



Biogenic amines and fish freshness assessment using a multisensor system based on voltammetric electrodes. Comparison between CPE and screen-printed electrodes

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ARTICLE INFO

Article history:

Received 4 June 2009

Received in revised form 8 July 2009

Accepted 9 July 2009

Available online 16 July 2009

Keywords:

Voltammetric sensor

Phthalocyanine

Biogenic amine

Electronic tongue

Fish freshness

ABSTRACT

The present work relates to a method for determining the degree of freshness of fish by monitoring the production of spoilage products. This method comprises the global detection of the spoilage products including biogenic amines resulting from the degradation of fish by using arrays of voltammetric sensors chemically modified with phthalocyanines. The performance of an array of screen-printed electrodes (SPE) has been compared with that of an array formed by classic carbon paste electrodes (CPE). The sensors have shown good sensitivity towards model solutions of biogenic amines (ammonia, dimethylamine, trimethylamine, cadaverine and histamine). It has been evidenced that the electroactivity and basic character of amines influences considerably the electrochemical behaviour of the electrodic material.

The pattern of responses provided by the array has been successfully used to evaluate fish freshness and to determine the post-mortem period. An increase of the signals associated to biogenic amines and other spoilage products is observed with increasing storage days. Signals provided by classical CPE are better resolved and show better sensor-to-sensor reproducibility than SPE. However, the modification of SPE enables simple mass-production of low-cost and miniaturised sensing units with good sensibility and repeatability. The capability of discrimination demonstrated by Principal Component Analysis (PCA) and the capability of prediction of fish freshness calculated by Partial Least Squares-Discriminant Analysis (PLS-DA) is similar in both cases.

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1. Introduction

Amines play an important role in the degradation pathways of amino acids in biological systems. For this reason, they are widely recognised as indicators of spoilage in foods [1,2]. In the case of fishes and seafoods, biogenic amines include dimethylamine (DMA), trimethylamine (TMA), ammonia, putrescine, cadaverine, dopamine, and histamine among others. The most common method used to determine amine compounds is the high-performance liquid chromatography (HPLC) [3–5]. Electrochemical methods have also demonstrated to be useful to detect biogenic amines. The direct detection of various amines in water solutions by voltammetric methods using bare diamond [6,7] and metal electrodes [8,9] has been reported. Chemically modified electrodes (CME) containing

surface-confined chemical functionalities exhibit some important advantages over conventional bare electrodes in terms of catalytic activity, selectivity and protection from fouling effects [10]. Determination of biogenic amines by using biosensors also represents an interesting alternative [11–19].

Although the measurement of a single amine can provide important information about fish freshness [20–22], the assessment of fish spoilage requires the use of technologies capable to provide global information about the biogenic amines and other spoilage subproducts present in the sample. Nowadays, fish freshness monitoring is determined by using specific chemical and biological analysis (determination of total volatile basic amines, biogenic amines, oxidative rancidity, and bacterial counts, etc.) as well as a trained panel that can evaluate sensory attributes such as appearance, texture, smell, colour, etc. [23]. These methods are generally expensive, time-consuming and not suitable for in situ or on site monitoring.

Evaluation of fish freshness through the global assessment of volatile amines using multisensor arrays (the so-called electronic noses) has attracted considerable interest [24–28]. In contrast, elec-

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tronic tongues have rarely been used for detection of amines or for fish quality monitoring [14,29–33]. Usually electronic tongues are constructed using potentiometric sensors that measure the potential created across a membrane produced by the diffusion of ions. Arrays of metallic voltammetric sensors can detect electroactive species [32]. Voltammetric sensors chemically modified with electroactive materials can provide a certain advantage because they are sensitive to both the presence of ions and of electroactive substances [34,35].

In previous works, our group has developed a multisensor array formed by voltammetric carbon paste electrodes (CPE) chemically modified with electroactive materials (phthalocyanines). Using this approach, it has been possible to discriminate among simple ionic solutions [34], antioxidants [36], red wines and olive oils [37,38]. Although these sensors have showed excellent sensing properties, the current trends in technology require the development of miniaturised devices. In this work, disposable electrodes have been developed where phthalocyanine compounds have been deposited onto miniaturised platinum screen-printed electrodes (SPE). The electrochemical behaviour of these new sensors has been analysed and compared with that of the conventional carbon paste electrodes. The capability of the array to detect biogenic amines [including ammonia (NH_3), dimethylamine (DMA), trimethylamine (TMA), cadaverine (CAD) and histamine (HIS)] usually formed during food degradation processes has been evaluated using multivariate analysis. The capability of the system to monitor the spoilage of real fish samples has also been analysed by means of Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA).

2. Experimental

2.1. Sensor fabrication

The rare-earth bisphthalocyanine molecules (LnPc_2) The LnPc_2 included in the study were: lutetium (III) bisphthalocyaninate (LuPc_2), gadolinium (III) bisphthalocyaninate (GdPc_2) and the lutetium octa-*tert*-butyl substituted derivative (LuPc_2^t). They were synthesized and purified in their neutral radical state following previously published procedures [36]. Cobalt (II) monophthalocyanine (Fluka) was also used as electroactive modifier.

Carbon paste electrodes were prepared as previously reported by mixing graphite powder (Ultracarbon, Ultra F purity) and the corresponding bisphthalocyanine (15%, w/w). Nujol oil was used as the binder of the mixture. Pastes were packed into the body of a 1 mL plastic syringe and compressed. A metallic wire was used as a contact.

Modified screen-printed sensors were prepared by depositing the sensitive materials onto screen-printed electrodes from BVT technologies. The devices consist in a working electrode formed by platinum disk (1 mm diameter). A pseudo Ag/AgCl reference electrode and a platinum contra electrode are also integrated in the same device. The corresponding phthalocyanine was mixed with carbon ink (C2000802D2 purchased from Gwent Electronic Materials) in 85:15 (w:w) ratio. Cyclohexanone was then added until a paste of the adequate viscosity was obtained. The mixture was deposited onto the working electrode of the screen-printed device by using a semiautomatic fluid dosificator (I&J Fisnar Inc., model JBE1113).

2.2. Reagents and solutions

All reagents were of high purity and used without further purification (Sigma–Aldrich).

