparation and characterization of the films

As it has been previously reported by our group, lutetium bisphthalocyanine nanowires can be formed onto ITO electrodes by applying a voltage pulse between two electrodes [33]. In this paper, the work has been extended to two other lanthanoid bisphthalocyanines, the dysprosium and the gadolinium bisphthalocyanines. In order to obtain nanowires with similar thickness and length, the electrodeposition conditions must me adapted to each material. Because the molecular weight increases when advancing in the lanthanoid series (Gd > Dy > Lu), and according to the Faraday's law, the formation of $DyPc_2$ and $GdPc_2$ required milder conditions than $LuPc_2$ in terms of deposition time and concentration of the starting solutions. The concentration of the bisphthalocyanines was 5×10^{-5} mol L^{-1} for $GdPc_2$ and $DyPc_2$ and 1×10^{-4} mol L^{-1} for the $LuPc_2$. The deposition time in the EPD experiment was 90 s for $GdPc_2$ and 180 s for $DyPc_2$ and $LuPc_2$. Under these conditions, nanowires of $GdPc_2$ and $DyPc_2$ with similar structure to that previously published for $LuPc_2$ in [33] were obtained (Fig. 1).

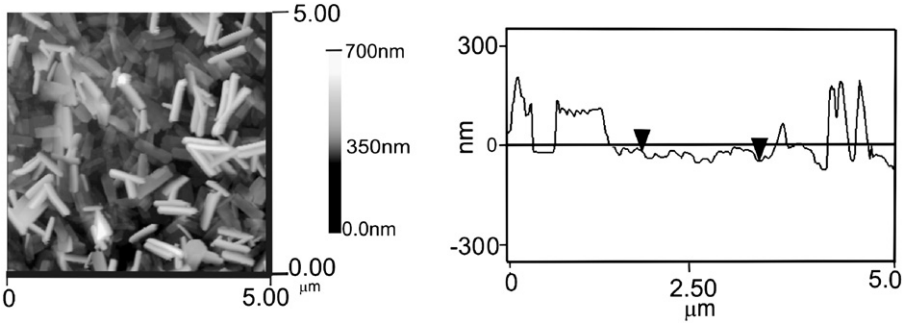

Fig. 1. (Left) AFM image and (right) profile of the EPD-$GdPc_2$ film.

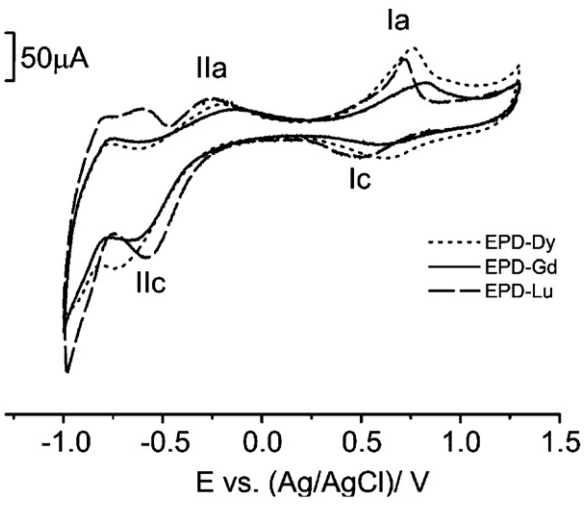

Fig. 2. Cyclic voltammograms in KCl 0.1 mol L^{-1} of the array of EPD sensors. Sweep rate 0.05 V s^{-1}.

3.2. Electrochemical response of the sensors in KCl

In order to evaluate the electrochemical behavior of the EPD-$LnPc_2$ sensors, the electrodes covered with nanowires were immersed in a KCl solution and cyclic voltammetry was performed. Cyclic voltammograms of the EPD-sensors in KCl are illustrated in Fig. 2. For the all three bisphthalocyanines, voltammograms consist of two quasi-reversible processes corresponding to one electron oxidation $Ln(III)Pc_2/Ln(III)Pc_2^+$ (peak I) and the one electron reduction $Ln(III)Pc_2/Ln(III)Pc_2^-$ (peak II) of the phthalocyanine ring. The $E_{1/2}$ of peak II (reduction of the phthalocyanine ring) is independent of the nature of the central ion ($E_{1/2} = -0.40$ V), indicating that the energy of the LUMO is essentially insensitive to the change in the central ion of the macrocycle. In contrast, the $E_{1/2}$ values of the oxidation (peak I) follow the sequence: $GdPc_2$ ($E_{1/2} = 0.72$ V) > $DyPc_2$ ($E_{1/2} = 0.69$ V) > $LuPc_2$ ($E_{1/2} = 0.59$ V) which is in agreement with previously published results for other nanostructured films [18,36].

