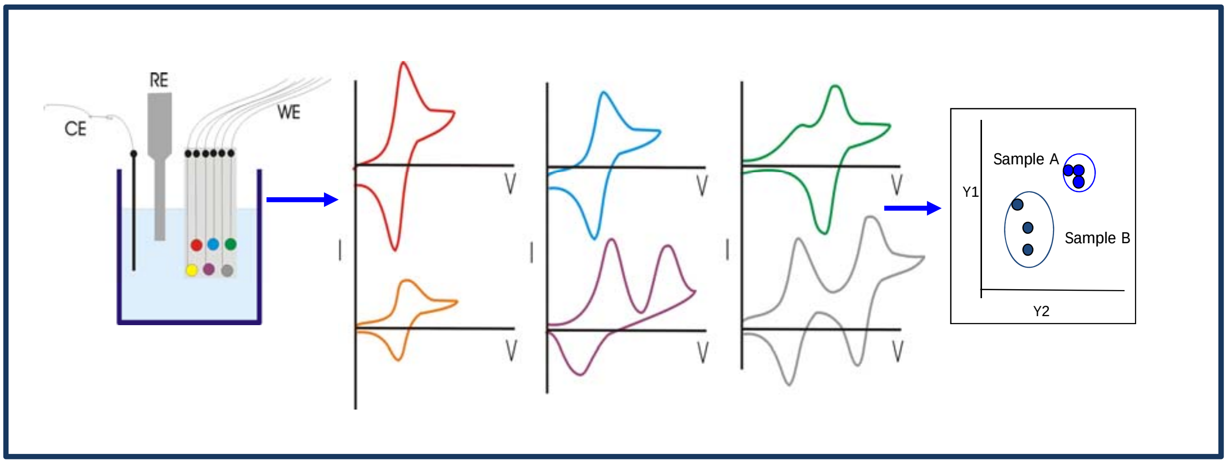
Universidad de Valladolid
Facultad de Ciencias
Departamento de Física de la Materia Condensada, Cristalografía y Mineralogía

TESIS DOCTORAL

“NUEVOS AVANCES EN SENSORES VOLTAMÉTRICOS NANOESTRUCTURADOS Y MINIATURIZADOS. APLICACIÓN EN UNA LENGUA ELECTRÓNICA EN EL SECTOR ALIMENTARIO.”



Mónica Gay Martín
Julio 2012

Universidad de Valladolid

FACULTAD DE CIENCIAS

DEPARTAMENTO DE FÍSICA DE LA MATERIA CONDENSADA,
CRISTALOGRAFÍA Y MINERALOGÍA

TESIS DOCTORAL:

NUEVOS AVANCES EN SENSORES VOLTAMÉTRICOS NANOESTRUCTURADOS Y MINIATURIZADOS. APLICACIÓN EN UNA LENGUA ELECTRÓNICA EN EL SECTOR ALIMENTARIO.

Presentada por Mónica Gay Martín para optar al grado de
Doctora por la Universidad de Valladolid

Dirigida por:
Prof. Dra. María Luz Rodríguez Méndez

Agradecimientos

No pensaba que este momento fuera a llegar tan pronto. Lo he deseado durante mucho tiempo, y ahora que me encuentro delante de estas páginas en blanco, echo la vista atrás y me vienen miles de recuerdos a la mente.

En primer lugar quiero dar las gracias a mi directora de Tesis, María Luz Rodríguez, por haberme dado la oportunidad de incorporarme en su grupo de trabajo. Por su acertada orientación en mi investigación, y por haberme enseñado a trabajar con rigor y seriedad. Gracias por alentarme siempre a la consecución de objetivos que a primera vista me parecían imposibles. A tu lado han aflorado aspectos muy positivos de mí que desconocía en un principio. Gracias por confiar en mí y saber que llegaría hasta donde he llegado ahora. Escribiendo estas líneas siento el orgullo y satisfacción de haber pertenecido a tu grupo de investigación. Al profesor D. José Antonio de Saja, por haberme posibilitado igualmente mi entrada en el Grupo de Sensores. Oportunidad que aprecio enormemente en estos momentos y que ha permitido que además de desarrollar mi carrera como investigadora durante estos años, lo haya hecho también como persona. Por su cercanía y saber escuchar en los momentos más difíciles, por su excelente trato humano y por su carisma. Me llena de orgullo el trabajar con personas de tal categoría. Gracias a los dos por haber posibilitado la experiencia más significativa de mi vida.

Al profesor Ludovico Valli del Dipartimento di Ingegneria dell'Inovazione, por brindarme la oportunidad de trabajar con él en Lecce y por su amabilidad. Por extensión, a Gabrielle Giancane y Simona Bettini, por su excepcional acogida, sin los cuales mi estancia en el laboratorio no hubiera sido tan agradable. Es imposible no acordarme de mi gente italiana, de manera muy especial de mis compañeras de piso; Marcella, Mavi, Simona, y Debora. Aunque compañera de piso, también amiga, Alessandra Lacaita. Las palabras se quedan cortas en un intento de expresar el cariño, cercanía y amistad ofrecida durante mi estancia en Lecce y durante los años sucesivos. Grazie amica, non ti dimenticare che domani è già qui. Sin duda alguna esta experiencia cambió mi vida. Y lo continua haciendo.

A Constantin Apetrei, a quien agradezco infinitamente su ayuda por la cantidad de horas compartidas de trabajo codo con codo y por su absoluta disponibilidad en mis difíciles comienzos. También a Álvaro Arrieta y Vicente Parra por su apoyo y entusiasmo.

Me vienen a la mente todas y cada una de las personas que han pasado por el laboratorio durante estos años. Gracias a todos por haber dejado huella en mí. De manera muy especial, Gaby, Priscila y Romina. Porque las horas de trabajo se han convertido en una amistad. Gracias por todo lo que hemos compartido.

A Gloria Alcaide, por aportar esa dosis de diversión y alegría tan necesaria en el laboratorio. Por hacerme sentir siempre en casa. A María Jesús Baena, por los cafés y conversaciones compartidas durante todos los años de mi estancia en el laboratorio.

A todos los brasileños que han pasado por el Grupo de Sensores. A todos y cada uno de ellos. Por haber enriquecido tanto mi vida, por la experiencia compartida tan intensa y en algunos casos, inolvidable. Angelo, Patricia, Edson, Felippe, Casé y Priscila. Sin duda habéis marcado un antes y un después en esta andadura.

A mis amigos. Por estar a mi lado siempre. A Ana, por la amistad que nos une desde hace tantos años, por todos los momentos que hemos compartido, tantos. Por tu apoyo en los momentos más difíciles, no lo olvido. A Virginia, por su saber escuchar y contar siempre conmigo. A Mik, por haber aliñado los momentos duros con tu humor tan característico, por los momentos de locura vividos. A Anita, por nuestros años juntas durante la carrera, por momentos únicos e irrepetibles que no se me olvidarán nunca. Porque sé que la relación tan especial que tenemos no cambiará. A mis jesuitineras del alma, María y Aurora. Es imposible olvidar nuestros años en el cole y sentir que no ha pasado el tiempo después de tanto tiempo. Gracias niñas. A Miguel, por nuestro reencuentro. Por haber compartido conmigo la cantidad de cosas que nos han pasado este año. Todo. Por tu cariño, compresión y preocupación cuando más lo he necesitado.

A mis padres, por ser el motor de mi vida. Es imposible expresar la gratitud y admiración que siento hacia vosotros. Gracias por el apoyo incondicional que me habéis dado siempre, especialmente durante estos años. Por vuestra infinita paciencia y saber escuchar. Por vuestro cariño y confianza. Siempre me hacéis ver que no existen dificultades, que todo lo puedo conseguir, todo. Por vuestro esfuerzo, sacrificio y trabajo, gracias al cual hoy me encuentro aquí. Creo que nunca podréis haceros una idea de lo que significáis para mí y de lo orgullosa que estoy de tener unos padres como vosotros. Sois mis referentes y mí ejemplo a seguir. Sin ninguna duda este trabajo está dedicado íntegramente a vosotros. A mi hermana, por haberme dado fuerza cuando más lo necesitaba, por su manera de ver las cosas, tan diferente de mi, y que tanto me gusta. Eres muy especial para mí, mucho. A mis abuelos, por el amor que me transmiten, por su preocupación (a veces excesiva). Por ser unas personas de excepcionales valores humanos, por ser algo más que unos abuelos para mí. A mi tía Concha, por estar ahí siempre que la necesito, siempre. Por su admirable generosidad. A una personita que me toca el corazón con su dulzura y con su preocupación por cada cosa que hago, a pesar de ser una niña. Gracias Sara.

A toda la gente que, de una manera u otra ha contribuido a que esté escribiendo estas páginas hoy. Gracias.

Por último, agradecer la financiación que ha posibilitado la realización de mi Tesis Doctoral. Al Ministerio de Ciencia por la beca F.P.I.(BES-2007-14435/AGL2006-05501), y a la CICyT (Grant no. AGL2006-05501/ALI y AGL2009-12660/ALI).

Ahora se cierra una etapa y empieza otra. Llegados a este punto espero con cierta inquietud lo que me deparará la vida. Sé que cambiará en muchos aspectos. Estoy preparada pues para el cambio. La riqueza personal conseguida gracias a las tantas experiencias vividas durante mis años como doctoranda, la gente a la que he conocido, los lugares a los que he viajado, mis éxitos, mis fracasos…Gracias a todo ello he experimentado una transformación personal que sin duda me ha preparado para esperar con las puertas abiertas a lo que esté por venir. Adelante.

A mis padres

Presentación

Capítulo I: Estado del Arte

Capítulo II: Resultados

Capítulo III: Conclusiones y perspectivas futuras

Apéndice

Presentation

Chapter I: State of the art

Chapter II: Results

Chapter III: Conclusions and future trends

Appendix

Presentación

1. SUMMARY OF THE THESIS

The present Thesis entitled: “News advances in nanostructured and miniaturized voltammetric sensors. Applications of a voltammetric electronic tongue for the food industry” is framed within a research line focused on the electronic tongues, developed in the Group of Sensors in the Physic Chemistry and Inorganic Chemistry Department of the University of Valladolid.

This thesis is presented to obtain the degree of Doctor by the University of Valladolid with an *International Mention*, and for this reason it is written in two languages: English and Spanish. In addition, the dissertation is structured according to the modality named “*article compendium*”, thus, seven articles published in international journals are attached in the following chapters. Although the apparent productivity, still at least two more papers are expected to be sent to international journals as regular papers.

This thesis tackles the development of arrays of voltammetric sensors modified with electroactive materials to employ them as the sensitive part of an electronic tongue devoted to the analysis of different foods or beverages (mainly wines) or compounds of interest in the food industry such as antioxidants.

The first works presented here, were carried out using the electronic tongue that was developed by our research group before my incorporation to the team. This system based on carbon paste electrodes, had been successfully used to the analysis of wines with different organoleptic characteristics (variety of grape, monitoring of the ageing, etc). In this Thesis, the system was applied to the study of more complex wine samples than those studied in previous works. In this sense, the system was used to detect the use of alternative methods of ageing and to evaluate the oxidation state of wines. Good correlations between the signals provided by the sensors and the data obtained by means of traditional chemical analysis and a human panel of experts have been obtained.

On the other hand, new types of voltammetric sensors have been developed. In this way, new miniaturized screen printed electrodes (SPE) and nanostructured sensors have been developed and investigated as sensing units of electronic tongues. The performance of miniaturized electrodes (SPE) was compared with that of traditional carbon paste electrodes. The responses were similar in terms of intensity and reversibility. The small size of SPE devices makes possible their portability as well as the industrialization.

Finally, a novel nanostructured sensor based on lutetium bisphthalocyanine has been developed. It has been prepared by means of electrophoretic deposition technique (EPD). The characterization of their morphology as well as its structure is presented in this work accompanied with the appropriate discussion and justification. The electrochemical behavior has been compared with that observed when Langmuir-Blodgett (LB) films of the same material are used as working electrodes. The main advantage is that the preparation time of EPD nanowires is much shorter than that of LB films (seconds vs. hours)

The good success of these nanowire based electrodes lead us to extend the study to other bisphthalocyanines and to construct an electronic tongue formed by an array of three nanostructured electrodes. For this purpose, three bisphthalocyanines (lutetium, gadolinium and dysprosium bisphthalocyanines) have been successfully electrodeposited and investigated as voltammetric sensors. The three electrodes show a similar behavior, however, differences in the potential values of the redox processes related to the central metal are observed. The array formed by the three EPD sensors has been applied to the study of different phenolic antioxidants present in a wide variety of food products. This array of EPD sensors has shown a good capability to detect and discriminate the antioxidants according to their structure (number and position of the hydroxyl groups in the aromatic ring). The novel type of voltammetric sensor developed has shown to be competitive with the classical LB sensors.

2. JUSTIFICATION AND OBJECTIVES

This work has been carried out in the Group of Sensors of the University of Valladolid. This group has a large experience in the development of electronic noses and tongues and their application to the analysis of foods and beverages.

In particular, the Group has developed an innovative electronic tongue based on voltammetric electrodes chemically modified with electroactive materials (phthalocyanines, conducting polymers etc.). In the early works, the system was applied to the analysis of wines with different organoleptic characteristics (variety of grape, monitoring of the ageing, etc). Most of these works were carried out in collaboration with cellars and oenological centers.

At the moment of my incorporation to the Group of Sensors, they were involved in a Project financed by the Spanish Ministry of Science devoted to the analysis of wines by means of an electronic tongue and to the development of a methology able to detect the use of alternative methods of ageing that were not permitted by the Spanish Laws at that moment (ageing in stainless steel tanks instead in oak barrels). The work was carried out with Professor M. del Álamo and I. Nevares of the Oenological Faculty of the University of Valladolid (Palencia).

The success of this research made us to continue the work in the field of electronic tongues applied to the analysis of foods. On one hand, different works were undertaken to improve the performance of the sensors by designing miniaturized sensors and nanostructured sensors. On the other hand, decision was taken to apply the new sensors to form arrays and evaluate their sensing capabilities in the fields of fishes, wines and in the study of antioxidants. These works have also been financed by the Ministry of Science and in particular by a Scholarship (BES-2007-14435/AGL2006-05501). The collaboration of Tencas de Casaseca and Nomacorc that supplied the samples has been invaluable.

According to these general ideas, the specific objectives of this Thesis are the following:

1. To optimize the voltammetric electronic tongue based on carbon paste electrodes developed previously by the group and to apply it to the study of more complex liquids.
    a. To study red wines aged with different methods; the traditional method (using oak barrels), and alternative methods (using stainless steel tanks were oak chips and staves are added).
    b. To study the capability of the array of sensors to discriminate among the different wines according to the ageing method.
    c. To find correlations between the electrochemical signals and the parameters measured by means of traditional chemical methods.
    d. To find correlations between the electrochemical signals and the parameters obtained from a human panel.
2. To apply the electronic tongue based on carbon paste electrodes to the study of red wines bottled with different types of closures (collaboration with a Belgium Company called Nomacorc)
    a. To study the electrochemical profiles of the wines employing an array of sensors composed by electroactive molecules with complementary activity; bisphthalocyanines and perylenes.
    b. To find correlations between the electronic tongue and the polyphenolic content of wines.