Biogenic amine solutions were prepared by solving ammonia (NH_3), dimethylamine, trimethylamine, cadaverine (CAD—pentane-1,5-diamine) and histamine (HIS—2-(3H-imidazol-4-yl)ethanamine) in ultrapure water ($10^{-1} \text{ mol L}^{-1}$). To improve the conductivity, KCl was added to the solutions ($10^{-1} \text{ mol L}^{-1}$). All solutions were prepared with deionized water (18.3 M Ω resistivity, Milli-Q, Millipore).

2.3. Fish samples

The spoilage study was carried out using tenchs (*Tinca tinca*), a freshwater and brackish water fish of the Cyprinid family. Cultured tenchs were furnished by Tencas de Casaseca S.L., Zamora, Spain.

Few minutes after death, fishes were eviscerated and washed. Four fishes of the same weight were stored inside a plastic box at 4 °C during 12 days. Every day, 1 g of the muscle of each fish was cut. 25 mL of a 0.1 mol L^{-1} KCl solution was added to fish sample and the mixture was sonicated for 5 min. Then, the liquid phase was separated by filtration and used in voltammetric and pH measurements.

2.4. Chromatography

Chromatography was run in a Shimadzu GCMS QP5050 gas chromatographer equipped with a mass detector. A Supelcowax-10 (length: 30 m; inner diameter: 0.25 mm) column was used. The program of temperature was: an initial temperature of 40 °C during 4 min. Then the temperature was increased at a rate of 4 °C min^{-1} to 200 °C for 10 min. The control method used was splitless mode. The carrier gas was helium at 6 mL min^{-1} .

The injection was carried out using SPME (Solid Phase Microextraction). Samples were placed in a 10-mL vial that was kept at 40 °C during 15 min. A DVB/C/PDMS fiber was exposed to the headspace of the vial for 15 min. Then, the SPME fiber was placed in the heated injection port of a gas chromatograph that was kept at 250 °C.

2.5. Electrochemical measurements

The electrochemistry was carried out in an EG&G PARC 263A potentiostat/galvanostat using a conventional three-electrode cell. The chemically modified electrodes were used as working electrodes. In the case of CPE, the reference electrode was Ag|AgCl/KCl 3 mol L^{-1} and the counter electrode was a platinum wire. In the case of SPE, the reference and the counter electrode were integrated in the device.

Cyclic voltammograms were registered from -1.0 to $+1.3 \text{ V}$ (the scan started at 0 V) at a sweep rate of 0.1 V s^{-1} (except otherwise indicated). Square wave voltammetry (SWV) was performed at a potential scan ranging from -1.0 to $+1.3 \text{ V}$, by using the setup: $f = 15 \text{ Hz}$; $E_{\text{sw}} = 100 \text{ mV}$; $\Delta E_s = 7 \text{ mV}$.

2.6. Data analysis

A non-supervised multivariate method such as Principal Component Analysis was used for the analysis of the voltammetric signals. Voltammograms were mathematically pre-processed and used as data source for multivariate data analysis according to a previously published method [33,36–39]. A supervised method, the Partial Least Squares-Discriminant Analysis (PLS-DA) was used to evaluate the classification capability of the system. PLS1 regressions were used for estimating of correlations between electrochemical data and pH.

All computations and chemometric analysis were carried out using the software Matlab v5.3 (The Mathworks Inc., Natick, MA, USA) and The Unscrambler 9.1 (Camo, Norway).

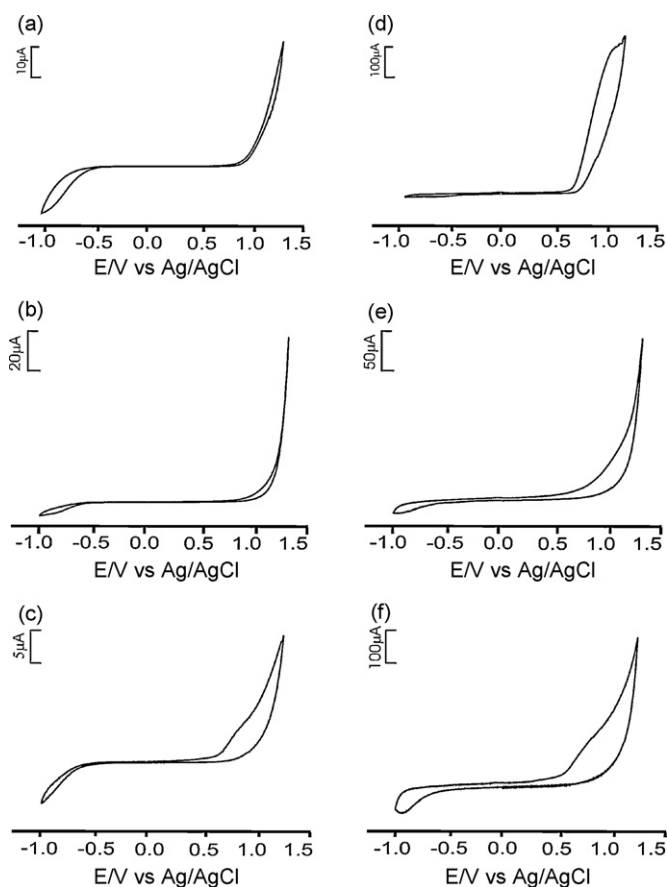


Fig. 1. Cyclic voltammogram of unmodified CPE (left side) and SPE (right side) immersed in $10^{-1} \text{ mol L}^{-1}$ (a and d) NH_3 ; (b and e) CAD; (c and f) HIS. All solutions were prepared in $10^{-1} \text{ mol L}^{-1}$ KCl.

3. Results

3.1. Sensing properties of CPE and SPE electrodes

The voltammetric behaviour of bare carbon paste electrodes and screen-printed carbon electrodes was tested towards a 0.1 mol L^{-1} KCl solution and towards solutions containing biogenic amines. The results are illustrated in Fig. 1 for electrodes immersed in NH_3 , cadaverine and histamine. When CPE or SPE electrodes were immersed in aliphatic DMA and TMA, a discernible voltammetric peak could be observed at ca. 1 V. ($E = 1.24 \text{ V}$ for DMA and $E = 1.03 \text{ V}$ for TMA). The CV curves did not evidence redox peaks in the case of NH_3 and CAD. These results are consistent with the expected redox behaviour of biogenic amines where the facility of oxidation decreases in order: tertiary amines, secondary amine, primary amines. In the case of histamine, a characteristic peak associated to the irreversible oxidation appears at 0.85 V. These results are consistent with those obtained by means of square wave voltammetry (Fig. 2) and are in agreement with previously published results [40,41].