The decrease of the oxidation potential when advancing in the lanthanoid series can be explained taking into account that the ring–ring distance becomes larger with the increasing size of the lanthanoid atom. For small values of the ionic radii, there is a large π–π interaction. This interaction gives a higher energy for the HOMO in the $LnPc_2$ molecule and lower energy is required to remove one electron from the phthalocyanine ring [36,37].

The reproducibility of the technique was evaluated by preparing nine identical sensors from each bisphthalocyanine. Then, cyclic voltammograms were recorded in KCl 0.1 mol L^{-1}. The standard deviations (SD) of the voltage value at which each peak appear were calculated (Table 1). The SD values were in the range 2–7% and were similar for the three $LnPc_2$ studied indicating that the oxidation potential of the $LnPc_2$ does not affect the reproducibility of the films. In order to evaluate the repeatability of the responses, five consecutive voltammograms were recorded for each sensor. For the three molecules, values of SD (calculated from the peak potential values) were lower than 2%. It has to be noticed that the repeatability of the EPD sensors is similar to that reported for nanostructured $LnPc_2$ LB films [18], but the preparation time (and the price) is drastically decreased in EPD films.

Table 1
Standard deviation of the four peaks for the array of sensors.[a]

	SD (%) Ia	SD (%) Ic	SD (%) IIa	SD (%) IIc
EPD-$GdPc_2$	2.19	4.56	5.88	3.86
EPD-$DyPc_2$	3.09	4.40	7.40	5.40
EPD-$LuPc_2$	2.94	8.70	4.54	1.64

[a] All the SD values are referred to the potential values (E vs Ag/AgCl) of the corresponding redox peak.

Kinetic studies were performed by registering cyclic voltammograms in KCl 0.1 mol L^{-1} at different scan rates, from 0.025 V s^{-1} to 1.2 V s^{-1}.

In all cases, the intensity of the four waves increased linearly with the square root of the scan rate, indicating the dominance of a diffusion controlled processes:

$$I = 2.687 \times 10^{-5} n^{3/2} v^{1/2} D^{1/2} AC \quad (1)$$

where I is the peak current, A is the electrode surface area (0.84 cm^2), D is the diffusion coefficient, and C is the bulk concentration (0.1 mol L^{-1}). Fig. 3 shows that the cathodic and anodic peak currents of the EPD-$GdPc_2$ are directly proportional to the square root of the rate, as predicted for a diffusion-controlled electron transfer process.

This result can be showy since during years in many theoretical studies dealing with thin film-modified electrodes it has been assumed that the kinetics is controlled by the electron exchange, excluding the accompanying ion transfer from the theoretical treatments [38]. Only recently, it has been established that the coupled electron–ion transfer reactions of these electrodes are quasireversible and controlled by the kinetics of ion transfer across the water|film interface [39]. Linear relationship has also been observed in previous works using $LnPc_2$ LB films [18]. In this case, it can be assumed that the electron transfer is accompanied by the simultaneous diffusion of a counterion present in the solution inside the film. This motion is necessary to maintain the electroneutrality of the film.

The diffusion coefficient (D) can be calculated from the I vs $v^{1/2}$ plot. Using the I vs $v^{1/2}$ plot for the first oxidation peak (Ia), the values obtained for the diffusion coefficients were 1.38×10^{-7} cm^2 s^{-1} for the $LuPc_2$ ($y = 8 \times 10^{-4}x - 1 \times 10^{-4}$, $r^2 = 0.9903$) and 1.75×10^{-7} cm^2 s^{-1} for $GdPc_2$ ($y = 9 \times 10^{-4}x - 2 \times 10^{-4}$, $r^2 = 0.9916$) and $DyPc_2$ ($y = 9 \times 10^{-4}x - 2 \times 10^{-4}$, $r^2 = 0.9853$). The diffusion coefficients calculated are consistent with those previously reported [33].