3. To develop miniaturized voltammetric sensors.
    a. To modify chemically the working electrode of miniaturized Screen Printed Electrodes (SPE) with different phthalocyanines.
    b. To compare the electrochemical profile of the miniaturized sensors with that obtained with their analogous, the carbon paste electrodes.
    c. To apply the SPE sensors to the study of biogenic amines and to study the discrimination capability of an array of SPE sensors in comparison with an array based on carbon paste electrodes.
    d. To extent the research to the study of real fish samples. To evaluate the capability of the SPE array to monitor the spoilage of fishes after death and the establish fish freshness.
4. To develop nanostructured sensors based on electroactive materials.
    a. To use the electrophoretic deposition technique to prepare nanostructured (nanowires) voltammetric sensors based on lutetium bisphthalocyanine.
    b. To characterize the nanostructure of the novel EPD sensor by means of UV-Vis, NIR, X-Ray, SEM and AFM.
    c. To investigate the electrochemical behavior, in simple electrolytes, of the new EPD sensor and to compare them with those showed by the LB films.
    d. To extent the study to antioxidants present in foods.

5. To develop an array of nanostructure sensors based on three different bisphthalocyanines by means of the EPD technique.
    a. To electrodeposit other bisphthalocyanines including gadolinium and dysprosium bisphthalocyanines.
    b. To study their electrochemical behavior in simple electrolytes and to compare it with that obtained using lutetium bisphthalocyanine as electroactive compound.
    c. To understand the different electrochemical profiles according to the different central metals of the molecules.
    d. To apply the nanostructure array in the study of several antioxidants present in wine.
    e. To investigate the capability of the nanoarray to discriminate the antioxidants according to their chemical structure; number and position of hydroxyl groups in the aromatic ring.

3. CONTENTS

The present dissertation consists of three chapters with the following information incorporated in each one:

- Chapter I: An overview about electronic tongues is presented to help to understand the basis of this thesis.
- Chapter II: The results obtained during this doctoral research are presented in form of a collection of published papers (original works). Each paper is preceded by a brief summary of the work and of the main conclusions. In table 1, the published papers are shown ordered by publication date.
- Chapter III: The main conclusions are listed and some hints of the future work are collected.

Table 1. Publications published in international journals appended in this dissertation.

Publications published in international journals	**Year**
Electronic tongue based on voltammetric electrodes modified with materials showing complementary electroactive properties. Applications. M.L. Rodríguez-Méndez, V. Parra, C. Apetrei, S. Villanueva, **M. Gay**, N. Prieto, J. Martínez, J.A. de Saja. Microchimica Acta. 163, 23-31.	2008
New insights into sensors based on radical bisphthalocyanines. M.L. Rodríguez-Méndez, **M. Gay,** J.A. de Saja. Journal of Porphyrins and Phthalocyanines. 13, 1159-1167.	2009
Biogenic amines and fish freshness assessment using a multisensory system based on voltammetric electrodes. Comparison between CPE and screen-printed electrodes. M.L. Rodríguez-Méndez, **M. Gay**, C. Apetrei, J.A. de Saja. Electrochimica Acta. 54, 7033-7041.	2009
Application of an electronic tongue to study the effect of the use of pieces of wood and micro-oxygenation in the ageing of red wine. **M. Gay**, C. Apetrei, I. Nevares, M. del Álamo, J. Zurro, N. Prieto, J.A. de Saja, M.L. Rodríguez-Méndez. Electrochimica Acta. 55, 6782-6788.	2010
Films of lutetium bisphthalocyanine nanowires as electrochemical sensors. **M. Gay Martín**, M.L. Rodríguez-Méndez, J.A. de Saja. Langmuir. 26 (24), 19217-19224.	2010
Analysis of the influence of the type of closure in the organoleptic characteristics of a red wine by using an electronic panel. N. Prieto, **M. Gay**, S. Vidal, O. Aagaard, J.A. de Saja, M.L. Rodríguez-Méndez. Food Chemistry. 129, 589-594.	2011
Multisensor System based on bisphthalocyanine nanowires for the detection of antioxidants. **M. Gay Martín**, J.A. de Saja, R. Muñoz, M.L. Rodríguez-Méndez. Electrochimica Acta. 68, 88-94.	2012
Using an electrochemical quartz crystal microbalance based on lutetium bisphthalocyanine for the investigation of antioxidant solutions. **M. Gay Martín**, E. Díez Arévalo, M.L. Rodríguez-Méndez and J.A. de Saja Sáez. *Pending to send*.	
Evaluation of the oxygen level and the polyphenolic content of red wines using a hybrid system formed by an electronic nose and an electronic tongue. N. Prieto, **M. Gay**, C. Apetrei, S. Vidal, O. Aagaard, J.A. de Saja, M.L: Rodríguez-Méndez. *Pending to send.*	

Capítulo I

Estado del Arte

1.1 INTRODUCCIÓN

Una lengua electrónica se puede definir como un sistema multisensorial formado por sensores no selectivos acoplados a herramientas quimiométricas multivariantes [1-7]. La parte más importante y que define la capacidad analítica de una lengua electrónica frente a la muestra líquida que se pretende valorar son los sensores que la componen. Por ello, existen numerosos trabajos de investigación dedicados al desarrollo de nuevos sensores. La trascendencia del sensor es tal que, para catalogar una lengua electrónica, necesariamente ha de hacerse referencia al tipo de sensores que la constituye.

Las metaloftalocianinas (MPc) poseen unas propiedades eléctricas, ópticas, másicas y redox que hacen de ellas uno de los materiales más interesantes en la fabricación de sensores químicos debido a que dichas propiedades se modifican cuando determinadas moléculas se adsorben sobre el material sensible, dando lugar a diferentes clases de sensores químicos basados en diferentes principios de trabajo [8-13].

Debido al rico comportamiento electroquímico y a sus propiedades electrocatalíticas, las ftalocianinas se presentan como materiales muy atractivos a la hora de emplearlos como modificadores químicos en sensores electroquímicos [12-18]. Dichos sensores se pueden preparar empleando diferentes técnicas. En este sentido, la nanotecnología ofrece nuevas herramientas para obtener sensores con mayor sensibilidad [13]. Una alternativa a los sensores electroquímicos convencionales son los biosensores electroquímicos, los cuales presentan la ventaja de la especificidad enzima-sustrato. En este tipo de sensores las ftalocianinas han demostrado ser eficientes mediadores electrónicos entre el enzima y el electrodo [15].

Con el fin de solventar el problema de la inespecificidad de este tipo de sensores, se han desarrollado redes que combinan las prestaciones de varios sensores individuales en un único dispositivo. Existen numerosos trabajos bibliográficos a cerca del desarrollo de lenguas electrónicas compuestas por redes de sensores basados en ftalocianinas utilizando técnicas electroquímicas tales como la potenciometría, voltametría o espectroscopía de impedancia [5, 16-18]. Debido a que en la presente Tesis Doctoral el tipo de sensores con los que se ha trabajado son voltamétricos, se hará especial hincapié en los mismos. Es importante conocer las propiedades de las ftalocianinas como materiales sensibles, ya que éstas constituyen el origen de su versatilidad y de sus propiedades electroquímicas y electrocatalíticas, las cuales hacen de estas moléculas unos materiales especialmente atractivos como modificadores químicos. Además, la posibilidad que ofrecen de procesarlas en forma de película delgada (films) y nanoestructurada es otra de las razones de su importancia como modificadores químicos en sensores electroquímicos.

A continuación se describirán los diferentes tipos de sensores electroquímicos; potenciométricos, amperométricos, voltamétricos e impedimétricos, de lenguas electrónicas basadas en ftalocianinas y sus principales aplicaciones.

1.2 FTALOCIANINAS

El compuesto conocido como ftalocianina (Pc) consiste en un macrociclo simétrico formado por cuatro unidades isoindólicas (Figura 1.1.).

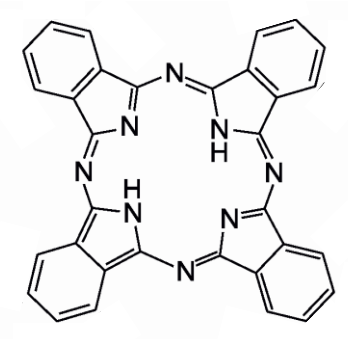


Figura 1.1. *Anillo de ftalocianina.*

La molécula más sencilla es la ftalocianina sin metal central (H_2Pc) cuya estructura ofrece múltiples posibilidades de modificación a partir de la introducción de diferentes iones metálicos, ligandos axiales o coordinación a sistemas aromáticos diferentes. La Figura 1.2. ilustra la gran cantidad de derivados de ftalocianina que se pueden obtener.

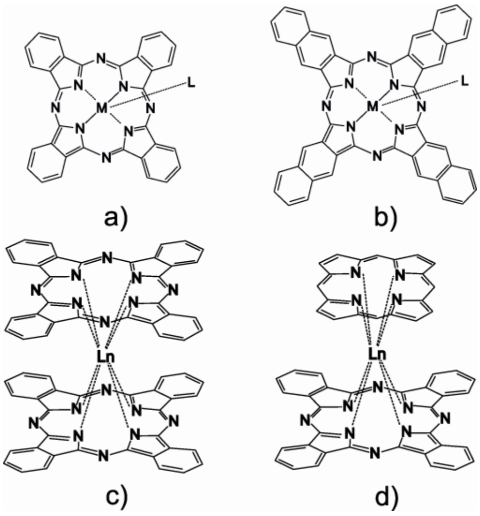


Figura 1.2. *(a) Metaloftalocianina (b) Naftalocianina (c) Bisftalocianina de lantánido (d) Ftalocianina-porfirina heteroléptica.*

Las metaloftalocianinas (MPc) consisten en un anillo de ftalocianina coordinado a un ión metálico (+2) situado en la cavidad central (Figura 1.2.a). Existen más de 70 elementos que pueden alojarse en dicha cavidad [8-11]. La Figura 1.2.b. ilustra la estructura de una familia de derivados de Pc denominada naftalocianinas (NPc), caracterizada por una estructura con conjugación extendida. Las ftalocianinas también pueden formar complejos tipo sándwich (*double decker*), en los cuales un ión lantanoide está coordinado a los dos anillos de ftalocianina ($LnPc_2$) (Figura 1.2.c.) [12, 19].

De la misma manera, los complejos triples (*triple decker*) consisten en la asociación de dos iones lantanoides con tres anillos de ftalocianina. Existen también derivados dobles y triples heterolépticos, que contienen diferentes ligandos tetrapirrólicos (Figura 1.2.d.) [20]. Los complejos denominados *double o triple decker* presentan fuertes interacciones π que dan lugar a interesantes propiedades electrónicas y ópticas. Existen estructuras más complejas donde los anillos de ftalocianina forman dímeros, trímeros o estructuras poliméricas, en las cuales los anillos de Pc se encuentran unidos mediante enlace covalente [11, 21]. En los últimos años se han sintetizado compuestos de ftalocianina mediante uniones covalentes con diferentes tipos de moléculas de interesantes propiedades, entre las que se incluyen fullerenos [22] y perilenos [23]. Además, se ha estudiado la posibilidad de ensamblar ftalocianinas con porfirinas a través de interacciones *host-guest* [24].

El amplio rango de posibilidades que existen para la obtención de derivados explica el vasto número de moléculas diferentes de ftalocianina sintetizadas hasta el momento y el número creciente de las mismas empleadas como materiales sensibles.

1.3 PROPIEDADES DE LAS FTALOCIANINAS

Las propiedades que hacen de las metaloftalocianinas compuestos susceptibles de empleo como materiales sensibles están íntimamente relacionadas con su comportamiento semiconductor, óptico y electroquímico [25]:

Semiconductividad: Las MPcs se comportan como semiconductores de tipo p cuando están dopadas con oxígeno, presentando conductividades en el rango 10^{-10} a 10^{-12} $S.cm^{-1}$ a 300K [26]. Las bisftalocianinas de lantánidos, $LnPc_2$, presentan una alta conductividad intrínseca ($\sigma=10^{-6}-10^{-3}$ $S.cm^{-1}$ a T=300K) [27] asociada al estado neutro radical y a la fuerte interacción electrónica entre los dos anillos de Pc. La conductividad de las moléculas Pc puede modificarse de manera reversible cuando éstas son expuestas a gases, lo cual constituye la base de los quimioresistores basados en ftalocianinas [12, 25].

Propiedades Ópticas: El sistema π conjugado que poseen las ftalocianinas da lugar a intensas bandas de absorción en la región 400-700 nm con coeficientes de extinción molar de aproximadamente 2×10^{5} $l.mol^{-1}.cm^{-1}$ en disolución [8-10]. Algunos derivados presentan también bandas en la región NIR, coincidentes con las longitudes de onda que se emplean en telecomunicación. La interacción de las ftalocianinas con compuestos aceptores y donadores de electrones originan cambios reversibles en el espectro de absorción electrónica [25, 28].

Propiedades Electroquímicas: Las ftalocianinas son materiales con unas interesantes propiedades electroquímicas, electrocatalíticas y electrocrómicas [9, 14]. El anillo Pc es un sistema aromático de 18 electrones, el cual en estado neutro posee dos cargas negativas Pc (2^-). La estructura electrónica de las ftalocianinas permite tanto la oxidación, dando lugar a las especies Pc (1^-) y Pc (0) por cesión de uno y dos electrones, respectivamente, como la reducción del anillo de Pc, dando lugar a las especies Pc (3^-), Pc (4^-), Pc (5^-) y Pc (6^-) en etapas que implican de uno a cuatro electrones. Además, se puede producir una variación en el estado de oxidación del ión del metal central. Un ejemplo de ello es la CoPc, donde el Co (II) puede oxidarse a Co (III) o reducirse a Co (I). Los potenciales electroquímicos a los cuales tienen lugar las oxidaciones y reducciones dependen de la naturaleza del ión del metal central, la clase de ftalocianina y la presencia de sustituyentes en el anillo aromático. Los cambios en el estado de oxidación están acompañados generalmente de cambios en el espectro electrónico, prueba de ello es que las $LnPc_2$ son materiales electrocrómicos.

Las MPcs muestran también una actividad electrocatalítica en la electrooxidación de numerosos compuestos tales como tioles, aminas, hidrógeno peróxido, etc [14, 25, 29-30]. Este comportamiento está asociado a la coordinación del anión analizado como ligando axial al metal central del anillo de ftalocianina.