As observed in the Fig. 1, both CPE and SPE electrodes yielded voltammograms with nearly identical features. However, SPE electrodes showed larger background currents; in addition, amine oxidation processes started at slightly higher potentials in CPE. For instance, in the case of HIS the anodic peak started at 0.49 V in SPE and at 0.57 V in CPE.

One of the most interesting differences between the CPE and the SPE sensors immersed in ammonia and histamine is that the peak current intensity observed in SPE was one order of magnitude higher than in CPE.

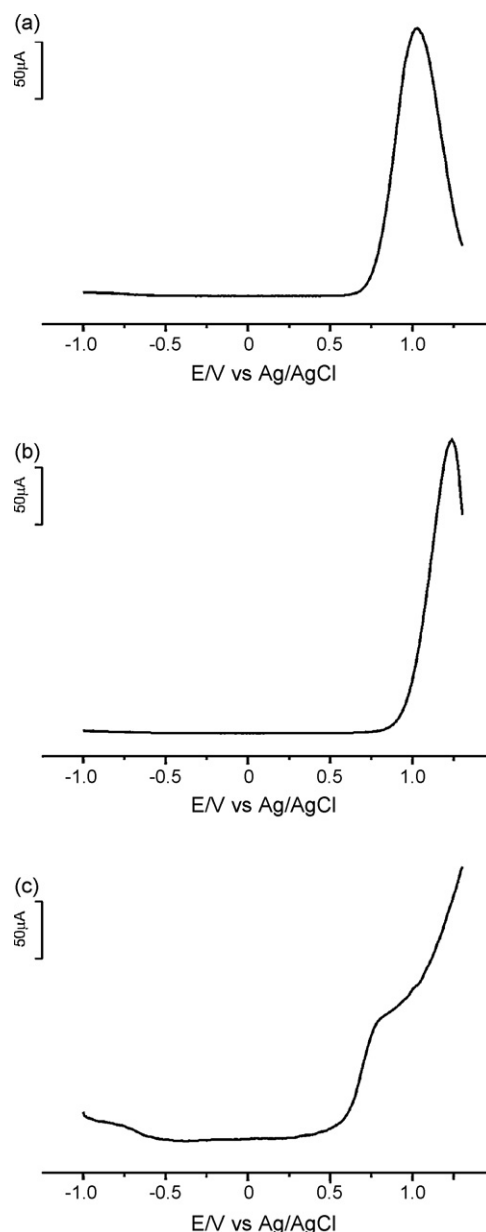


Fig. 2. SWV curves of an unmodified CPE towards 0.1 mol L^{-1} solution of (a) TMA; (b) DMA; (c) HIS. All solutions were prepared in $10^{-1} \text{ mol L}^{-1}$ KCl.

In both cases, the adhesion of oxidation products to the electrodes surface resulted in fouling of the electrodes [7].

Chemical modification of electrode surfaces with organic films has opened up new options in the development of electrochemical sensors. In this work, CPE and SPE have been modified with phthalocyanine derivatives and their sensing properties towards biogenic amines have been evaluated. The response towards KCl was used as a reference.

In good agreement with previously published results, the electrochemical response of LnPc_2 immersed in KCl consisted in two definite redox couples that are related to the one electron oxidation (peak I) and the one electron reduction (peak II) of the phthalocyanine ring. In the case of octa-*tert*-butyl substituted phthalocyanine derivative, peak II is less intense than peak I and appears at lower potential values than in the unsubstituted analogue. Electrodes modified with CoPc present one anodic and one cathodic peak that correspond to the oxidation and reduction of the cobalt ion.

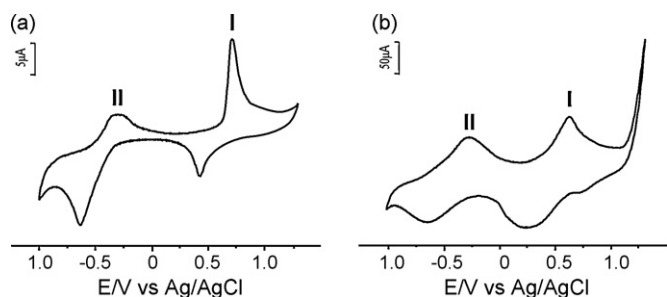


Fig. 3. The responses of a LuPc₂-CPE (a) and a LuPc₂-SPE (b) immersed in KCl 0.1 mol L⁻¹.

The responses obtained using the miniaturised SPE were similar to those obtained with CPE although some differences could be noticed. This is illustrated in Fig. 3 where the responses of a LuPc₂-CPE and a LuPc₂-SPE immersed in KCl are shown. In both cases the ΔE values obtained were larger than 59 mV, indicating the existence of a certain degree of irreversibility. The responses of SPE were characterised by a higher background current, the peaks were more intense and ΔE between anodic and cathodic peak potentials were also larger than in CPE. Because different preparation methods correspond to different structures (molecular aggregation states, surface, porosity, etc.) it can be assumed that the differences in the voltammograms are due to the different structures of the electrodes.

The electrochemical responses depend on the nature of the electroactive material used to modify the electrode and on the nature of the amine analysed.

Fig. 4 shows the voltammetric curves of CPE modified with the four phthalocyanines included in this study registered in 0.1 mol L⁻¹ NH₃ solution. The solutions were prepared in 10⁻¹ mol L⁻¹ KCl. As observed in the figure, the intensity and position of the peaks associated to the phthalocyanines followed the same general trends (related to the nature of the central metal atom and of the substituents) than those observed in KCl. However, the oxidation of the macrocycle rings occurs at higher potentials in the presence of ammonia. For instance, $E_{1/2}$ of peak I obtained with a LuPc₂ based CPE electrodes immersed in KCl appears at ca. 0.55 V, whereas, the same peak appear at $E_{1/2}$ = 0.57 V when the electrodes were immersed in a NH₃ solution.