3.3. Electrochemical response of the sensors toward antioxidants

The $LnPc_2$ nanowires-based sensors were used to detect four antioxidants present in red wines containing only one hydroxyl group (vanillic and ferulic acid) or hydroxyl groups in ortho positions (caffeic and gallic acid) in their structures. In order to mimic the wine environment, the antioxidants were solved in a hydroalcoholic solution prepared from a 12% (v/v) ethanol solution, where 0.033 mol L^{-1} tartaric acid and NaOH (to give a pH of 3.6) were added.

As a reference, the electrochemical responses of the sensors immersed in the hydroalcoholic solution were registered. Such voltammograms (at acidic pH) differ strongly from the responses observed in KCl (Fig. 4).

In acidic medium, one of the Pc rings of the $LnPc_2$ is protonated. For this reason, two redox processes can be observed at positive potentials, one associated with the already described oxidation of the phthalocyanine ring (peak I), and a new peak corresponding to the oxidation of the protonated Pc ring (peak III) [22,25,40]. The value of the $E_{1/2}$ of this new peak III varies with the central metal ion of the bisphthalocyanine (0.11 V for $GdPc_2$, 0.03 for $DyPc_2$ and 0.02 for $LuPc_2$) following the same trend than the variation observed for the non-protonated forms [36].

The voltammetric signals of electrodes immersed in hydroalcoholic solution containing antioxidants are illustrated in Fig. 5 for the EPD-$DyPc_2$ sensor. As a general trend, the voltammetric signals consist of the already described peaks I, II and III associated with

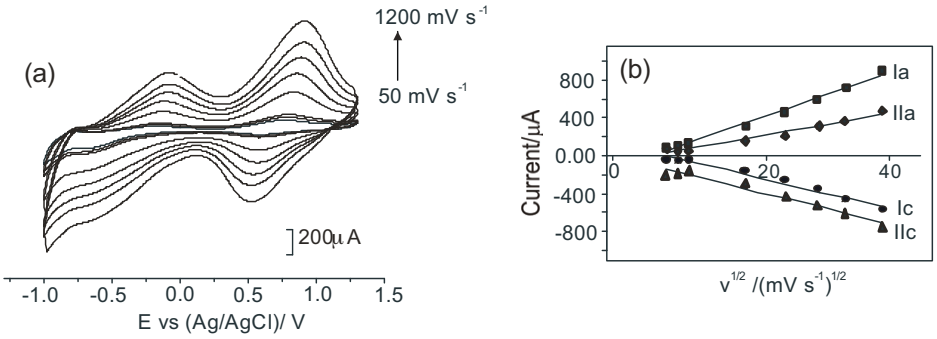

Fig. 3. (a) Cyclic voltammogram of EPD-DyPc$_2$ sensors in KCl 0.1 mol L^{-1} at increasing scan rates (50, 75, 100, 300, 500, 700, 900 and 1200 mV s^{-1}) and (b) linear dependence between the intensity and the square root of the scan rate.

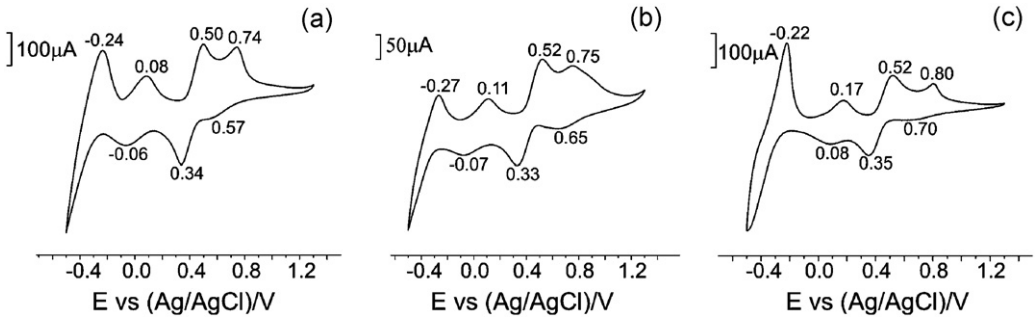

Fig. 4. Cyclic voltammograms of the array of sensors in the hydroalcoholic solution (12% (v/v) ethanol and pH = 3.6).

the phthalocyanine ring and of new redox processes associated with the oxidation of the corresponding phenol. In addition, the phthalocyanines show a strong electrocatalytic effect that results in a decrease of the oxidation potential of the phenolic compounds with respect to the voltage found using a bare ITO. For instance, caffeic acid measured using a bare ITO glass shows the anodic peak at 1.2 V [33] whereas when EPD-DyPc$_2$ is employed, the oxidation potential shifts to 0.52 V. Simultaneously, the presence of