Las propiedades electroquímicas y electrocatalíticas de las ftalocianinas otorgan a dichos materiales características muy atractivas en el desarrollo de sensores electroquímicos para el análisis de líquidos.

Otras Propiedades: Las ftalocianinas poseen alta estabilidad térmica y química. En vacío muchos complejos no se descomponen por debajo de los 500ºC. Además, los ácidos y bases fuertes no afectan a MPcs. Por último, probablemente una de las propiedades más interesantes es que se pueden preparar electrodos químicamente modificados con ftalocianinas mediante diferentes técnicas, dando lugar a gran variedad de sensores [31].

1.4 PREPARACIÓN DE SENSORES ELECTROQUÍMICOS BASADOS EN FTALOCIANINAS

Las ftalocianinas se pueden confinar en la superficie de un electrodo, dando lugar a electrodos modificados mediante una gran variedad de técnicas. El método empleado en la preparación de los sensores determina la morfología, la cual desempeña un papel decisivo en el comportamiento electroquímico de los sensores.

1.4.1 Métodos clásicos para preparar sensores basados en ftalocianinas

Los métodos clásicos para la preparación de sensores incluyen casting, adsorción, electrodos de pasta de carbono, películas preparadas por spin-coating, screen printing, método sol-gel, deposición electroforética, polimerización electroquímica y sublimación a vacío. Cada uno de estos métodos se describe brevemente a continuación:

Casting: Es un método muy sencillo que consiste en depositar una gota de disolución del material en cuestión sobre la superficie del electrodo. Una vez que el disolvente se evapora se obtiene una película del material sensible [9, 14, 25].

Adsorción: La adsorción de las ftalocianinas en materiales carbononos es uno de los métodos más conocidos debido a su simplicidad. La adsorción de las moléculas sobre la superficie del electrodo se consigue mediante la inmersión de éste en la disolución del compuesto correspondiente en un disolvente adecuado durante varios minutos, lográndose así la adsorción del mismo [9, 14, 25].

Electrodos de Pasta de Carbono (*Carbon Paste Electrode*, *CPE*): Se preparan mezclando grafito (u otro material carbonoso como por ejemplo nanotubos de carbono) con ftalocianina y aceite mineral [32, 33] o una resina epoxi. La mezcla se introduce en un tubo y se emplea un hilo metálico como contacto.

Spin-Coating: El material sensible se deposita en la superficie del electrodo empleando fuerzas centrífugas. La primera etapa consiste en la deposición de la ftalocianina disuelta encima del sustrato. Seguidamente se hace girar el sustrato a una velocidad constante, originándose de esta manera la dispersión de la disolución y un fluído gradual hasta que el disolvente se evapora completamente. El espesor de la película obtenida de esta manera depende de la concentración y viscosidad de la disolución así como de la velocidad de rotación del sustrato. Estas características hacen del spin-coating uno de los métodos más prometedores para ser empleado en sensores comerciales económicos [34, 35].

Screen Printing: El material sensible está serigrafiado sobre un soporte cerámico. Este método ha sido ampliamente utilizado para la preparación de sensores electroquímicos [36]. Los electrodos comerciales screen printed contienen en el mismo dispositivo el electrodo de trabajo, el electrodo de referencia y el contraelectrodo [37, 38].

Método Sol-Gel: La técnica sol-gel se ha empleado ampliamente para incorporar moléculas orgánicas y organometálicas en el interior de moléculas inorgánicas denominadas “huésped” (*host*). La principal ventaja en el uso de los materiales inorgánicos como matriz es que la ftalocianina puede ser “atrapada” en el gel, obteniendo un material con una alta estabilidad [39].

Deposición electroforética (*Electrophoretic Deposition, EPD*): En la primera etapa, las moléculas en disolución migran hacia el electrodo correspondiente mediante la aplicación de un campo eléctrico a la disolución (electroforesis). A continuación, las moléculas se depositan en la superficie de dicho electrodo. El mecanismo de nucleación es instantáneo [40].

Polimerización electroquímica: Es un método efectivo para el desarrollo de superficies electroactivas con un control cuidadoso de la velocidad y de la extensión del proceso de polimerización así como de las propiedades químicas y físicas del polímero resultante [41, 42]. Uno de los posibles métodos para la modificación de un electrodo es la electropolimerización de monómeros (por ejemplo, tetraaminoftalocianina) sobre una superficie conductora.

Sublimación a vacío (*Vacuum sublimation*): El método consiste en calentar las ftalocianinas a temperaturas del orden de 300-400ºC bajo vacío. En estas condiciones, las ftalocianinas subliman y se pueden depositar sobre sustratos adecuados [25]. Las películas evaporadas están formadas generalmente por microcristalitos. Los parámetros de deposición tales como la temperatura del sustrato y velocidad de deposición, así como el calentamiento post-deposición, determinan la estructura (la anchura, tamaño de los microcristales y la fase del cristal) y la morfología de la superficie de la película, la cual determina las características del material sensible.

1.4.2 Preparación de sensores nanoestrucuturados basados en ftalocianinas

Las películas nanoestructuradas han demostrado poseer un gran potencial en la mejora de la sensibilidad de los sensores químicos. La primera razón es que las películas delgadas poseen una alta uniformidad de la superficie y una mayor relación superficie/volumen, la cual permite que las moléculas de analito puedan adsorberse y desadsorberse de los sitios activos más rápidamente. La segunda razón es que la organización estructural a nivel nanométrico hace que las propiedades de la película sean diferentes de aquellas obtenidas con los mismos materiales en forma de película gruesa [20, 43]. Los métodos típicos empleados en la preparación de películas de ftalocianinas nanoestructuradas incluyen las técnicas de self-assembled, layer by layer y Langmuir-Blodgett.

Monocapas autoensambladas (*Self-Assembled Monolayers*, *SAMs*): El término s*elf-assembling* se refiere a la formación espontánea de monocapas autoorganizadas (*self-organized*) adsorbidas en sustratos sólidos inorgánicos. Se han preparado monocapas autoensambladas de ftalocianinas en las cuales un grupo terminal del anillo aromático (generalmente un grupo tiol) muestra una afinidad específica y reversible hacia un sustrato. De esta manera, se han obtenido SAM basadas en ftalocianinas empleando oro, vidrio, silicio o grafito como sustratos [42, 44, 45].

Método Layer by Layer (LbL): Las monocapas se preparan por deposición alterna de compuestos polielectrolitos catiónicos y aniónicos [43, 46, 47]. Este método es sencillo y rápido. Además la estructura, composición de la capa y espesor de la película, puede ser controlada con precisión. La técnica LbL proporciona una herramienta útil para la obtención de nanoestructuras interesantes a partir de la combinación de rectivos tales como nanopartículas de oro, polialilamina y ftalocianina [48].

Langmuir-Blodgett (LB): Las películas LB se obtienen a partir de la dispersión de una disolución de la molécula objetivo en la superficie de agua contenida en una bañera Langmuir. Una vez transcurrido el tiempo necesario para la evaporación del disolvente se realizan compresiones de las moléculas en la interfase aire/agua con el fin de provocar su orientación en la misma, dando lugar a una monocapa flotante altamente ordenada. Dicha monocapa puede transferirse a un sustrato sólido mediante la inmersión del mismo (*dipping*) de manera perpendicular en la subfase del agua. Llevando a cabo numerosas inmersiones del sustrato se obtienen multicapas ordenadas del material electroactivo sobre el sustrato, donde el espesor se puede controlar mediante el número de ciclos de inmersión [13, 49].

Además de estos tres métodos específicos, algunas técnicas clásicas se han modificado para obtener films nanoestructurados. La deposición electroforética se puede emplear para depositar películas nanoestructuradas constituídas por nanohilos (*nanowires*) y nanocilíndros (*nanorods*) de MPcs [50] y $LnPc_2$ [51]. La deposición a vacío puede emplearse también para preparar nanohilos de MPc mediante la adecuada selección de la molécula de partida, de la temperatura del sustrato, y tratamiento del sustrato [52]. Los sensores nanoestructurados pueden prepararse también mediante la inmovilización de ftalocianinas en la superficie de nanopartículas de oro, o nanopartículas coloidales en la superficie de un electrodo [53], o combinando MPcs con nanotubos de carbono [54].

1.5 PRINCIPALES TIPOS DE SENSORES ELECTROQUÍMICOS BASADOS EN FTALOCIANINAS

Los sensores electroquímicos modificados con ftalocianinas pueden operar bajo diferentes principios. a) Electrodos selectivos de iones (*Ion Selective Electrodes, ISE*). En este caso, la respuesta potenciométrica implica la coordinación del ión analizado como ligando axial del metal central del anillo de ftalocianina. b) Muchos de los sensores electroquímicos presentan como ventaja las propiedades electrocatalíticas de los electrodos modificados con MPcs que pueden alterar el potencial de oxidación de la molécula objetivo. Esta actividad electrocatalítica se ha empleado en el desarrollo de sensores amperométricos, voltamétricos o potenciométricos para una gran variedad de sustancias. c) Los cambios inducidos por la disolución analizada en el comportamiento electroquímico de las películas de ftalocianina han abierto la posibilidad de usar dichas películas como sensores voltamétricos. d) En los sensores impedimétricos, se aplica al electrodo recubierto con MPc una corriente alterna. e) Por último, las ftalocianinas pueden ser incorporadas en biosensores como mediadores para facilitar la transferencia electrónica entre el enzima y el electrodo.

1.5.1 Electrodos selectivos de iones: Sensores potenciométricos basados en ftalocianinas

En este tipo de sensores, la concentración de determinados iones en disolución se puede cuantificar a través de la medida del potencial que se genera a través de una membrana selectiva de iones. Uno de los elementos de mayor importancia en el desarrollo de membranas selectivas de iones implica interacciones específicas metal-ligando. En este sentido, las MPc se pueden incorporar en la membrana de sensores selectivos de aniones. La respuesta potenciométrica de estos electrodos se cree que está basada en la coordinación axial del analito en cuestión (anión) al metal central de la molécula presente en la membrana del electrodo [29]. Existen numerosas publicaciones que estudian la utilidad de MPcs como materiales modificadores en electrodos selectivos de iones [13]. Pueden prepararse electrodos selectivos de CN^-, F^-, Cl^-, y Br^-, I^-, SO_4^{2-}, tiocianato, sulfato, azida, salicilato, surfactantes o cisteína mediante la incorporación de MPcs (M=Cu, Zn, Pb, Sn, ZrO) en membranas plásticas de policloruro de vinilo o poliacrilamida, las cuales recubren directamente la superficie de electrodos de grafito [55-58].

1.5.2 Electrodos electrocatalíticos basados en ftalocianinas

Los electrodos modificados con complejos de metales de transición con propiedades redox tienen la habilidad de catalizar la oxidación o reducción de determinados compuestos en disolución mediante la disminución del potencial requerido para el sistema redox en cuestión cuando se compara con el del electrodo sin modificar. Las metaloftalocianinas de iones de metales de transición poseen alta estabilidad y excelentes propiedades redox, además de exhibir cierta actividad electrocatalítica hacia una amplia variedad de sistemas redox. Así, los sensores de ftalocianina se han aplicado en la determinación electrocatalítica amperométrica, potenciométrica y voltamétrica de numerosos compuestos orgánicos e inorgánicos [14]. El número de trabajos publicados en este campo es muy amplio e incluye gran variedad de compuestos de ftalocianina, diseño de electrodos, y moléculas analizadas. Por ejemplo, se ha demostrado que los electrodos modificados con MPcs son útiles para la determinación electrocatalítica de numerosos compuestos de importancia tales como nitrito, cianuro, peróxido de hidrogeno, óxido nítrico (medio biológico), hidracina, tioles y compuestos orgánicos de azufre, glucosa y polisacáridos, aminas alifáticas, ácido ascórbico, glutatión, fenoles o serotonina. Debe señalarse que estas aplicaciones son sólo una pequeña ilustración de la gran variedad de moléculas analizadas que aparecen en la bibliografía (ver referencia [14] para más detalles).

Entre toda la variedad de MPcs que se han aplicado como modificadores en sensores electrocatalíticos, la CoPc y sus derivados constituyen una de las MPcs más empleadas. Esto se debe a la alta reactividad de dicha molécula, la cual, dependiendo de las condiciones experimentales, implica los procesos redox de los pares $Co^{III}/Co^{II}Pc^{2-}$ o $Co^{II}/Co^{I}Pc^{2-}$ (cabe mencionar que generalmente, en las MPcs, los procesos redox están asociados al anillo aromático y no al metal central) [59]. Otras MPcs, como NiPc o FePc, presentan también una buena actividad electrocatalítica, observándose el proceso redox M^{II}/M^{III} [14]. La actividad catalítica de las metaloftalocianinas en la oxidación de numerosas moléculas puede modularse introduciendo grupos apropiados en el macrociclo.

El método de preparación del sensor juega un papel decisivo en la sensibilidad y límite de detección de los electrodos [59]. En trabajos clásicos, las técnicas empleadas incluyen la adsorción directa en la superficie del electrodo o CPEs [60]. La electropolimerización de metaltetraaminoftalocianinas (p-MTAPc) (M=Cu o Ni) da lugar a la obtención de sensores con excelentes propiedades electrocatalíticas hacia especies orgánicas e inorgánicas, por ejemplo H_2O_2, tioles, S^{2-} o NO_2^- [14, 58-61]. La deposición de capas de MPc sobre carbón y microfibras de grafito es un método para diseñar microsensores.

Por ejemplo, la ftalocianina tetrasulfonada de nickel electrodepositada sobre fibra de carbón, grafito o microelectrodos metálicos puede emplearse para detectar óxido nítrico in vivo (dentro de una célula) [62]. Las MPcs pueden encapsularse durante la preparación de la superficie empleando el método sol-gel. Se ha desarrollado un sensor de glutatión mediante la incorporación de CoPc usando este método [63]. Se han investigado también electrodos screen-printed modificados con CoPc como sensores desechables para la medida de ácido nítrico [64].

1.5.2.1 Electrodos con nanopartículas y nanotubos

El número de trabajos dedicados al estudio de la mejora de las propiedades electrocatalíticas de electrodos nanoestructurados basados en ftalocianinas crece de forma significativa cada año. Algunos de estos trabajos combinan ftalocianinas con nanopartículas. A continuación se describen algunos ejemplos:

Los electrodos de pasta de carbono (CPE) impregnados con nanopartículas de ftalocianina de hierro (nanoFePc) combinan las conocidas propiedades electrocatalíticas de FePc con las propiedades características de las nanopartículas (gran área superficial) en un entorno de pasta de carbono [65]. Además, las MPcs se han combinado con nanopartículas de oro (AuNPs). La combinación de estas dos extraordinarias unidades catalíticas (MPcs y AuNPS) puede aumentar significantemente las propiedades catalíticas de los electrodos [66]. De manera similar, se han desarrollado una gran variedad de sensores electroquímicos combinando ftalocianinas y nanotubos de carbono (*CNT*) [54]. El método es sencillo, ya que los electrodos de pasta de carbono se pueden modificar fácilmente mediante la adsorción de MPcs y CNT sobre su superficie. El sistema es capaz de catalizar la reacción de NO, mercaptoetanol o carbaryl [67]. Se pueden emplear métodos más complejos para combinar MPcs y CNT, a través de electrodos serigrafiados de oro para inmovilizar de manera covalente nanotubos de carbono de pared simple (*Single-Walled Carbón Nanotubes*, *SWCNT*) y metal tetra-amino ftalocianinas empleando reacciones base de Schiff [68]. Un estudio comparativo del comportamiento de ftalocianinas con nanopartículas de oro o con SWCNT hacia bisphenol A ha demostrado que los nanotubos dan lugar a una mejor catálisis además de una menor pasivación de la superficie electródica [69].