The responses produced by other biogenic amines are illustrated in Fig. 5 for an electrode based on GdPc₂ immersed in the five amines under study. The response towards each amine is different due to the particular interactions occurring between the electroactive material and the corresponding amine. Such interactions are dominated by the response of the phthalocyanines towards electron donor biogenic amines. Another important effect is related to diffusion of ions inside the film that counterbalance the electrical charges formed in electrode during oxidation and reduction.

Some general features observed for all the amines analysed include the following (a) in electrodes immersed in ammonia, the response is similar to that observed in KCl, but the electron donor character of ammonia caused a shift of the peak associated to the oxidation of the phthalocyanine ring to higher potentials; (b) cadaverine produces a strong interaction with the electrodes that makes impossible the oxidation of the phthalocyanine ring; (c) the voltammograms of electrodes immersed in TMA, DMA or histamine are dominated by a broad intense peak at ca. 0.7–0.9 V that is the result of the overlapping of the peak associated to the oxidation of the phthalocyanine ring and the peak associated to the oxidation of the amine.

This general behaviour was observed in both CPE and SPE sensors. However, some differences could be noticed. Table 1 collects

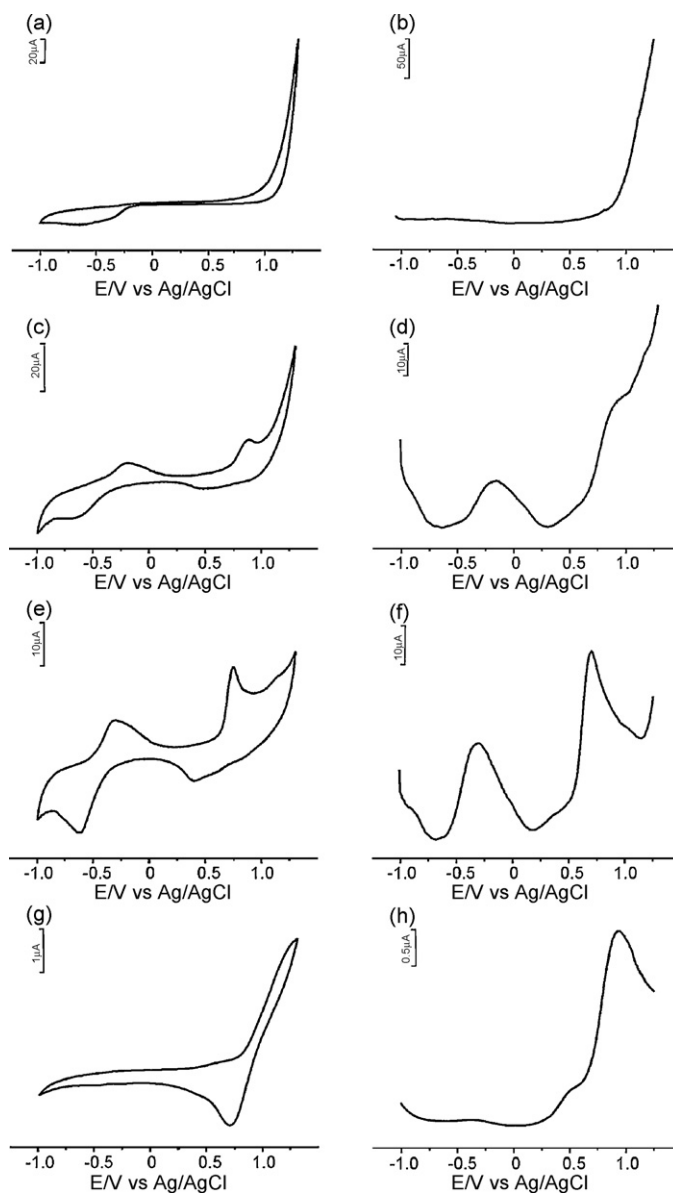


Fig. 4. CV and SWV curves in NH₃ solution 0.1 mol L⁻¹ of (a and b) CoPc-CPE; (c and d) GdPc₂-CPE; (e and f) LuPc₂-CPE; (g and h) LuPc₂-CPE. All solutions were prepared in 10⁻¹ mol L⁻¹ KCl.

Table 1
Peak potentials of LuPc₂ electrodes immersed in different aqueous solutions.

Electrode	Solution	Peak I (V)			Peak II (V)		
		Cathodic	Anodic	$E_{1/2}$	Cathodic	Anodic	$E_{1/2}$
LuPc ₂ -CPE	KCl	-0.64	-0.29	-0.46	0.42	0.69	0.55
LuPc ₂ -SPE		-0.640	-0.27	-0.45	0.26	0.63	0.45
LuPc ₂ -CPE	TMA	-0.62	-0.28	-0.45	0.45	0.74	0.59
LuPc ₂ -SPE		-0.63	-0.20	-0.41	0.22	0.71	0.46
LuPc ₂ -CPE	DMA	-0.65	-0.26	-0.45	0.44	0.73	0.58
LuPc ₂ -SPE		-0.60	-0.13	-0.36	0.24	0.63	0.44
LuPc ₂ -CPE	NH ₃	-0.64	-0.30	-0.47	0.40	0.74	0.57
LuPc ₂ -SPE		-0.60	-0.23	-0.42	0.34	0.64	0.49
LuPc ₂ -CPE	CAD	-0.65	-0.23	-0.44	0.34	0.84	0.59
LuPc ₂ -CPE		-0.67	-0.14	-0.41	0.30	0.78	0.54
LuPc ₂ -CPE	HIS	-0.68	-0.30	-0.49	0.39	0.81	0.60
LuPc ₂ -CPE		-0.54	-0.14	-0.34	0.31	0.74	0.52