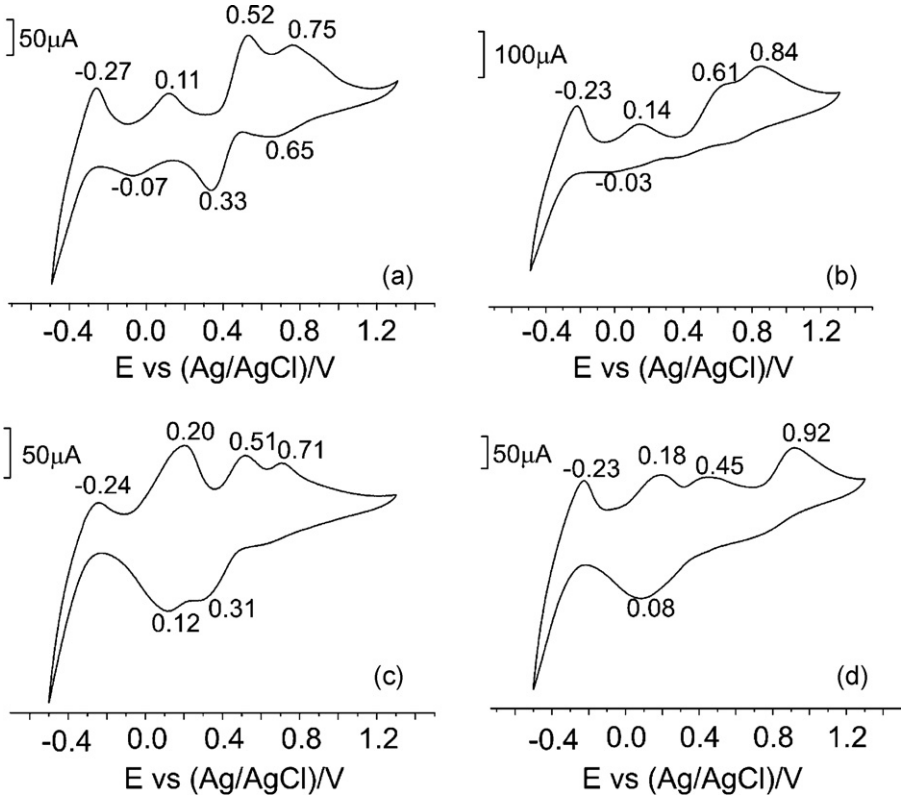

Fig. 5. Cyclic voltammograms of EPD-DyPc$_2$ sensor immersed in 0.5 mmol L^{-1} (a) caffeic, (b) gallic, (c) vanillic and (d) ferulic acid.

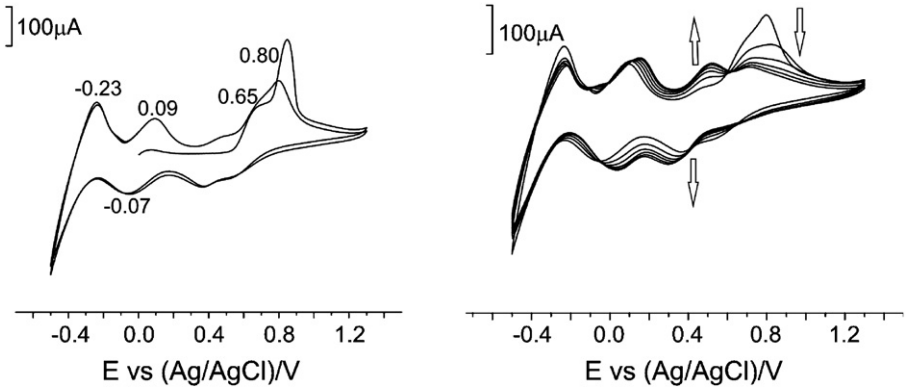

Fig. 6. CV of a $DyPc_2$ electrode immersed in a hydroalcoholic solution containing ferulic acid. Left curve shows the first and second voltammograms. Right curve shows scans from 2 to 7. Scan rate 50 mV s^{-1}.

antioxidants produces important changes in the electrochemistry of the bisphthalocyanines, mainly related to shifts in the potential value of peak III.

In summary, the interactions between the solution and the working electrode (EPD-$LnPc_2$) affect to the value of the redox potentials and to the shape of the curve. Due to the complexity of the signals each electrode provides a specific response toward each compound analyzed.