Los sensores nanoestructurados electrocatalíticos se pueden preparar también mediante la inmovilización de MPc en materiales mesoporosos preparados mediante el proceso sol-gel [70] o por electrodeposición en un líquido iónico funcionalizado [71] el cual ha sido empleado de forma exitosa para la detección de una gran variedad de sustancias tales como ácido oxálico.

1.5.2.2 Electrodos electrocatalíticos basados en películas nanoestructuradas

Las técnicas LbL y SAM se han empleado extensivamente en la obtención de sensores nanoestructurados con propiedades electrocatalíticas.

El método Layer-by-Layer (LbL) es una buena elección para la fabricación de electrodos nanoestructurados en los que se alternan ftalocianinas con diferentes materiales catiónicos y aniónicos [72]. Esta técnica ha permitido la obtención de electrodos basados en ftalocianina de cobalto tetrasulfonada sobre un sustrato de plata y su empleo en la detección de hidracina [73]. También se han empleado de forma exitosa sensores LbL basados en nanohojas (*nanosheets)* de hidróxido y ftalocianina de cobalto para la detección de dopamina [74].

Los sensores LbL compuestos por polianilina (PANI) y ftalocianinas metálicas tetrasulfonadas han demostrado ser capaces de distinguir entre dopamina y ácido ascórbico, el cual actúa como un interferente en fluídos biológicos [75]. La técnica LbL también se puede emplear para producir una interacción sinérgica entre nanopartículas de oro y ftalocianina de hierro. Dicha sinergia provoca un aumento en la actividad electrocatalítica hacia el peróxido de hidrógeno [48].

Las monocapas autoensambladas (SAM) de derivados tiólicos o carboxílicos de ftalocianina depositados sobre una superficie de oro han demostrado poseer una excelente actividad catalítica con una respuesta rápida hacia cisteína u otros tioles [20, 76] y nitrito [77]. Se ha comprobado que el comportamiento electrocatalítico de las SAM de MPc está fuertemente influenciado por su orientación sobre la superficie de oro [20].

Cabe señalar que los trabajos descritos en la sección anterior corresponden a sensores amperométricos. En dichos trabajos, la voltametría se ha empleado como etapa preliminar para investigar el comportamiento electroquímico del analito. Una vez establecido el potencial al cual el analito se oxida, se lleva a cabo la determinación amperométrica [78].

1.5.3 Sensores voltamétricos basados en ftalocianinas

En los sensores voltamétricos se aplica un voltaje al electrodo de trabajo a la vez que se registra la corriente producida en el sistema. Al aplicar un determinado rango de potencial, los compuestos electroactivos se oxidan o reducen sobre el electrodo de trabajo, registrándose una corriente en el sistema. Los voltamogramas muestran picos redox asociados a la oxidación y reducción de las moléculas presentes en disolución. La intensidad y posición de los picos depende de las condiciones experimentales (pH, fuerza iónica) y de la naturaleza del electrodo de trabajo. Además, se pueden aplicar diferentes señales de excitación (voltametría cíclica, voltametría de pulsos, voltametría de onda cuadrada). Dependiendo de la técnica empleada se obtienen diferentes tipos de información [79].

Cuando los sensores voltamétricos se modifican con ftalocianinas electroactivas (que muestren actividad redox en el rango de potencial estudiado), se mejora la selectividad del sistema [44, 80]. En este caso, los voltamogramas muestran picos redox asociados tanto al electrodo de trabajo como a la disolución analizada (si hay presentes analitos susceptibles de sufrir procesos redox). La característica importante de este tipo de sensores modificados es que la interacción que se produce entre el material sensible y la disolución puede mejorar extraordinariamente la selectividad de los electrodos (Figura 1.3.), haciendo que se obtenga una respuesta única y diferente para el mismo electrodo cuando se introduce en disoluciones distintas.

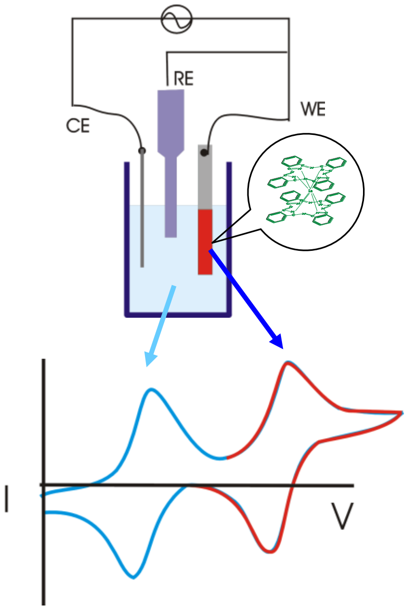


Figura 1.3. *Principio de trabajo de electrodos voltamétricos modificados con un material electroactivo.*

1.5.3.1 Ftalocianinas electroactivas

La respuesta electroquímica de un sensor basado en ftalocianinas electroactivas se puede modificar cambiando el metal central o introduciendo sustituyentes en el anillo de ftalocianina [44]. Entre las MPcs, la CoPc presenta la ventaja de poseer electroactividad asociada al metal central.

En comparación con las metaloftalocianinas, las bisftalocianinas de lantanoides ($LnPc_2$), en las cuales los anillos de Pc están coordinados con un ión de las tierras raras, han suscitado un gran interés en su aplicación como sensores voltamétricos [12, 19]. Dicho interés está asociado al rico comportamiento electroquímico que poseen estos materiales, caracterizado por dos pares redox monoelectrónicos reversibles asociados a la oxidación y reducción del anillo de ftalocianina respectivamente [10, 81]. Se han estudiado una gran cantidad de $LnPc_2$ como materiales sensibles en electrodos voltamétricos [12, 18, 80]. La Figura 1.4. ilustra las respuestas electroquímicas de derivados de $LnPc_2$ con diferentes iones de metales centrales y sustituyentes.

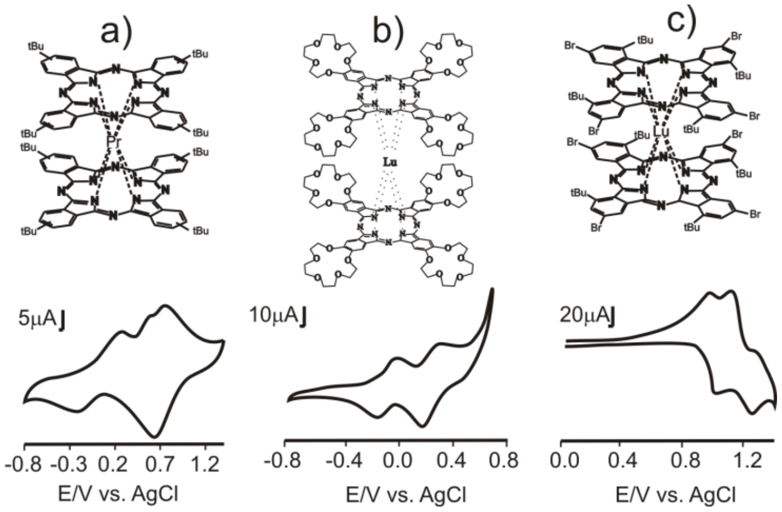


Figura 1.4. *Voltamogramas cíclicos de a) Bisftalocianina de praseodimio octatercbutil sustituída, b)Bisftalocianina de lutecio sustituída con éter corona c) Bisftalocianina de lutecio sustituída con bromo.*

Es posible obtener una gran variedad de respuestas electroquímicas empleando bisftalocianinas con diferentes estructuras como materiales modificadores en sensores voltamétricos.

1.5.3.2 Respuesta a disoluciones iónicas

Las reacciones redox que tienen lugar en el electrodo de trabajo están acompañadas por un flujo de iones entre la disolución y el material electródico con el fin de preservar la electroneutralidad del mismo. Por ello, la posición de los picos en el voltamograma así como su anchura dependen de la naturaleza y de la concentración de los iones en la disolución bajo estudio. Dicha influencia se ilustra en la Figura 1.5., donde se recoge la respuesta electroquímica de un electrodo de $GdPc_2$ inmerso en a) KCl y b) $MgCl_2$ [44].

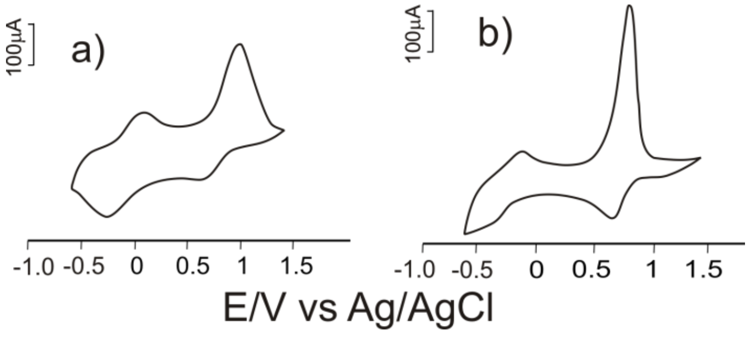


Figura. 1.5. *Voltamogramas cíclicos de un sensor de $GdPc_2$ expuesto a 0.1 mol.L^{-1} (a) KCl (b) $MgCl_2$.*

Existen trabajos bibliográficos en los cuales se han estudiado sensores voltamétricos modificados con derivados de $LuPc_2$ disueltos en una película delgada de nitrobenceno para recubrir un electrodo de grafito o como micropartículas inmovilizadas en la superficie de un electrodo de grafito impregnado con una parafina [82, 83].

1.5.3.3 Respuesta a disoluciones electroactivas

Cuando los electrodos modificados con ftalocianinas electroactivas se introducen en disoluciones que contienen especies químicas con actividad redox, los voltamogramas muestran picos redox de dos orígenes diferentes: respuestas asociadas al material del electrodo y picos asociados a la oxidación-reducción de los analitos presentes en disolución. Además, las interacciones entre el electrodo y la disolución pueden mejorar extraordinariamente la selectividad de los sensores. Dichas interacciones incluyen los siguientes aspectos: i) el carácter oxidante o reductor de la disolución puede modificar el potencial de oxidación del material electródico; ii) la actividad electrocatalítica del material del electrodo puede facilitar la oxidación de compuestos disueltos en la disolución bajo estudio; iii) el carácter ácido o básico de la disolución puede protonar/deprotonar el electrodo [44, 80]. Todos estos procesos redox e interacciones dan lugar a complejos voltamogramas con un alto grado de selectividad.

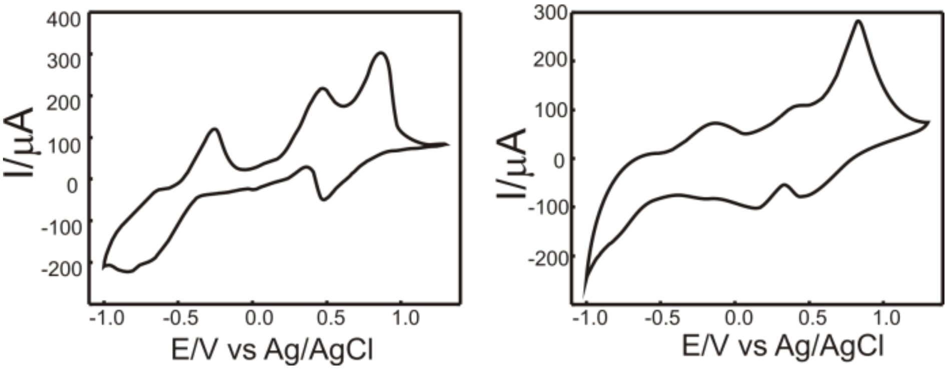


Figura 1.6. *Voltamograma cíclico de un electrodo LB de* $GdPc_2$ *inmerso en una disolución* 10^{-2}*mol.*L^{-1} *KCl y* 10^{-3} *mol.*L^{-1} *de a) Ácido ascórbico; b) Catequina. Velocidad de barrido 0.1* $V.s^{-1}$.

En la Figura 1.6. se muestra la respuesta de un electrodo químicamente modificado con bisftalocianina de gadolinio ($GdPc_2$) en diferentes disoluciones de antioxidantes presentes en alimentación y bebidas. En la Figura 1.6.a. se ilustra el voltamograma de un electrodo de $GdPc_2$ inmerso en KCl, el cual muestra los dos pares de picos correspondientes a la oxidación y reducción reversibles del anillo de ftalocianina. Cuando dicho electrodo se introduce en disoluciones que contienen antioxidantes, se observan nuevos picos asociados a los compuestos electroactivos presentes en disolución. Además, se observan desplazamientos de los picos redox de la $GdPc_2$ hacia valores mayores de potencial debido al carácter antioxidante de la disolución.

Las películas nanoestructuradas LB y LbL se caracterizan por poseer una alta relación superficie/volumen, la cual hace que los sensores preparados de esta manera posean una mayor sensibilidad. Los sensores voltamétricos basados en películas LB de composites

CNT/LnPc$_2$ muestran una mejora en la actividad electrocatalítica cuando se exponen a disoluciones de antioxidantes [84].

La cinética de este tipo de sensores es más rápida, además las películas nanoestructuradas dan lugar a procesos redox más reversibles. Sin embargo, los electrodos de pasta de carbono son más fáciles de preparar y su tiempo de vida es mayor [44].

1.5.4 Sensores impedimétricos

La espectroscopía electroquímica de impedancia ofrece información sobre los mecanismos de reacción del proceso electroquímico que ocurre en el electrodo en disolución. En este caso, el electrodo cubierto con el material sensible se somete a una señal eléctrica alterna de diferente frecuencia.

Como sensores impedimétricos se han empleado electrodos interdigitados recubiertos con películas delgadas de ftalocianinas (FePc) para la detección de trazas de iones en agua. La respuesta de las películas del mismo material pero preparadas por evaporación, por las técnicas de LB o LbL indican que la respuesta eléctrica depende de la organización molecular [85].