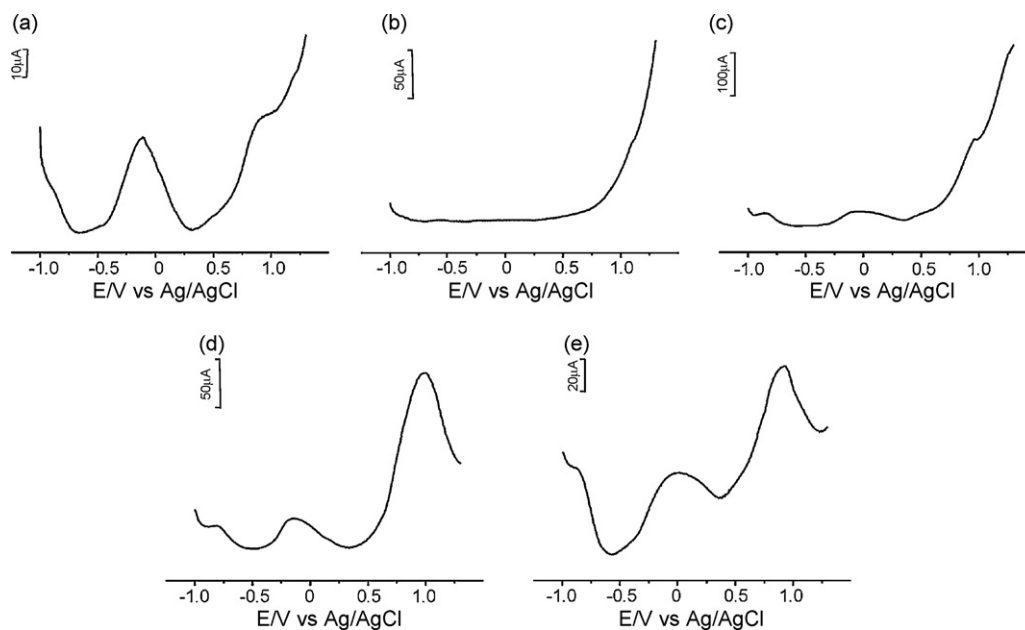


Fig. 5. SWV curves of GdPc₂-CPE towards 0.1 mol L⁻¹ solution of (a) NH₃; (b) CAD; (c) DMA; (d) TMA; (e) HIS. All solutions were prepared in 10⁻¹ mol L⁻¹ KCl.

the peak potentials of LuPc₂-CPE and -SPE electrodes, immersed in different amine solutions. In all cases, ΔE between anodic and cathodic peak potentials were larger in SPE than in CPE. But the $E_{1/2}$ values were lower in SPE. This indicates that even if the reversibility of is improved in CPE, the oxidation is facilitated in SPE.

The dynamic character of the electrode process for both types of electrodes was further examined. For this purpose, the effect of the sweep rate on the performance of CPE and SPE electrodes was studied.

Fig. 6 shows the cyclic voltammograms of LuPc₂-CPE and -SPE at various scan rates (200–1000 mV s⁻¹) in TMA solution. The plots of the anodic and cathodic peak currents were linearly dependent

on $v^{1/2}$. This linear relationship was similar in SPE and CPE and demonstrated that in both cases the process is diffusion controlled.

This figure also demonstrates that the peaks associated to both the oxidation of the phthalocyanine (at ca. 0.7 V) and of the amine (at ca. 1.1 V) are better resolved in CPE than in SPE.

Two of the main characteristics that electrochemical sensors have to fulfil are good repeatability and good long-term stability. In order to evaluate the repeatability of CPE and SPE, consecutive cycles were recorded in 0.1 mol L⁻¹ KCl solution. The first scan was slightly different from the second cycle; subsequent scans were highly repeatable. The values of the peak potentials for the anodic wave of peak I measured in KCl over a two weeks period, showed a similar coefficient of variation (lower than 9%) When the cyclic

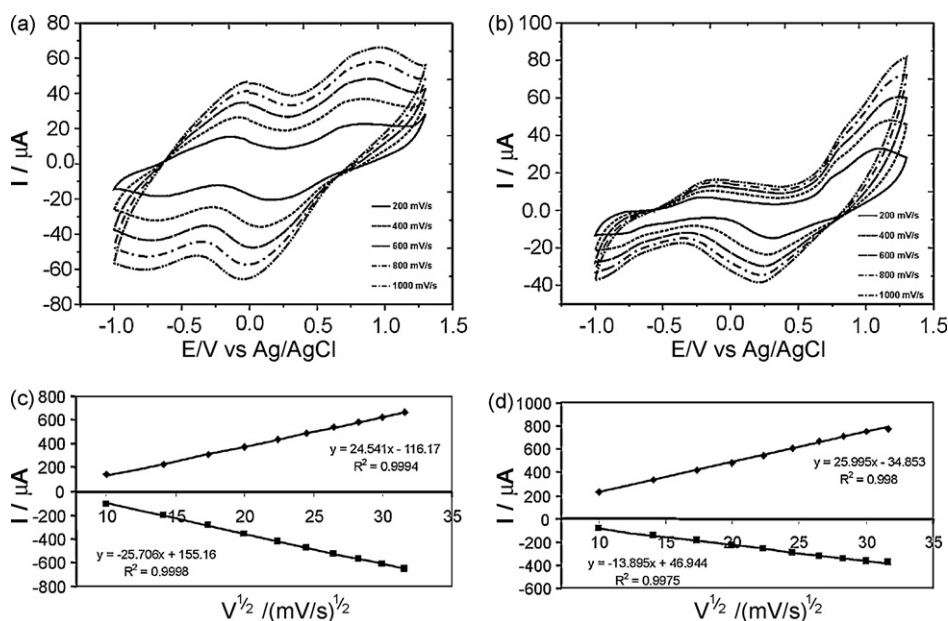


Fig. 6. Cyclic voltammograms of (a) LuPc₂-SPE and (b) LuPc₂-CPE, respectively, at various scan rates: (200–1000 mV s⁻¹) in 0.1 mol L⁻¹ TMA solution. Plot of I_{pa} versus $v^{1/2}$, respectively I_{pc} versus $v^{1/2}$ of (c) LuPc₂-SPE and (d) LuPc₂-CPE. All solutions were prepared in 10⁻¹ mol L⁻¹ KCl.