After this general description, some particular features observed for each voltammogram will be described in the following paragraphs.

The response of caffeic acid (a diphenol) consists of one anodic peak at 0.52 V corresponding to the two-electrons oxidation of the hydroxyl ortho-groups of the aromatic ring, generating the corresponding quinone (3,4-dioxocinnamic acid). The cathodic peak observed at 0.33 V is due to the reduction of the quinone to the polyphenolic form (Fig. 5a). This behavior coincides with the electrochemical responses obtained for the same antioxidants in a hydroalcoholic solution when glassy carbon or ITO electrodes are employed as the working electrode [33,41,42].

According to the literature, the oxidation of gallic acid occurs in two steps. The first oxidation peak is also associated with the oxidation of the hydroxyl ortho-groups, generating the quinone form. The second peak, which appears at higher potentials, is related to the oxidation of the third phenol group adjacent to the ortho-diphenol groups already oxidized [39]. In our case (Fig. 5b), the first oxidation peak is observed at 0.61 V and the second one (at ca. 0.8 V) overlaps with the peak produced by the oxidation of the phthalocyanine ring (peak I). This overlapping gives rise to an intense peak at 0.84 V.

Ferulic acid has one hydroxyl group and a $-OCH_3$ group in an ortho position. Its cyclic voltammogram is characterized by two oxidation processes one at ca. 0.67 V which corresponds to the oxidation of the hydroxyl group, while the second peak at ca 0.80 V corresponds to the oxidation of the $-OCH_3$ group [41]. This peak is again overlapped with the oxidation of the phthalocyanine ring. A similar overlapping was observed for vanillic acid (Fig. 5c), where the oxidation process occurs at a similar potential than the oxidation of the phthalocyanine ring (0.92 V).

It is important to notice that the electrochemical responses of caffeic and gallic acid are highly reproducible. The main noticeable change observed upon cycling is a small gradual change of the intensity. In contrast, in the case of vanillic and ferulic acids, the first scan is different from the subsequent ones. This is illustrated in Fig. 6, where the first scans registered using a $DyPc_2$ electrode toward ferulic acid is shown.

Both compounds possess a phenol and a $-OCH_3$ group in an ortho position. Upon oxidation, the first scan shows the two expected oxidation processes described above giving rise to the formation of a quinone, similar to the quinone produced by caffeic acid (in the case of ferulic acid it is the same compound) [43]. This newly formed species can be reduced during the reverse scan forming the polyphenolic form (caffeic acid in case of ferulic acid). The formation of the polyphenolic form in every new scan, explains the appearance of a peak at ca. 0.50 V that progressively increases its intensity upon cycling, while a simultaneous progressive decrease of the intensity of the oxidation peak associated to ferulic or vanillic acid (at ca. 0.70 and 0.9 V, respectively) is observed.

On the other hand, antioxidants produce a remarkable effect in the electrochemical behavior of the bisphthalocyanines, making difficult the oxidation of the phthalocyanine ring. This is particularly noticeable in the presence of ferulic and vanillic acids (Table 2).

Table 2
$E_{1/2}$ values for the peak III obtained with the three EPD sensors immersed in the antioxidant solutions.

	$E_{1/2}$ vs (Ag/AgCl)/V			
	Caffeic	Gallic	Vanillic	Ferulic
EPD-$GdPc_2$	0.13	0.13	0.17	0.21
EPD-$DyPc_2$	0.02	0.06	0.13	0.16
EPD-$LuPc_2$	0.01	−0.01	0.12	0.12

3.4. Detection limit

As expected, the intensity of the peaks associated to phenols increased with the concentration of the antioxidant. In order to establish the detection limits, the maximal current of the peaks associated to phenols (indicated with an arrow) was measured and plotted against the concentration of antioxidants. Fig. 7 shows the cyclic voltammograms of EPD-$GdPc_2$ immersed in the four antioxidants at different concentrations and the corresponding calibration curves. The calculated detection limits are listed in Table 3.

The detection limits attained (10^{-5}–10^{-6} mol L^{-1}) are of the same order of magnitude as those published using electrodes based

Table 3
Detection limit for the four antioxidants achieved with the EPD-$LnPc_2$ sensors.