1.5.5 Biosensores

El empleo de enzimas para la preparación de sensores bioelectroquímicos puede ayudar a mejorar la selectividad de los mismos. Se ha demostrado que la incorporación en los electrodos de mediadores de electrones que faciliten la transferencia electrónica entre el enzima y el electrodo puede mejorar la sensibilidad del biosensor electroquímico. Las propiedades catalíticas de ftalocianinas combinadas con su biocompatibilidad con enzimas, hacen de estos compuestos macrocíclicos materiales llave para el desarrollo de novedosos dispositivos biosensores para glucosa [86], etanol [87], ácido úrico [88], fenoles [89], etc.

Para preparar biosensores amperométricos, las enzimas pueden inmovilizarse en diferentes soportes electródicos, incluyendo superficies nanoestructuradas de carbón y oro [90, 91], electrodos modificados con polímeros conductores [92] o electrodos modificados con sol-gel [93]. Los coloides de oro, partículas inorgánicas de tamaño nanométrico [91], constituyen materiales atractivos para la obtención de biocomposites con proteínas, facilitando la transferencia electrónica a la superficie del sensor. De manera similar, los nanocomposites de nanopartículas de metaloftalocianinas y enzimas muestran transferencias electrónicas mucho mayores que los biocomposites formados con partículas [94].

Las técnicas de Langmuir-Blodgett (LB) [95, 96], Layer-by-Layer (LbL) [72, 97, 98] y películas autoensambladas (SAMs) [99,100] son de especial interés para la inmovilización de la enzima, ya que que permiten preparar sistemas biocompatibles donde el enzima se adsorbe en una capa lipídica imitando la membrana celular (Figura 1.7.). Este entorno biomimético puede aumentar la actividad enzimática. Con estas técnicas, la preparación de la película delgada se lleva a cado bajo condiciones que permiten preservar la actividad de las biomoléculas en una amplia diversidad de materiales. Las técnicas LB, LbL y SAM permiten también la incorporación en las películas de gran variedad de mediadores electrónicos, incluyendo ftalocianinas, mejorando la sensibilidad del sensor electroquímico [95-100].

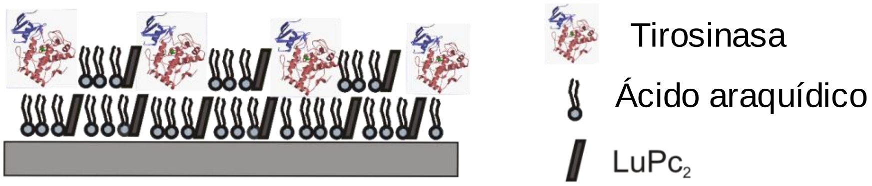


Figura 1.7. *Biosensor biomimético nanoestructurado preparado por la técnica de LB con tirosinasa, ácido araquídico y ftalocianina.*

Recientemente se ha descrito un biosensor electroquímico de ADN que consiste en un electrodo de pasta de carbono cuya superficie se ha modificado mediante el ensamblaje de grupos amino. Se han realizado medidas electroquímicas en una disolución de ftalocianina de cobre tetrasulfonada, observándose un aumento significativo de la corriente del pico de reducción de la ftalocianina en el proceso de hibridación [101].

1.6 LENGUAS ELECTRÓNICAS BASADAS EN FTALOCIANINAS

La aplicación más prometedora de los sensores químicos no específicos descrita en los apartados anteriores consiste en el empleo de los mismos como redes de sensores. De acuerdo con la definición de la IUPAC [1], una lengua electrónica (*Electronic Tongue*, *ET*) es un sistema multisensorial formado por un conjunto de sensores con selectividad cruzada y que emplea procedimientos matemáticos avanzados para procesar las señales, basados en el reconocimiento de patrones y/o análisis de datos multivariantes. En todos los casos, los elementos sensibles deben presentar una especificidad parcial para responder ampliamente a un rango de líquidos, en lugar de a un compuesto químico específico. Por lo tanto, una lengua electrónica es un sistema analítico ideado para la obtención de información global de la muestra estudiada. Gracias al empleo de herramientas estadísticas tales como el Análisis de Componentes Principales (*Principal Component Analysis*, *PCA*) es posible discriminar entre muestras con diferentes características.

Las técnicas de clasificación desempeñan un papel fundamental en el desarrollo de redes de sensores, ya que abordan el problema de la identificación de muestras desconocidas y las asignan a un cierto grupo de muestras previamente entrenadas.

Los modelos típicos de clasificación empleados en lenguas electrónicas son análisis discriminante lineal (*Linear Discriminant Analysis, LDA*), modelización suave e independiente por analogía de clases (*Soft Independent Modelling of Class Analogy, SIMCA*) o redes neuronales (*Artificial Neural Networks, ANN*). El estado del arte de las lenguas electrónicas ha sido objeto de numerosos reviews [1-7], siendo los sensores electroquímicos (potenciométricos, amperométricos, voltamétricos o impedimétricos) los más utilizados en dichos sistemas electrónicos [102-105].

1.6.1 Lenguas electrónicas potenciométricas

La mayoría de los trabajos en este campo están basados en la generación de señales eléctricas a partir de sensores potenciométricos, donde se mide el potencial de membrana creado por la difusión de los iones en disolución a través de la misma [6, 104, 105]. Dichas membrana pueden prepararse con diferentes materiales, proporcionando de esta manera sensores con diferente selectividad. Actualmente se investiga activamente en la búsqueda de ionóforos que puedan reconocer iones específicos y mejorar la selectividad hacia diferentes iones.

Las redes de sensores potenciométricos basadas en metaloftalocianinas y porfirinas disueltas en membranas poliméricas y electrodos de pasta de carbono modificados con porfirinas pueden coordinar aniones al ión del metal central, demostrando de esta manera que son ionóforos eficientes para detectar una gran variedad de iones y analizar bebidas alcohólicas tales como vinos [106-108]. Recientemente se ha descrito el empleo de una red de sensores potenciométricos basados en MPcs y redes neuronales para determinar perclorato e iones sulfuro en aguas contaminadas [109]. Como sensores se han empleado electrodos con membranas de PVC, donde se han incorporado diferentes metaloftalocianinas (GaPc, ZnPc y CoPc) como ionóforos. Dicho estudio ilustra la ventaja del empleo de sistemas multisensoriales para contrarrestar los efectos interferentes entre los aniones bajo estudio (ClO_4^- y S^{2-}).

La Figura 1.8. ilustra la respuesta de una red de sensores potenciométricos basados en sensores de MPc.

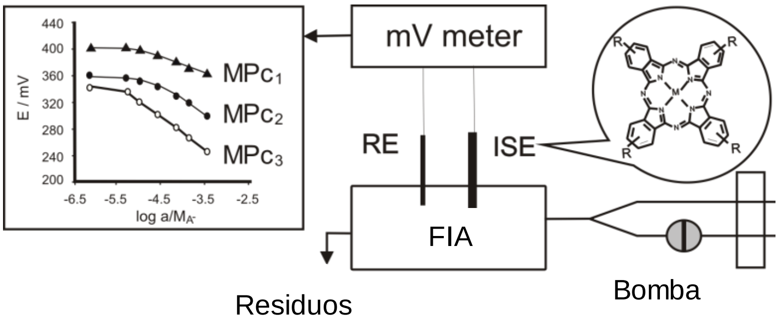


Figura 1.8. *Respuestas de los electrodos ISEs que componen la red de sensores potenciométrios basados en metaloftalocianinas hacia diferentes iones primarios.*

1.6.2 Lenguas electrónicas voltamétricas

Las lenguas electrónicas voltamétricas clásicas consisten en un número de electrodos de trabajo fabricados a partir de diferentes metales en una configuración estándar de tres electrodos. Las medidas se realizan empleando la técnica de voltametría de pulsos [2, 79]. La red de sensores puede mejorarse incluyendo electrodos fabricados con otros materiales (acero inoxidable o pasta de carbón), combinando electrodos voltamétricos con potenciométricos [110] o adaptando un sistema FIA (*Flow Injection Analysis*) [111, 112].

El Grupo de Sensores de la Uva ha sido pionero en el desarrollo de sensores voltamétricos modificados químicamente con ftalocianinas y su empleo en lenguas electrónicas. El principio de funcionamiento de este tipo de sensores se ha comentado en el apartado 1.5.3. Cuando se aplica un determinado voltaje al electrodo de trabajo, el voltamograma que se obtiene refleja los picos asociados a la oxidación y reducción de los analitos presentes en la disolución bajo estudio y los procesos redox relacionados con la ftalocianina empleada como modificador. Además, las interacciones que tienen lugar entre la disolución y la ftalocianina hacen que se obtengan voltamogramas de gran complejidad [44]. Las interacciones incluyen:

- El efecto electrocatalítico de las ftalocianinas puede modificar el potencial de oxidación y la intensidad de los picos asociados a la disolución estudiada.
- El pH o el carácter oxidante o reductor de la disolución puede modificar las propiedades redox de las ftalocianinas depositadas en el electrodo de trabajo.
- La coordinación de iones presentes en la disolución al metal central puede modificar el potencial redox de la ftalocianina.
- Las reacciones redox que tienen lugar en los electrodos modificados con ftalocianinas están acompañadas por movimientos de iones entre la disolución y el material electródico con el fin de preservar la electroneutralidad del mismo.

Las unidades sensibles formadas por derivados de diferentes ftalocianinas pueden proporcionar selectividad cruzada (*cross-selectivity*) debido a su versatilidad, posibilidad de coordinación de diferentes iones y propiedades electrocatalíticas. Además, la presencia de $LnPc_2$ como material sensible es de especial interés debido a su rico comportamiento electroquímico [12, 44, 113]. El principio de trabajo de una lengua electrónica voltamétrica basada en diferentes ftalocianinas como materiales modificadores se ilustra en la Figura 1.9.

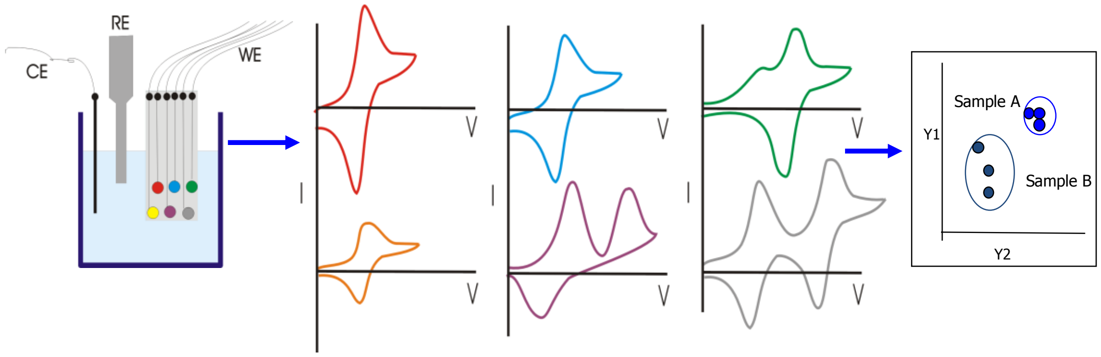


Figura 1.9. *Esquema de una lengua electrónica voltamétrica.*

A parte de su versatilidad, otra ventaja que presentan las ftalocianinas como materiales sensibles para sensores voltamétricos es que los electrodos pueden prepararse empleando diferentes técnicas, las cuales proporcionan sensores con diferentes estructuras y en consecuencia, con diferentes propiedades. Las redes de electrodos voltamétricos basadas en ftalocianinas se han preparado por métodos clásicos tales como electrodos de pasta de carbono [44, 113], incluyendo en algunos casos enzimas [114]. También se ha empleado la técnica de Langmuir-Blodgett (LB) [44], las películas delgadas obtenidas de esta manera poseen una alta relación superficie/volumen, la cual facilita la difusión de los iones en disolución hacia la película, mejorando la cinética.

Cuando se emplean electrodos modificados con ftalocianinas como sensores voltamétricos, las señales que se obtienen presentan una gran complejidad. Los voltamogramas reflejan numerosos picos redox en diferentes posiciones y con diferentes intensidades. Lo que a primera vista puede parecer una desventaja de este tipo de sensores, constituye precisamente el gran potencial que presentan frente a otro tipo de sensores electroquímicos. La complejidad intrínseca de dichos voltamogramas, la cual se traduce en la riqueza y selectividad cruzada de las señales generadas por la red de sensores voltamétricos, representa la gran ventaja de este tipo de sensores, ya que el conjunto de datos obtenido encierra una gran cantidad de información acerca de la muestra estudiada.

Sin embargo, el hecho de que dicho conjunto de datos contenga información significativa a cerca de la disolución bajo estudio puede dificultar el procesado de los mismos. Con el fin de reducir el número de variables sin que ello conlleve pérdida de información significativa es necesario realizar una etapa de pre-tratamiento de la señal obtenida.

Una solución es simplificar la alta dimensionalidad, la cual en parte puede ser redundante o estar relacionada con el ruido, empleando técnicas WT (*feature extraction stage such as the wavelet transformation*) [2]. Otro procedimiento para reducir el número de variables consiste en pre-procesar los voltamogramas mediante técnicas de reducción de datos basadas en el empleo de una función matemática denominada “kernel” [113, 115].

La técnica de pre-procesado consiste en multiplicar los valores de la curva voltamétrica por 10 coeficientes kernel, cuyos resultados constituyen unos coeficientes que representan la información contenida en la señal (Figura 1.10.). Dichos coeficientes se usan como variables de entrada, dando forma a la matriz de datos de entrada para el posterior análisis estadístico.

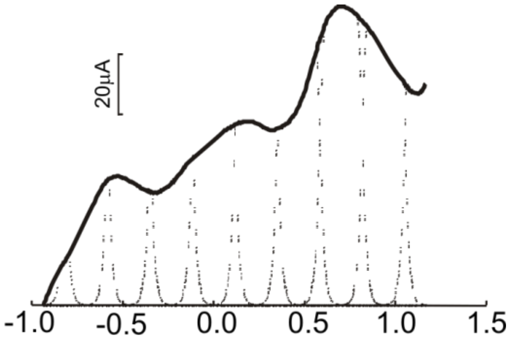


Figura 1.10. *Voltamograma de onda cuadrada y kernels para obtener diez parámetros por curva.*

El empleo de redes de sensores voltamétricos modificados con bisftalocianinas ha permitido discriminar entre disoluciones modelo de sabores básicos (ácido cítrico para producir acidez; salado con NaCl o KCl; $MgCl_2$ o quinina para amargor; dulce con sucrosa o glucosa y umami con glutamato monosódico) [44]. Dicho sistema ha sido también capaz de detectar disoluciones modelo de amargor [116]. Las sustancias bajo estudio incluyen $MgCl_2$, quinina y cuatro compuestos polifenólicos extraídos de aceites de oliva, los cuales son responsables de su amargor. Otros estudios aplican una red de sensores formada por bisftalocianinas y derivados heterolépticos para discriminar antioxidantes presentes en alimentación tales como ácido ascórbico, ácido vanílico o pirogalol [80].