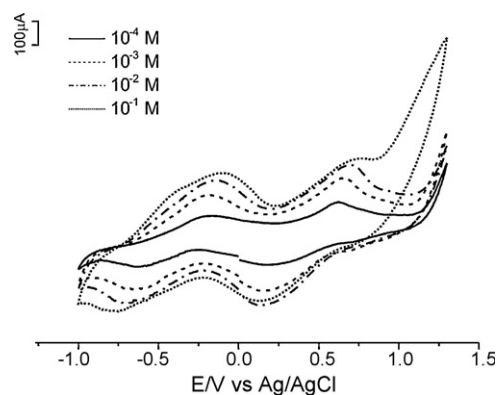


Fig. 7. CVs of a LuPc₂-SPE immersed in NH₃ solution with different concentration. All solutions were prepared in 10⁻¹ mol L⁻¹ KCl.

voltammetry experiments were carried out in biogenic amines and the CPE and SPEs were withdrawn from the solution, some changes in the voltammograms were observed. Variation coefficients increased up to 20%. The results confirm that the products of the amine oxidation remain partially captured at the electrode surface. For this reason, sensors must be considered as single-use electrodes. In the case of CPE electrodes, the surface can be renewed by smoothing the electrode surface using a filter paper, while new SPE must be prepared for each measurement. In disposable electrodes, the reproducibility during fabrication is a key step. To evaluate the reproducibility of the preparation method used to fabricate the electrodes, seven identical electrodes based on LuPc₂ were prepared and the voltammograms were registered in KCl solutions. The calculated variation coefficient using peak I was 4% for CPE and 10% for SPE.

Electrodes modified with LuPc₂ were used to evaluate the detection limit by registering voltammetric measurements in solutions containing increasing amounts of TMA and NH₃ concentrations in order to obtain an analytical curve.

Fig. 7 illustrates the response of the electrodes towards increasing concentrations of biogenic amines. In this example, the response of a LuPc₂-SPE immersed in NH₃ solved in KCl medium is shown. As observed in the figure, an increase of the ammonia concentration produced a linear increase of the intensity of the peak until a plateau is reached. It is important to notice that high concentrations of ammonia produce a splitting of the peak II associated to the reduction of the phthalocyanine ring.

In CPE, the anodic peak I was linearly dependent on the TMA concentration in the range from 2×10^{-4} to 2×10^{-3} mol L⁻¹ with a detection limit of 4×10^{-4} mol L⁻¹ in KCl medium. In the case of NH₃ the detection limit was 3×10^{-4} mol L⁻¹ in KCl medium. These results are in agreement with previously results reported that obtain similar detection limit for ammonium in simulated fermentation media. [42,43]. Due to the larger signal intensities produced by SPE electrodes immersed in ammonia, the detection limit was improved in one order of magnitude (detection limit of 5×10^{-5} mol L⁻¹).

Compared with enzyme-based sensors previously published [11–18], chemical modified sensors used in this study have a longer lifetime and do not require special conditions of temperature, humidity or light for conservation. In general, the stability of the enzyme-based sensors is lower than CPEs, and unless they are kept under specific conditions, their stability is guarantee only for a week [17]. In terms of selectivity, biosensors show higher selectivity and specificity, while the sensors described in this work are unspecific. As shown in the next section, the use of arrays on unspecific sensors can provide global information of the sample instead of information of particular compounds.

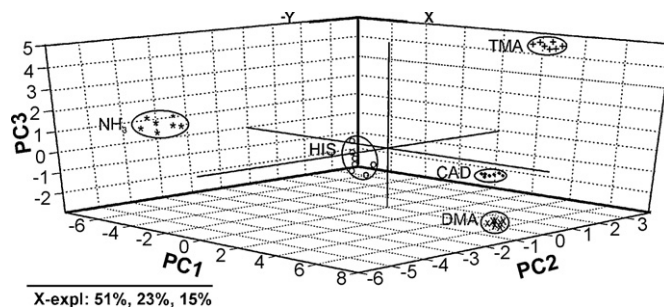


Fig. 8. Scores plot of a PCA on amine using the voltammetric sensors array.

3.2. Array of sensors based on CPE and SPE

3.2.1. Discrimination of model solutions containing biogenic amines

As stated before, the responses obtained when using CPE and SPE modified with phthalocyanines showed a high degree of complexity, since transient responses observed in the voltammograms are related to the electrode material and to the nature and concentration of the amine molecules present in the solutions (and to the interactions electrode-solution).

This makes possible to use the sensors in an array configuration. The pattern of responses generated by the array is a fingerprint of the sample studied. This pattern can be related with certain features or characteristics of the samples by means of chemometrics.

In order to evaluate the discrimination capabilities of the array of voltammetric sensors, Principal Component Analysis was conducted using the information obtained from an array formed by CPE or by SPE sensors. Fig. 8 shows the PCA results as a three-dimensional scores plot of principal components that allow obtaining well-defined and separated clusters. PCA has been validated by full cross validation method and an optimal number of 4 principal components have been used. In the case of CPE, first three principal components explain the 89% of the information (PC1 = 51%; PC2 = 23%; PC3 = 15%). The capability of discrimination obtained using an array of SPE sensors was similar and the captured information was 80% (PC1 = 35%; PC2 = 23%; PC3 = 22%).

The separated clusters indicate that the five solutions could be clearly discriminated from each other. In addition, the positions of the clusters are related to the electrochemical properties of the tested solutions. It has to be noticed that the cluster corresponding to the ammonia, appears in the left side of the diagram (negative PC1), far apart from the rest of the amines. Aliphatic amines appear in the right side of the diagram (positive PC1). In turn, the tertiary amine (TMA) appears far apart from the clusters corresponding to primary (CAD) and secondary amines (DMA). The aromatic amine, histamine has a particular electrochemical behaviour that permits to discriminate it from aliphatic amines and ammonia.

3.2.2. Fish freshness monitoring

Fish freshness has been monitored through the global assessment of spoilage products (including biogenic amines) using a multisensor array. For this purpose, fishes were eviscerated and washed and stored at 4 °C during 12 days. Everyday, muscle samples were prepared and measured with the CPE and the SPE sensors.

A characteristic pattern of the deterioration of fish stored in ice can be divided into four phases: (a) fish is fresh and has a sweet, seaweed and delicate taste (highly fresh); (b) there is a loss of the characteristic odour and taste. The flesh becomes neutral but has no off-flavours (fresh); (c) there is sign of spoilage and a range of volatile, unpleasant-smelling substances are produced (degraded); (d) fish is spoiled and has a putrid odour (spoiled).