	Detection limit/mol L^{-1}			
	Caffeic	Gallic	Vanillic	Ferulic
EPD-$GdPc_2$	2.03×10^{-6}	1.39×10^{-6}	2.14×10^{-5}	3.18×10^{-5}
EPD-$DyPc_2$	1.10×10^{-5}	1.88×10^{-5}	1.00×10^{-5}	2.19×10^{-6}
EPD-$LuPc_2$	6.21×10^{-5}	3.29×10^{-5}	1.48×10^{-5}	6.91×10^{-6}

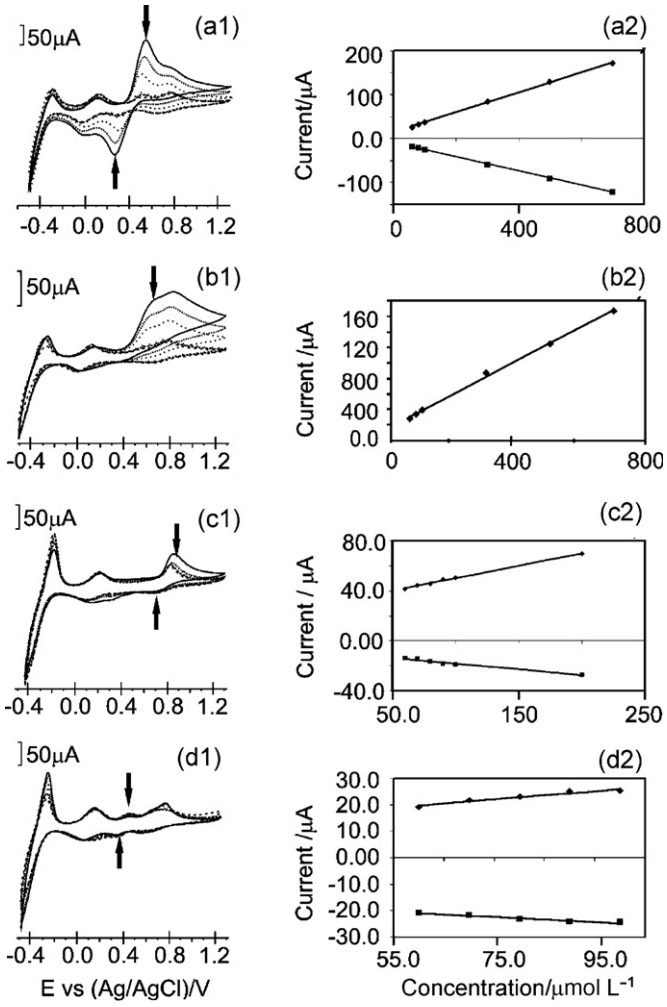

Fig. 7. Cyclic voltammograms (left) and calibration curves (right) of EPD-$GdPc_2$ immersed in (a) caffeic, (b) gallic, (c) vanillic and (d) ferulic acids.

on the LB technique, but with the advantage of the lower preparation times and a reduced cost [33,44]. In addition, the levels of antioxidants detected are lower than the concentrations found in wines.

3.5. Array of electrodes: data treatment

As demonstrated in the above sections, the EPD sensors show complex voltammograms that contain global information about the solution analyzed. In terms of specificity, this can be an advantage vs conventional amperometric or potentiometric electrodes that provide information in a more simple way (intensity measured at a single potential or membrane potential).

In previous works, our group has developed a method that allows the use of the information of the whole curve instead of particular peaks. The method consists of slicing the anodic curve in ten sections (kernels) that provide ten parameters per curve [17–21]. Since each sensor provides a specific pattern when immersed in each antioxidant solution, it can be expected that the voltammograms could be used to discriminate and classify the selected samples by using multivariate data analysis. This is the basis of the so-called electronic tongues [2].

With the aim of evaluating the capability of the array of EPD sensor to discriminate the antioxidants under study, each EPD sensor was immersed in each antioxidant [0.5 mmol L^{-1} hydroalcoholic solution] and 6 replicates were registered. Since a cyclic voltammogram is a bi-valuated curve, the anodic scan was selected and the kernel method was applied. 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 built the matrix used for the pattern recognition techniques.

Fig. 8 illustrates the PCA score plot corresponding to the four antioxidants analyzed with the array of EPD sensors. The First Principal Component (PC1) explains the 53% of the information and Second Principal Component (PC2) the 31%. It is important to notice that with only two principal components, the percentage of explained variance was 84%. It can be observed that the sensor array is able to distinguish between the four antioxidants. PC1 that represents the major correlation percentage discriminates according the number of hydroxyl groups. The clusters are displaced to the left when increasing the number of —OH groups. The monophenol appears at positive values, diphenols at values close to zero and the triphenol at negative PC1 values. The clusters corresponding to caffeic and ferulic acid appear close, indicating that their electrochemical responses are similar (see Fig. 4).