Las redes de sensores basadas en ftalocianinas se han aplicado con éxito también en la discriminación de aceites de oliva de diferentes características [117] y cervezas de diferentes calidades. Las lenguas electrónicas híbridas pueden estar constituídas también a partir de la fusión de sensores voltamétricos y amperométricos. Este tipo de sistema híbrido se ha empleado con éxito en el reconocimiento de cervezas [118].

1.6.2.1 Lenguas electrónicas voltamétricas aplicadas al estudio de vinos

El Grupo de Sensores de la Uva atesora una amplia experiencia en el análisis de vinos empleado redes de sensores voltamétricos modificados con ftalocianinas.

El vino es una compleja disolución hidroalcohólica de pH ácido en la cual se encuentran disueltos una gran cantidad de compuestos de diferente naturaleza. Los polifenoles, los cuales desempeñan un papel fundamental en las características organolépticas del vino, son compuestos electroactivos. Su carácter antioxidante así como el pH ácido del vino influyen de manera notable en el comportamiento electroquímico de los sensores voltamétricos, obteniéndose de esta manera una señal electroquímica característica para cada tipo de vino, reflejo del material modificador (ftalocianina), de las especies disueltas en disolución con propiedades redox y de las interacciones existentes entre ambos.

Las lenguas electrónicas voltamétricas han sido capaces de discriminar vinos tintos con diferentes características organolépticas [115].

El empleo de lenguas electrónicas voltamétricas híbridas formadas por diferentes materiales sensibles (bisftalocianinas, perilenos y polímeros conductores) ha permitido discriminar entre vinos elaborados con diferentes variedades de uva [113] (Figura 1.11.), detectar adulteraciones [119] o seguir el envejecimiento de vinos tintos empleando diferentes métodos [120]. El empleo de una red de biosensores modificados con ftalocianinas ha servido para discriminar vinos cava o cervezas [114].

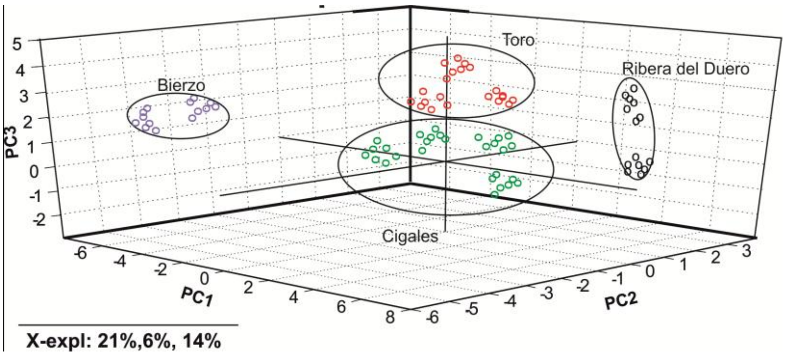


Figura 1.11. *Discriminación entre vinos tintos elaborados con diferentes variedades de uva.*

Uno de aspectos más interesantes en este tipo de sistemas, consiste en establecer correlaciones entre los resultados obtenidos a partir de la lengua electrónica y los obtenidos a partir de análisis químicos tradicionales. Este objetivo es de especial interés ya que los sistemas electrónicos proporcionan información global de las muestras mientras que los análisis químicos proporcionan información sobre compuestos específicos.

Uno de los principales logros alcanzados por nuestro grupo de investigación ha sido la excelente correlación entre las señales proporcionadas por la lengua electrónica voltamétrica basada en ftalocianinas y los parámetros químicos obtenidos empleando técnicas analíticas tradicionales.

Se han encontrado correlaciones excelentes entre los resultados obtenidos con la lengua electrónica y el contenido polifenólico o acidez de los vinos empleando un análisis de regresión por mínimos cuadrados parciales (*Partial Least Squares Regression Analysis*, *PLS2*) [113, 119]. Las excelentes corrrelaciones obtenidas demuestran que las lenguas electrónicas basadas en ftalocianinas se puedan emplear como herramientas analíticas para evaluar el contenido de estos compuestos presentes en vinos con buena precisión.

Por último, cabe mencionar que la combinación de narices y lenguas electrónicas puede aumentar la cantidad de información extraída de una determinada muestra. La combinación de una nariz electrónica (basada en sensores MOX) y una lengua electrónica (basada en bisftalocianinas) ha sido capaz de discriminar vinos con diferentes características organolépticas [121].

1.6.3 Lenguas electrónicas impedimétricas

La espectroscopía de impedancia se ha empleado también como método de transducción en lenguas electrónicas para el análisis de una gran variedad de líquidos. De esta manera, se han empleado redes de sensores impedimétricos recubiertos con diferentes materiales orgánicos, incluyendo ftalocianinas, preparados mediante la técnica de LB y LbL para detectar trazas de compuestos fenólicos en agua [122] o analizar disoluciones complejas tales como cafés o vinos [123]. Se ha demostrado que la red de sensores formada por películas delgadas fabricadas a partir de tres métodos diferentes, evaporación, LB y LbL es capaz de discriminar disoluciones que contienen iones cobre [85].

1.6.4 Lenguas bioelectrónicas

Una lengua biolectrónica es un sistema formado por una red de biosensores con sensibilidad cruzada acoplado a un software adecuado para procesar los datos. Estos sistemas poseen la ventaja de combinar la especificidad de la reacción enzima-sustrato, característica de los biosensores, con la habilidad de la detección simultánea de una gran cantidad de compuestos, característica de los sistemas multisensoriales, proporcionando una amplia información de la muestra en una única etapa. El número de trabajos publicados en este campo ha crecido de manera significativa en los últimos años, e incluye lenguas bioelectrónicas potenciométricas, amperométricas y voltamétricas [124].

La combinación de biosensores con enzimas redox (generalmente oxidasas o deshidrogenasas) se han empleado para analizar una gran variedad de muestras con contenido de glucosa, urea, compuestos fenólicos, etc [114, 124]. Como se introdujo en la sección 1.5.5, las ftalocianinas se pueden incorporar a los biosensores como mediadores electrónicos facilitando la transferencia electrónica entre el enzima y el electrodo. Se han empleado lenguas bioelectrónicas en el campo de las cervezas formadas por sensores de tirosinasa con diferentes ftalocianinas, demostrando que dichos sistemas son capaces de discriminar entre cervezas de diferentes características además de predecir el contenido alcohólico y polifenólico de las mismas [125].

La versatilidad de estos sistemas se puede ilustrar mediante un interesante trabajo donde se ha empleado una red de puntas de platino modificadas con ftalocianina para la detección de óxido nítrico (NO) liberado por poblaciones de células de tejidos umbilicales [126].

1.6.5 Otras lenguas electrónicas

Existen otros métodos de transducción, además de los comentados con anterioridad, que se han empleado en redes de sensores basados en ftalocianinas como materiales modificadores.

En la bibliografía existen trabajos en los cuales se ha descrito lenguas electrónicas basadas en redes de microbalanzas de cuarzo (*Quartz Crystal Microbalances*, *QCM*) recubiertas con ftalocianinas. El cambio en la masa producido cuando el conjunto de sensores se expone a diferentes muestras se ha empleado para estudiar la composición de las mezclas gaseosas analizadas. Se espera que la red de electrodos QCM se aplique en un futuro próximo al análisis de líquidos [127].

La interacción de las ftalocianinas con compuestos donadores o aceptores de electrones da lugar a cambios reversibles en el color de las películas de dichos materiales. Estos cambios de color pueden ser empleados para la construcción de redes de sensores colorimétricos a partir de diferentes conjuntos de pigmentos, los cuales pueden incluir ftalocianinas serigrafiadas en una membrana hidrofóbica, Las imágenes digitales de la red antes y después de la inmersión en un determinado líquido proporciona un perfil de cambio de color que constituye una huella dactilar única para cada analito específico [128].

REFERENCIAS

1. Vlasov, Y., Legin, A., Rudnitskaya, A., Di Natale, C. and D'Amico, A. (**2005**). Nonspecific sensor arrays ("electronic tongue") for chemical analysis of liquids (IUPAC Technical Report), *Pure Appl. Chem.*, 77, pp. 1965-1983.
2. Winquist, F. (**2008**). Voltammetric electronic tongues-basic principles and applications, Microchim. Acta., 163, pp. 3-10.
3. Hayashi, K., Yamanaka, M., Toko, K: and Yamafuji, K. (**1990**). Multichannel taste sensor using lipid-membranes, *Sens. Actuators B.*, 2, pp. 205-213.
4. Escuder-Gilabert, L. and Peris, M. (**2010**). Review: Highlights in recent applications of electronic tongues in food analysis, *Anal. Chim. Acta*, 665, pp. 15-25.
5. Riul, A. Jr., Dantas, C.A.R., Miyazaki, C.M. and Oliveira, O.N. Jr. (**2010**). Recent advances in electronic tongues, *Analyst*, 135, pp. 2481-2495.
6. Ciosek, P. and Wroblewski, W. (**2011**). Potentiometric electronic tongues for foodstuff and biosample recognition-An overview, *Sensors*, 11, pp. 4688-4701.
7. Del Valle, M. (**2011**). Bioinspired sensor systems, *Sensors*, 11, pp. 10180-10186.
8. McKeown, N.B. (**1998**) Phthalocyanine materials: Synthesis, structure and function, (Cambridge University Press, Cambridge)
9. Leznoff, C.C. and Lever, A.B.P. (**1989-1996**) Phthalocyanines, Properties and applications, 1-4, (VCH Publishers, New York)
10. Kadish, K.M., Smith, K.M. and R. (**2000-2003**) The porphyrin handbook, 1-20, (Academic Press, San Diego)
11. de la Torre, G., Nicolau, M. and Torres, T. (**2001**) Phtalocyanines: Synthesis, Supramolecular organization and Physical Properties, (Academic Press, New York)
12. Rodríguez-Méndez, M.L., Gay, M. and de Saja, J.A. (**2009**). New insights into sensors based on radical bisphthalocyanines, *J. Porph. Phthaloc.*, 13, pp. 1159-1167.
13. Valli, L. (**2005**). Phthalocyanine-based Langmuir–Blodgett films as chemical sensors, *Adv. Coll. Interf. Sci.*, 116, pp. 13-44.
14. Zagal, J.H., Griveau, S., Silva, J.F., Nyokong, T. and Bedioui, F. (**2010**). Metallophthalocyanine-based molecular materials as catalysts for electrochemical reactions, *Coord. Chem. Rev.*, 254, pp. 2755–2791.
15. Eggins B.R. (**2002**) Chemical sensors and biosensors. (Wiley).
16. Vlasov, Y.G., Ermolenko, Y.E., Legin, A.V., Rudnitskaya, A.M. and Kolodnikov, V. (**2010**). Chemical sensors and their systems, *J. Anal. Chem.*, 65, pp. 880-890.
17. Del Valle, M. (**2010**). Electronic tongues employing electrochemical sensors, *Electroanal.*, 22, pp. 1539–1555.
18. Rodríguez-Méndez, M.L., Parra, V., Apetrei, C., Villanueva, S., Gay, M., Prieto, N., Martínez, J. and de Saja, J.A. (**2008**). Electronic tongue based on voltammetric electrodes modified with materials showing complementary electroactive properties. Applications, *Microchim. Acta*, 163, pp. 23-31.
19. Weiss R and Fischer J. (**2003**) Porphyrin handbook 16, eds. Kadish, K.M., Smith, K.M. and Guilard, R. Chapter 105 "Lanthanide phthalocyanine complexes", (Academic Press: New York) pp. 171-246.
20. Jiang, J. (**2010**) Functional Phthalocyanine Molecular Materials. Series: Structure and Bonding 135,.1st Ed., (Springer).
21. Maya, E.M., García-Frutos, E.M., Vázquez, P., Torres, T., Martín, G., Rojo, G., Agulló-López, F., González-Jonte, RH., Ferro, V.R., García de la Vega, J.M.G., Ledoux, I. and Zyss. J. (**2003**). Novel push-pull phthalocyanines as targets for second-order nonlinear applications, *J. Phys. Chem.*, 107, pp. 2110-2117.
22. Tkachenko, N.V., Efimov, T.A. and Lemmetyinen, H. (**2011**). Covalent phthalocyanine-fullerene dyads: synthesis, electron transfer in solutions and molecular films, *J. Porph. Phthaloc.*, 15, pp. 780-790.
23. Fukuzumi, S., Ohkubo, K., Ortiz, J., Gutiérrez, A.M., Fernández-Lázaro, F. and Sastre-Santos, A. (**2008**). Control of photoinduced electron transfer in zinc phthalocyanine–perylenediimide dyad and triad by the magnesium ion, *J. Phys. Chem.*, 112, pp. 10744-10752.