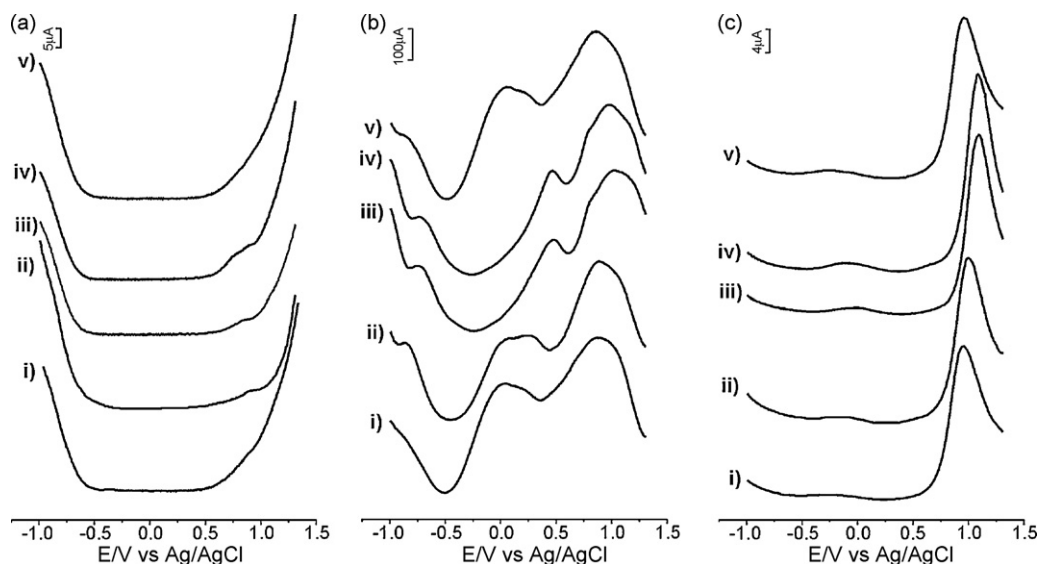


Fig. 9. SWV curves of (a) unmodified-CPE, (b) GdPc₂-CPE and (c) LuPc₂⁺-CPE during storage time. (i) Day 0; (ii) day 4; (iii) day 5; (iv) day 7; (v) day 10.

The variations observed in the voltammetric responses during fish storage are presented in Fig. 9 (SWV curves correspond to days 0, 4, 5, 7 and 10 of storage at 4 °C).

In the case of unmodified electrodes, a new peak appears at ca. 0.75 V after 2 days of storage (Fig. 9a). This peak can be associated to the generation of biogenic amines, mainly histamine during degradation of fish flesh proteins [1,2].

In the next days, the peak increased its intensity. After the seventh day, the peak decreased its intensity and after 10 days the peak was not longer observed in the working range. Such effect has been confirmed using chromatography. Chromatograms registered during the spoilage demonstrate that during the first days, the levels of biogenic amines increased. After the 7–10th day, the biogenic amines decompose and the intensity of the peaks decreased drastically in the chromatogram (Fig. 10).

Bisphthalocyanine modified electrodes showed similar trends (Fig. 9b). When the spoilage progressed, the peak potentials related to the oxidation of the phthalocyanine ring (peak I) increased the intensity and shifted to higher potential values. At the same time, splittings and changes in peak II also occur. These changes are consistent with an increase of the concentration of biogenic amines. It is also remarkable that a new peak appears at ca. 0.3 V, which increased its intensity and shifted to higher potential when the spoilage increased.

In good agreement with the chromatograms after the seventh day, the concentration of biogenic amines decreased and a partial recovery of the original signals was observed. A similar behaviour is observed in the case of LuPc₂⁺ (Fig. 9c)

CoPc modified electrodes were also able to detect the formation of biogenic amines that was followed by a shift of the peak at ca. 1 V to higher potentials during the first days. A partial recovery of the original signal was also observed in CoPc electrodes.

Principal Component Analysis was used to analyse the degradation process measured with the array of sensors. Fig. 11 shows the PCA obtained using the electrochemical signals registered every day using SPE sensors. The PCA score plot of the three first principal components accounts for 69% of variance. Clearly discriminated clusters can be observed. The first cluster, that appears in the left side of the figure corresponds to samples analysed days 1 and 2 and correspond to a highly fresh product. Samples analysed in days 3 and 4 did not show any off odour and could be classified as fresh product. The clusters appear in the central part of the figure. Samples collected days 4 and 5 showed off odours (degraded product).

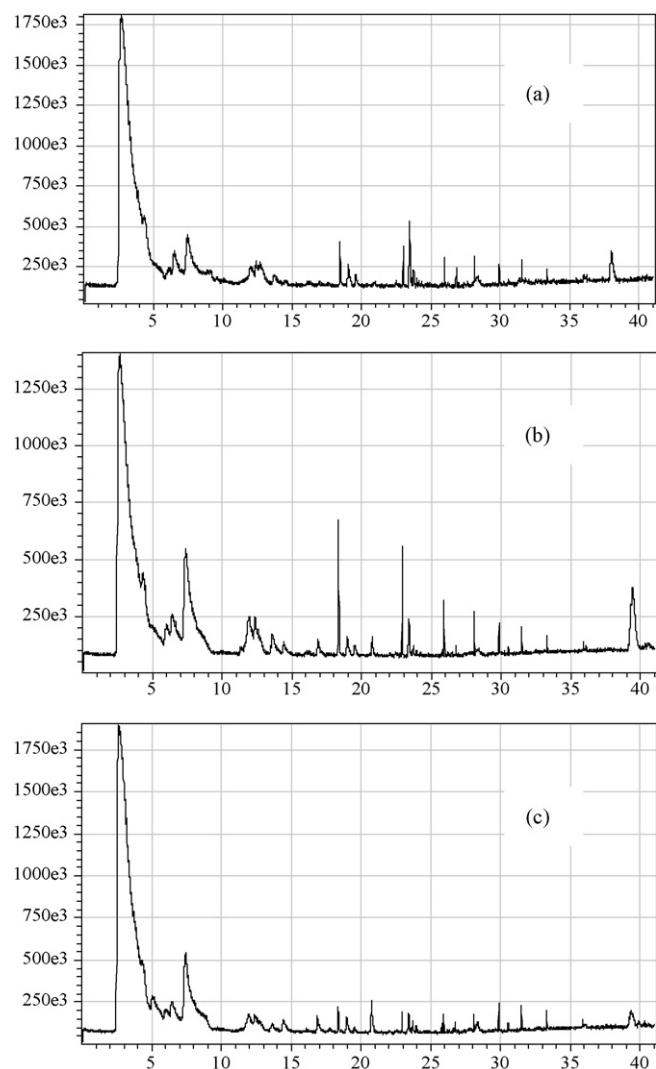


Fig. 10. Gas chromatograms of fish samples collected the (a) first (b) fifth and (c) tenth day after death.