The second principal component (PC2) allows also discriminating the antioxidants according to the number of hydroxyl groups present their structure. For instance, antioxidants with an ortho diphenol group (gallic and caffeic acids) appear apart from antioxidants with only one hydroxyl group (ferulic and vanillic acids).

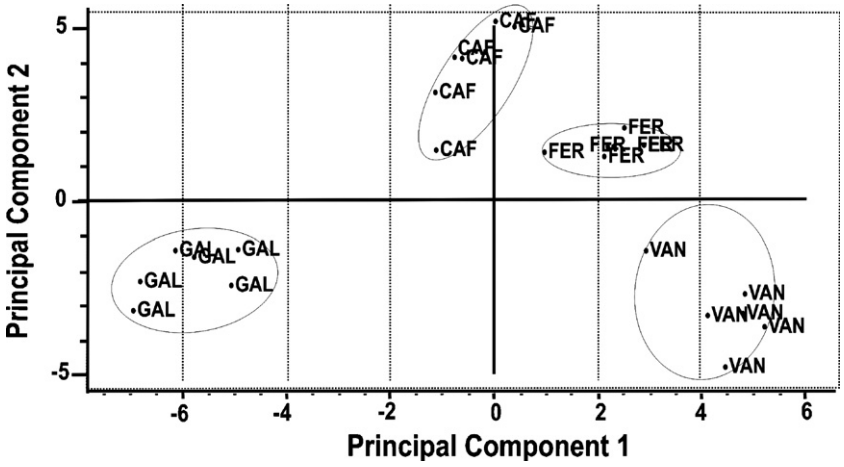

Fig. 8. PCA score plot corresponding to the classification of the antioxidants.

Table 4
PLS-DA prediction model using the EPD array of sensors.

Classes	Slope		Offset		Correlation		RMSEC	RMSEP
	Calibration	Validation	Calibration	Validation	Calibration	Validation		
Caffeic	0.9709	0.9620	0.0072	0.0073	0.9853	0.9790	0.0738	0.0882
Gallic	0.9596	0.9516	0.0101	0.0120	0.9795	0.9704	0.0871	0.1045
Ferulic	0.9308	0.9145	0.0173	0.0241	0.9648	0.9490	0.1139	0.1367
Vanillic	0.9410	0.9284	0.0147	0.0174	0.9700	0.9590	0.1052	0.1228

In order to support this high-quality performance in the classification of antioxidants, pattern recognition techniques based on supervised algorithms can be applied such as Partial Least Squares Discriminant Analysis (PLS-DA). In this data treatment the multivariate variables corresponding to observations (cyclic voltammograms) were related to the class membership for each antioxidant using a cross validation method. A quantitative evaluation of the regression model is given in Table 4.

As observed, both the calibration and the validation values involved a good quality model performance; slope near 1, offset near 0 and large correlations between the voltammetric signals and the classes established. Additionally, low RMSEC (root mean square error of calibration) and RMSEP (root mean square error of prediction) values were accomplished. These results indicate that this new array of sensors based on bisphthalocyanines electrodeposited is able to carry out a significant classification of the antioxidants.

4. Conclusions

An array formed by three EDP films of $LnPc_2$ nanowires ($LuPc_2$, $DyPc_2$ and $GdPc_2$), has been successfully used for the detection of four antioxidants: caffeic, gallic acid (with an ortho diphenol group in their structure), vanillic and ferulic acids (with only one hydroxyl group in their structure).

A multivariate data treatment was made to explore the capability of discrimination of the array. PCA shows a good discrimination according to the number of —OH groups present in the structure of the antioxidants. The percentage of variance explained using two principal components was 84%, almost the total information of the model. PLS-DA analysis was carried out to make a quantitative evaluation of the discrimination. Good values for the correlations coefficients were obtained with a slope of nearly 1 and offset close to 0.

The sensing characteristics of the films in terms of repeatability, reproducibility, and detection limits were similar to those found in other nanostructured sensors based on phthalocyanines. However, these sensors have the important advantage of the easiest preparation.

These results confirm the possibility of using this new array of sensors to the study the antioxidants in hydroalcoholic solutions typically present in wines.

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