24. Leng, X., Choi, C-F., Lo, P-C. and Ng, D.K.P. (**2007**). Assembling a mixed phthalocyanine-porphyrin array in aqueous media through host-guest interactions, *Org. Lett.*, 9, pp. 231-234.
25. Rodríguez-Méndez, M.L. (**2006**) Encyclopedia of Sensors 9, eds. Grimes, C. S., Dickey, E.C. and Pishko, M.V., "Sensing properties of phthalocyanines" (America Scientific Publishers. California. USA) pp 111-134.
26. Bouvet, M. (**2003**) Porphyrin handbook 19, eds. Kadish, K.M., Smith, K,M, and Guilard, R. Chapter 118, "Radical Phthalocyanines and Intrinsic semiconduction" (Academic Press: New York) pp. 37-104.
27. Muzicante, I., Fovans, E., Silinsh, E., Fretigny, C., Bouvet, M., Simon J. and Spirkovitch, S. (**1998**). Charge carrier transport properties of thin films of an intrinsic molecular semiconductor: Lutetium bisphthalocyanine, *Mol. Materials.*, 9, pp. 301-331.
28. Basova, T., Vladimir, P., Aseel, H., Gumus, G.A.G. and Ahsen, G.V. (**2009**). Phthalocyanine films as active layers of optical sensors for pentachlorophenol detection, *Sens. Actuators B.*, 139, pp. 557-562.
29. Lever A.B.P. (**1999**) The phthalocyanines-Molecules of enduring value; a two-dimensional analysis of redox potentials, *J. Porph. Phthaloc.* 3, pp. 488-499.
30. Bedioui, F., Griveau, S., Nyokong, T., Appleby, A.J., Caro, C.A., Gulppi, M., Ochoa, G. and Zagal, J.H. (**2007**). Tuning the redox properties of metalloporphyrin and metallophthalocyanine based molecular electrodes for the highest electrocatalytic activity for the oxidation of thiols, *Phys. Chem. Chem. Phys.*, 9, pp. 3383-3396.
31. Cook, J. and Chambrier, I. (**2003**) Porphyrin handbook 17, Eds. Kadish K.M., Smith, K.M. and Guilard, R. Chapter 108 "Phthalocyanines thin films: Deposition and structural studies" (Academic Press) pp.37-127.
32. Apetrei, C., Apetrei, I., de Saja, J.A. and Rodriguez-Méndez, M.L. (**2011**). Carbon paste electrodes made from different carbonaceous materials: application in the study of antioxidants, *Sensors*, 11, pp. 1328-1344.
33. Gooding, J. J. (**2005**). Electrochemistry at nanoscale nanostructuring electrodes with carbon nanotubes: A review on electrochemistry and applications for sensing, *Electrochim. Acta*, 50, pp. 3049-3060.
34. Roberts, M.E., Mannsfeld, S.C.B., Queraltó, N., Reese, C., Locklin, J., Knoll, W. and Bao, Z. (**2008**). Water-stable organic transistors and their application in chemical and biological sensors, *Proc. Natl. Acad. Sci.*, 105, pp. 12134-12139.
35. Rella, R., Spadavecchia, J., Ciccarella, G., Siciliano, P., Vasapollo, G. and Valli, L. (**2003**). Optochemical vapour detection using spin coated thin films of metal substituted phthalocyanines, *Sens. Actuators B.*, 89, pp. 86-91.
36. Hart, J.P., Crew, A., Crouch, C., Honeychurch, K.C. and Roy, M. (**2005**). Some recent designs and developments of screen-printed carbon electrochemical sensors/biosensors for biomedical, environmental, and industrial analyses, *Anal. Lett.*, 37,pp. 789-830.
37. www.dropsens.com
38. www.bvt.cz
39. Wang, J., Pamidi, P.V.A., Parrado, C., Su Park, D. and Pingarron, J. (**1997**). Sol-gel-derived cobalt phthalocyanine-dispersed carbon composite electrodes for electrocatalysis and amperometric flow detection, *Electroanalysis*, 9, pp. 908–911.
40. Ilangovan, G., Zweier, J.L. and Kuppusamy, P. (**2000**). Electrochemical preparation and EPR studies of lithium Phthalocyanine: Evaluation of the nucleation and growth mechanism and evidence for potential-dependent phase formation, *J. Physical Chemistry*, 104, pp. 4047-4059.
41. Cosnier, S., Karyakin, A., Alpatova, N.M. and Ovsyannikova, E.V. (**2010**) Electropolymerization: Concepts, materials and applications, eds. Cosnier, S. and Karyakinn, A. Chapter 6 "Electropolymerization of Phthalocyanines" (WILEY-VCH Verlag GmbH) pp. 111-130.
42. Obirai, J., Rodrigues, N.P., Bedoui, F. and Nyokong, T. (**2003**). Synthesis, spectral and electrochemical properties of a new family of pyrrole substituted cobalt, iron, manganese, nickel and zinc phthalocyanine complexes, *J. Porph. Phthaloc.*, 7, pp. 508-520.

43. Ferreira, M., Zucolotto, V., Oliveira, O. N. Jr. and Wohnrath, K. (**2003**). Encyclopedia of nanoscience and nanotechnology 4, ed. Nalwa, H.S. (American Scientific Publishers, Los Angeles) pp. 441-460.
44. Wang, Y.F., Zhang, X.R., Ye, Y.C., Liang, D.J., Wang, Y. and Wu, K. (**2010**). Self-assemblies of Oxovanadium Phthalocyanine Molecules and Nanoclusters on Highly Oriented Pyrolytic Graphite, *Acta Physico-Chimica Sinica*, 26, pp. 933-938.
45. Yang, Z.Y., Gan, L.H., Lei, S-B., Wan, L-J., Wang, C. and Jiang, J.Z. (**2005**). Self-Assembly of PcOC8 and Its sandwich lanthanide complex Pr (PcOC8)2 with oligo (Phenylene-ethynylene) molecules, *Phys. Chem. Chem. Phys.*, 109, pp. 19859-19865.
46. Santos, A.C., Zucolotto, V., Constantino, C.J.L., Cunha, H.N., dos Santos, J.R.Jr. and Eiras, C. (**2007**). Electroactive LBL films of metallic-phthalocyanines and poly (o-methoxyaniline) for sensing, *J. Solid State Electrochem.*, 11, pp. 1505-1510.
47. Mashazi, P., Antunes, E. and Nyokong, T. (**2010**). Probing electrochemical and electrocatalytic properties of cobalt (II) and manganese (III) octakis(hexylthio) phthalocyanine as self-assembled monolayers, *J. Porph. Phthalocyan.*, 14, pp. 932-947.
48. Alencar, W.S., Crespilho, F.N., Martins, M.V.A., Zucolotto, V., Oliveira, O.N.Jr. and Silva, W.C. (**2009**). Synergistic interaction between gold nanoparticles and nickel phthalocyanine in layer-by-layer (LbL) films: evidence of constitutional dynamic chemistry (CDC), *Phys. Chem. Chem. Phys.*, 11, pp.5086-5091.
49. Arrieta, A., Rodriguez-Mendez, M.L. and de Saja, J.A. (**2003**). Langmuir–Blodgett film and carbon paste electrodes based on phthalocyanines as sensing units for taste, *Sens. Actuators B.*, 95, pp. 357-365.
50. Tong, W.Y., Djurisic, A.B., Xie, M.H., Ng, A.C.M., Cheung, K.Y., Chan, W.K., Leung, Y.H., Lin, H.W. and Gwo, S. (**2006**). Metal phthalocyanine nanoribbons and nanowires, *J. Phys. Chem. B.*, 110, pp. 17406-17413.
51. Chen, H.Z., Cao, L., Zhou, H.B., Ronh, Y. and Wang, M. (**2005**). Fabrication of gadolinium bisphthalocyanine nano/microwires by electrophoretic deposition, *J. Cryst. Growth*, 281, pp. 530-537.
52. Borras, A., Groning, O., Koeble, J. and Groning, P. (**2009**). Connecting organic nanowires, *Adv. Mat.*, 21, pp. 4816.
53. Wang, K., Xu, J.J., Tang, K.S. and Chen, H.Y. (**2005**). Solid-contact potentiometric sensor for ascorbic acid based on cobalt phthalocyanine nanoparticles as ionophore, *Talanta*, 67, pp. 798-805.
54. Zagal J.H.; Griveau S.; Ozoemena K.I.; Nyokong, T. and Bedioui, F. (**2009**). Carbon nanotubes, phthalocyanines and porphyrins: Attractive hybrid materials for electrocatalysis and electroanalysis, *J. Nanosci. Nanotechnol.*, 9, pp. 2201-2214.
55. Xu, W.J., Chai, Y.Q., Yuan, R. and Liu, S.L. (**2006**). A novel thiocyanate-selective electrode based on a zinc–phthalocyanine complex, *Anal. Bioanal. Chem.*, 385, pp. 926–930.
56. Hassan, S.S.M., Kelany, A.E. and Al-Mehrezi, S.S. (**2008**). Novel polymeric membrane sensors based on Mn(III) porphyrin and Co(II) phthalocyanine ionophores for batch and flow injection determination of azide, *Electroanalysis*, 20, pp. 438-443.
57. Ganjali, M.R., Pourjavid, M.R., Shamsipur, M., Poursaeri, T., Rezapour, M., Javanbakht, M. and Shargi, H. (**2003**). Novel membrane potentiometric sulfate ion sensor based on zinc-phtalocyanine for the quick determination of trace amounts of sulphate, *Anal. Sci.*, 19, pp. 995-999.
58. El-Nemma, E.M., Badawi, N.M. and Hassan, S.S.M. (**2009**). Cobalt phthalocyanine as a novel molecular recognition reagent for batch and flow injection potentiometric and spectrophotometric determination of anionic surfactants, *Talanta*, 78, pp. 723-729.
59. Kozub, B.R. and Compton, R.G. (**2010**). Voltammetric studies of the redox mediator, cobalt phthalocyanine, with regard to its claimed electrocatalytic properties, *Sens. Actuators B.*, 147, pp. 350-358.
60. Zagal, J.H. and Henriquez, J.J.H. (**2000**). Potentiometric response of a graphite electrode modified with cobalt phthalocyanine for thiols and disulfides, *Bol. Soc. Chil. Quím.*, 45, pp. 237-242.
61. Wem, Z.H. and Kang, T.F. (**2004**). Determination of nitrite using sensors based on nickel phthalocyanine polymer modified electrodes, *Talanta*, 62, pp. 351-355.

62. Pereira-Rodrigues, N., Albin, V., Koudelka-Help, V., Auger, V., Pailleret, A. and Bedoui, F. (**2002**). Nickel tetrasulfonated phthalocyanine based platinum microelectrode array for nitric oxide oxidation, *Electrochem. Comm.*, 4, pp. 922-927.
63. Guro, Y. and Guadalupe, A.R. (**1998**). Screen-printable surfactant-induced sol–gel graphite composites for electrochemical sensors, *Sens. Actuators. B.*, 46, pp. 213-219.
64. Honeychurch, K.C., Gilbert, L. and Hart, J.P. (**2010**). Electrocatalytic behaviour of citric acid at a cobalt phthalocyanine-modified screen-printed carbon electrode and its application in pharmaceutical and food analysis, *Anal. Bioanal.Chem.*, 396, pp. 3103-3111.
65. Siswana, M., Ozoemena, K.I. and Nyokong, T. (**2006**). Electrocatalytic behaviour of carbon paste electrode modified with iron(II) phthalocyanine (FePc) nanoparticles towards the detection of amitrole, *Talanta*, 69, pp. 1136-1142.
66. Xhauke, V.P., Chidawanyika, W. and Nyokong, T. (**2001**). The electrochemical behavior of gold nanoparticle-tantalum(V) phthalocyanine composites: Applications towards the electroanalysis of bisphenol A, *Electroanalysis*, 23, pp. 487-496.
67. Silva, J.F., Griveau, S., Richard, C., Zagal, J.H. and Bedoui, F. (**2007**). Glassy carbon electrodes modified with single walled carbon nanotubes and cobalt Phthalocyanine and nickel tetrasulfonated Phthalocyanine: Highly stable new hybrids with enhanced electrocatalytic performances, *Electrochem. Comm.*, 9, pp. 1629-1634.
68. Mashazi, P., Mugadza, T., Sosibo, N., Mdluli, P., Vilakazi, S. and Nyokong, T. (**2011**). The effects of carbon nanotubes on the electrocatalysis of hydrogen peroxide by metallo-phthalocyanines, *Talanta*, 85, pp. 2202-2211.
69. Chauke, V.P., Antunes, E. and Nyokong, T. (**2011**). Comparative behavior of conjugates of tantalum phthalocyanines with gold nanoparticles or single walled carbon nanotubes towards bisphenol A electrocatalysis, *J. Electroanal. Chem.*, 661, pp. 1-7.
70. Rahim, A., Barros, S.B.A., Arenas, L.T. and Gushikem,Y. (**2011**). In situ immobilization of cobalt phthalocyanine on the mesoporous carbon ceramic SiO_2/C prepared by the sol-gel process. Evaluation as an electrochemical sensor for oxalic acid, *Electrochim. Acta*, 56, pp.1256-1261.
71. Wang, K., Dai, L.N., Liu, Q., Li, H.N., Ju, C., Wu, J. And Li, H.M. (**2011**). Electrodeposition of unsubstituted iron phthalocyanine nano-structure film in a functionalized ionic liquid and its electrocatalytic and electroanalysis applications, *Analyst*, 136, pp. 4344-4349.
72. Crespilho, F.N., Zucolotto, V., Oliveira O.N.Jr. and Nart, F.C. (**2006**). Electrochemistry of layer-by-layer films: a review, *Int. J. Electrochem. Sci.*, 1, pp. 194-214.
73. Li, X., Zhang, S. and Sun, C. (**2003**). Fabrication of a covalently attached multilayer film electrode containing cobalt phthalocyanine and its electrocatalytic oxidation of hydrazine, *J. Electroanal. Chem.*, 553, pp. 139-145.
74. Han, J.B., Xu, X.Y., Rao, X.Y., Wei, M., Evans, D.G. and Duan, X.J. (**2011**). Layer-by-layer assembly of layered double hydroxide/cobalt phthalocyanine ultrathin film and its application for sensors, *Mater. Chem.*, 21, pp. 2126-2130.
75. Zucolotto, V., Ferreira, M., Cordeiro, M.R., Constantino, C.J.L., Moreira, W.C. and Oliveira, O.N.Jr. (**2006**). Nanoscale processing of polyaniline and phthalocyanines for sensing applications, *Sens. and Actuators B.*, 113, pp. 809-815.
76. Ponce, I., Silva, J.F., Oñate, R., Rezende, M.C., Paez, M.A., Pavez, J. and Zagal, J.H. (**2011**). Enhanced catalytic activity of Fe phthalocyanines linked to Au(111) via conjugated self-assembled monolayers of aromatic thiols for O_2 reduction, *Electrochem. Comm.*, 13, pp. 1182-1186.
77. Matemadombo, F., Durmus, M., Togo, C., Limson, J. and Nyokong, T. (**2009**). Characterization of manganese tetraarylthiosubstituted phthalocyanines self assembled monolayers, *Electrochim. Acta*, 54, pp. 5557-5565.
78. Wring, S.A., Hart, J.P. and Birch, B.J. (**1990**). Voltammetric behaviour of ascorbic acid at a graphite-epoxy composite electrode chemically modified with cobalt phthalocyanine and its amperometric determination in multivitamin preparations, *Anal. Chim. Acta.*, 229, pp. 63-70.