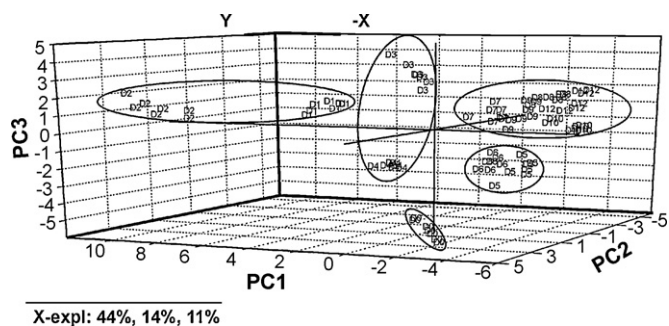


Fig. 11. PCA score plot as a function of time. The days 0, 1–2, 3–4, 5–6, and 7–12 form grouped clusters.

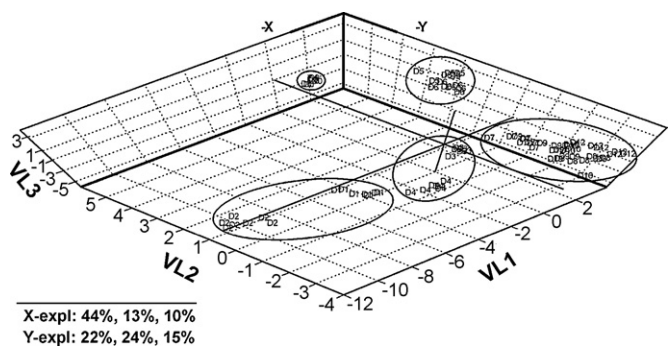


Fig. 12. PLS-DA score plot corresponding to fish freshness monitoring.

The last cluster that appears on the right side of the figure corresponds to samples collected on days 7–12 (spoiled fish).

PLS-DA was used to classify the day of fish degradation from the sensor array response. As shown in Fig. 12, the score plots of the three latent variables, of the fully cross-validated PLS-DA model (using an optimal number of 7 latent variables), revealed a clear identification of the fish degradation phases.

Table 2 collects the quantitative data derived from the PLS-DA regression model. As observed, both the calibration and the validation values involved a good-quality model performance (slope near 1, off set near 0 and large correlation between sensors and categorised variables). Additionally, low RMSEC (root mean square error of calibration) and RMSEP (root mean square error of prediction) values were obtained. These results indicate that this methodology is able to real time monitor the fish freshness during storage.

Changes in pH in the fish samples throughout the storage time are summarised in Table 3.

Partial least squares (PLS1) regressions were performed to model the relationships between CPE and SPE signals and the pH values of the samples. The initial pH of fish flesh was close to 7.49, which is a typical value in very fresh products. In the solution obtained by procedure before mentioned the pH is 6.81, which

Table 3
pH evolution during fish storage at 4 °C.

Time/days	pH
0	6.81
1	6.57
2	6.49
3	6.48
4	6.42
5	6.28
6	6.38
7	6.43
8	6.53
9	6.84
10	6.88
12	6.91

indicate that the KCl aqueous solution do not influence the pH of the fish flesh.

During the storage is registered a decrease in the pH as a consequence of the enzymatic and chemical processes occurred in the beginning the post-mortem stage. After 5 days the pH of the solution started to increase as consequence of the biogenic amine generation.

An excellent correlation was obtained using a model with 5 latent variables and validated by full cross validation method. The regression coefficient was 0.973 in calibration and 0.961 in validation. Additionally, low values of RMSEC (0.042) and RMSEP (0.051) were accomplished.

4. Conclusions

Two multisensory systems based on carbon paste or screen-printed electrodes modified with phthalocyanines have been developed and applied to the analysis of biogenic amines. Both systems have been used to monitor fish spoilage with similar results. An increase of the signals associated to biogenic amines was observed with increasing storage days. PCA and PLS-DA have been successfully used to monitor the fish freshness and to classify the day of fish degradation. Using the sensor responses it has been possible to build prediction models (PLS1 method) that have allowed estimating quantitative chemical parameters such as the pH.

Signals provided by classical CPE are better resolved and show better sensor-to-sensor reproducibility. The screen-printing technology allowed the preparation of miniaturised electrodes, which are promising for the mass-production of low-cost and single-use sensors, with significant advantages (higher sensibility) compared to the corresponding modified carbon paste electrodes.

Acknowledgments

Financial support from CICYT (Grant no. AGL2006-05501/ALI) and the Junta de Castilla y León. (VA-052A06) is gratefully acknowledged.

Table 2
Results of the calibration and validation of PLS-DA.

Group	Slope		Offset		Correlation coefficient		RMSEC	RMSEP
	Calibration	Validation	Calibration	Validation	Calibration	Validation		
D0	0.952	0.938	0.004	0.007	0.976	0.969	0.060	0.067
D1–2	0.963	0.954	0.006	0.008	0.981	0.974	0.071	0.083
D3–4	0.928	0.908	0.011	0.014	0.963	0.949	0.099	0.117
D5–6	0.935	0.907	0.010	0.016	0.966	0.955	0.094	0.109
D7–12	0.951	0.929	0.020	0.025	0.975	0.967	0.108	0.124

RMSEC: "root mean square of error of calibration". RMSEP: "root mean square of error of prediction".

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