79. Ivarsson, P., Holmin, S., Höjer, N.E., Krantz-Rülcker, C. and Winquist, F. (**2001**). Discrimination of tea by means of a voltammetric electronic tongue and different applied waveforms, *Sens. Actuators B.*, 76, pp. 449-454.
80. Apetrei, C., Casilli, S., De Luca, M., Valli, L., Jiang, J., Rodríguez-Méndez, M.L. and de Saja, J.A. (**2006**). Spectroelectrochemical characterisation of Langmuir-Shaeffer films of heteroleptic phthalocyanine complexes. Potential applications, *Coll. Surf. A*, 284, pp. 574-582.
81. Yilmaz, I., Nakanishi, T., Gurek, A. and Kadish, K.M. (**2003**) Electrochemical and spectroscopic investigation of neutral, oxidized and reduced double-decker lutetium(III) Phthalocyanines, *J. Porph. Phthaloc.*, 7, pp. 227-238.
82. Njanja, E., Nassi, A., Ngameni, E., Elleouet, C., Quentel, F. and L'Her, M. (**2007**). Lu(III) bisphthalocyanines as mediators for redox reactions at thin-organic-film modified electrodes, *Electrochem. Commun.*, 9, pp. 1695-1699.
83. Komorsky-Lovric, S., Quentelm F., L'Herm M. and Scholzm F. J. (**2008**). Voltammetry of microparticles of lutetium bisphthalocyanine, *Sol. State Electrochem.*, 12, pp. 165-169.
84. Apetrei, C., Nieto, M., Rodríguez-Méndez, M.L. and De Saja, J.A. (**2011**). Development of lutetium bisphthalocyanine/carbon nanotube Langmuir-Blodgett films. Sensing properties, *J. Porph. Phthaloc.*, 15, pp. 908-917.
85. Volpati, D., Alessio, P., Zanfolim, A.A., Storti, F.C., Job, A.E., Ferreira, M., Riul, A. Jr., Oliveira, O.N.Jr. and Constantino, C.J.L. (**2008**). Exploiting distinct molecular architectures of ultrathin films made with iron phthalocyanine for sensing, *J. Phys. Chem. B*, 112, pp. 15275-15282.
86. Rosen-Margalit, I., Bettelheim, A. and Rishpon, J., (**1993**). Cobalt phthalocyanine as a mediator for the electrooxidation of glucose oxidase at glucose electrodes, *Anal. Chim. Acta*, 281, pp. 327-333.
87. Boujtita, M., Hart, J.P. and Pittson, R. (**2000**). Development of a disposable ethanol biosensor based on a chemically modified screen-printed electrode coated with alcohol oxidase for the analysis of beer, *Biosens. Bioelectron.*, 15, pp. 257-263.
88. Gilmartin, M.A.T., Birch, B.J. and Hart, J.P. (**1994**). Development of an amperometric sensor for uric acid based on chemically modified graphite-epoxy resin and screen-printed electrodes containing cobalt phthalocyanine, *Analyst*, 119, pp. 243-252.
89. Portaccio, M., Di Martino, S., Maiuri, P., Durante, D., De Luca, P., Lepore, M., Bencivenga, U., Rossi, S., De Maio, A. and Mita, D.G. (**2006**). Biosensors for phenolic compounds: The catechol as a substrate model, *J. Mol. Catal. B-Enzym.*, 41, pp .97–102.
90. Liu, S.Q., Yu, S.H. and Ju, H.X. (**2003**). Renewable phenol biosensor based on a tyrosinase-colloidal gold modified carbon paste electrode, *J. Electroanal. Chem.*, 540, pp. 61-67.
91. Schnorr, J.M. and Swager, T.M. (**2001**). Emerging Applications of Carbon Nanotubes, *Chem. Mater.*, 23, pp. 646-657.
92. Rajesh and Kaneto, K. (**2005**). A new tyrosinase biosensor based on covalent immobilization of enzyme on N-(3-aminopropyl) pyrrole polymer film, *Current Appl. Phys.*, 5, pp. 178-183.
93. Liu, Z.J., Deng, J.Q. and Li, D. (**2000**). A new tyrosinase biosensor based on tailoring the porosity of Al2O3 sol–gel to co-immobilize tyrosinase and the mediator, *Anal. Chim. Acta.*, 407, pp. 87-96.
94. Wang, K., Xu, J. and Chen, H. (**2005**). A novel glucose biosensor based on the nanoscaled cobalt phthalocyanine–glucose oxidase biocomposite, *Biosens. Bioelectron.*, 20, pp. 1388-1396.
95. Apetrei, C., Alessio, P., Constantino, C.J.L., de Saja, J.A., Rodriguez-Mendez, M.L., Pavinatto, F.J., Fernandes, E.G., Zucolotto, V. and Oliveira, O.N.Jr. (**2011**). Biomimetic biosensor based on lipidic layers containing tyrosinase and lutetium bisphthalocyanine for the detection of antioxidants, *Biosens. Bioelectron.*, 26, pp. 2513-2519.
96. Cabaj, J., Sołoducho, J. and Nowakowska-Oleksy, A. (**2010**). Langmuir–Blodgett film based biosensor for estimation of phenol derivatives, *Sens. Actuators B.*, 143, pp. 508-515.

97. Iost, R.M., Madurro, J.M., Brito-Madurro, A.G., Nantes, I.L., Caseli, L. and Crespilho, F.N. (**2011**). Strategies of nano-manipulation for application in electrochemical biosensors, *Int. J. Electrochem. Sci.*, 6, pp. 2965-2997.
98. Zampa, M.F., Araújo, I.M.S., dos Santos, J.R.Jr., Zucolotto, V., Leite, J.R.S.A. and Eiras, C. (**2012**). Development of a Novel Biosensor Using Cationic Antimicrobial Peptide and Nickel Phthalocyanine Ultrathin Films for Electrochemical Detection of Dopamine, *Int. J. Anal. Chem.*, doi:10.1155/2012/850969.
99. Vieira, N.C.S., Figueiredo, A., de Queiroz, A.A.A., Zucolotto, V. and Guimaraes, F.E.G. (**2011**). Self-Assembled films of dendrimers and metallophthalocyanines as FET-Based glucose biosensors, *Sensors*, 11, pp. 9442-9449.
100. Campuzano, S., Serra, B., Pedrero, M., Villena, J.M. and Pingarron, J.M. (**2003**). Amperometric flow-injection determination of phenolic compounds at selfassembled monolayer-based tyrosinase biosensors, *Anal. Chim. Acta.*, 494, pp. 87-197.
101. Mehrgardi, M.A. and Daneshtalab, R. (**2011**). Electrochemical detection of different types of single-base mismatches in DNA using copper-phthalocyanine tetrasulfonic acid, *J. Electroanal. Chem.*, 650, pp. 214-218.
102. Del Valle, M. (**2010**). Electronic tongues employing electrochemical sensors. *Electroanalysis*, 22, pp. 1539-1555.
103. Scampicchio, M., Ballabio, D., Arecchi, A., Cosio, S.M. and Mannino, S. (**2008**). Amperometric electronic tongue for food analysis, *Electrochim. Acta*, 163, pp. 11-21.
104. Gallardo, J., Alegret, S. and del Valle, M. (**2005**). Application of a potentiometric electronic tongue as a classification tool in food analysis, *Talanta*, 66, pp.1303-1309.
105. Bratov, A., Abramova, N. and Ipatov, A. (**2010**). Recent trends in potentiometric sensor arrays-A review, *Anal. Chim. Acta*, 678, pp. 149-159.
106. Legin, A., Makarychev-Mikhailov, S., Goryacheva, O., Kirsanov, D. and Vlasov, Y. (**2002**). Cross-sensitive chemical sensors based on tetraphenylporphyrin and phthalocyanine, *Anal. Chim. Acta*, 457, pp. 297-303.
107. Paolesse, R., Di Natale, C., Burgio, M., Martinelli, E., Mazzone, E., Palleschi, G. and D’Amico, A. (**2003**). Porphyrin-based array of cross-selective electrodes for analysis of liquid samples, *Sens. Actuators B.*, 95, pp. 400-405.
108. Lvova, L., Paolesse, R., Di Natale, C., Burgio, M., Martinelli, E., Pennazza, G. and D'Amico, A. (**2006**). Detection of alcohols in beverages: An application of porphyrin-based electronic tongue, *Sens. Actuators B.*, 118, pp. 439-447.
109. Wilson, D., Abbas, M.N., Radwan, A.L.A. and del Valle, M. (**2011**). Potentiometric electronic tongue to resolve mixtures of sulfide and perchlorate anions, *Sensors*, 11, pp. 3214-3226.
110. Holmin, S., Bjorefors, F., Eriksson, M., Krantz-Rulcker, C. and Winquist, F. (**2002**). Investigation of electrode materials as sensors in a voltammetric electronic tongue, *Electroanalysis*, 14, pp. 839-847.
111. Winquist, F., Rydberg, E., Holmin, S., Krantz-Rülcker, C. and Lundström, I. (**2002**). Flow injection analysis applied to a voltammetric electronic tongue, *Anal. Chim. Acta*, 471, pp. 159-172.
112. Gutes, A., Ibañez, A.B., del Valle, M. and Cespedes, F. (**2006**). Automated SIA e-tongue employing a voltammetric biosensor array for the simultaneous determination of glucose and asorbic acid, *Electroanalysis*, 18, pp. 82-88.
113. Parra, V., Arrieta, A., Fernández-Escudero, J.A., García, H., Apetrei, C., Rodríguez-Méndez, M.L. and de Saja, J.A. (**2006**). E-tongue based on a hybrid array of voltammetric sensors based on phthalocyanines, perylene derivatives and conducting polymers: Discrimination capability towards red wines elaborated with different variety of grapes, *Sens. Actuators B.*, 115, pp. 54-61.
114. Ceto, X., Cespedes, F., Pividori, M.I., Gutierrez, J.M. and del Valle, M. (**2012**). Resolution of phenolic antioxidant mixtures employing a voltammetric bio-electronic tongue, *Analyst*, 137, pp. 349-356.
115. Parra, V., Hernando, T., Rodríguez-Méndez, M.L. and de Saja, J.A. (**2004**). Electrochemical sensor array made from bisphthalocyanine modified carbon paste electrodes for discrimination of red wines, *Electrochim. Acta*, 49, pp. 5177-5185.

116. Apetrei, C., Rodríguez-Méndez, M.L., Parra, V., Gutierrez, F. and de Saja, J.A. (**2004**). Array of voltammetric sensors for the discrimination of bitter solutions, *Sens. Actuators. B.*, 103, pp. 145-152.
117. Apetrei, C., Rodríguez-Méndez, M.L. and De Saja, J.A. (**2008**). Evaluation of the polyphenolic content of extra virgin olive using an array of voltammetric sensors, *Electrochim. Acta*, 53, pp. 5867-5872.
118. Haddi, Z., Amari, A., Bouchikhi, B., Gutierrez, J.M., Ceto, X., Mimendia, A. and del Valle, M. (**2011**). Data fusion from voltammetric and potentiometric sensors to build a irbid electronic tongue applied in classification of beers, Proc. Of the 14th Intl. Symposium on olfation and electronic nose, 1362, pp. 189-190.
119. Parra, V., Arrieta, A., Fernández-Escudero, J.A., Rodríguez-Méndez, M.L. and de Saja, J.A. (**2006**). Electronic tongue based on chemically modified electrodes and voltammetry for the detection of adulterations in wines, *Sens. Actuators B.*, 118, pp. 448-453.
120. Apetrei, C., Apetrei, I., Nevares, I., del Álamo, M., Parra, V., Rodríguez-Méndez, M.L. and De Saja, J.A. (**2007**). Using an e-tongue based on voltammetric electrodes to discriminate among red wines aged in oak barrels or aged using alternative methods. Correlation between electrochemical signals and analytical parameters, *Electrochim. Acta*, 52, pp. 2588-2594.
121. Rodríguez-Méndez, M.L., Arrieta, A., Parra, V., Vegas, A., Villanueva, S., Gutierrez-Osuna, R. and de Saja, J.A. (**2004**). Fusion of three sensory modalities for the multimodal characterization of red wines, IEEE Sensors J., 4, pp. 348-354.
122. Olivati, C.A., Riul, A. Jr., Balogh, D.T., Oliveira O.N. Jr. and Ferreira, M. (**2009**). Detection of phenolic compounds using impedance spectroscopy measurements, *Bioprocess Biosyst Eng.*, 32, pp. 41–46.
123. Ferreira, E.J., Pereira, R.C.T., Mattoso, L.H.C., Delbem, A.C.B. and Oliveira, O.N. (**2007**). Random subspace method for analysing coffee with electronic tongue, *Electron. Lett.*, 43, pp. 1138-1139.
124. Zeravik, J., Hlavacek, A., Lacina, K. and Sklàdal, P. (**2009**). State of the art in the field of electronic and bioelectronic tongues - Towards the analysis of wines, *Electroanal.*, 21, pp. 2509-2520.
125. Ghasemi-Vamankhasti, M., Rodríguez-Méndez, M.L., Mohtasebi, S.S., Apetrei, C., Lozano, J., Razavi, S.H., Ahmadi, H. and de Saja, J.A. (**2012**). Monitoring the aging of beers using a bioelectronic tongue, *Food Control*, 25, pp. 216-224
126. Isik, S., Berdondini, L., Oni, J., Blöchl, A., Koudelka-Hep, M. and Schuhmann, W. (**2005**). Cell-compatible array of three-dimensional tip electrodes for the detection of nitric oxide release, *Biosen. Bioelec.*, 20, pp. 1566-1572.
127. Ozturk, Z.Z., Kilinc, N., Atilla, D., Gurek, A.G. and Ahsen,V. (**2009**).Recent studies chemical sensors based on phthalocyanines, *J. Porph. Phthaloc.*, 13, pp. 1179-1187.
128. Zhang, C. and Suslick, K.S. (**2007**). Colorimetric sensor array for soft drink analysis, *J. Agric. Food Chem.*, 55, pp. 237-242.

Capítulo II

Resultados

2.1 INTRODUCCIÓN

En este capítulo se resumen los resultados que se han obtenido durante la realización de la presente Tesis Doctoral. La investigación realizada en el Grupo de Sensores de la Universidad de Valladolid por parte de la autora de esta Tesis, ha dado lugar a siete publicaciones científicas, que se recogen a continuación, precedidas de un resumen en el que se describen los objetivos y conclusiones alcanzadas en cada uno de los trabajos.

Las dos primeras publicaciones aportan una visión general de la investigación realizada en el Grupo de Sensores de la UVa. En el Review publicado en el Journal of Porphyrins and Phthalocyanines (*New Insights Into Sensors Based on Radical Bisphthalocyanines. M.L. Rodríguez-Méndez, M. Gay Martín and J.A. de Saja. 13, 1159-1167. 2009*) se resumen las propiedades más interesantes de las $LnPc_2$ como materiales sensibles en diferentes tipos de sensores; resistivos, ópticos y electroquímicos. El trabajo publicado en Microchimica Acta (*Electronic Tongue Based on Voltammetric Electrodes Modified With Materials Showing Complementary Electroactive Properties. Applications. M.L. Rodríguez-Méndez, V. Parra, C. Apetrei, S. Villanueva, M. Gay, N. Prieto, J. Martínez and J.A. de Saja. 163, 23-31. 2008*) recoge una visión global de las ventajas que presentan los sensores voltamétricos modificados con bisftalocianas y perilenos, tomando como ejemplo sistemas sencillos, tales como la discriminación de sabores básicos para seguidamente, abordar sistemas más complejos como vinos blancos.

Los trabajos posteriores se basan en los avances y novedades logradas durante la investigación llevada a cabo. En primer lugar, uno de los avances logrados está relacionado con el estudio de muestras de vino de mayor complejidad que las estudiadas anteriormente por nuestro grupo de investigación. En este sentido, se han estudiado vinos tintos envejecidos con sistemas alternativos al tradicional (depósitos de acero inoxidable a los que se añaden virutas de madera, en lugar de barricas) y, como variable adicional, se ha realizado una microoxigenación de las muestras, con el fin de simular la difusión del oxígeno que tiene lugar a través de los poros de las barricas de roble.

Esta investigación ha dado lugar a un trabajo publicado en la revista Electrochimica Acta (*Application of an Electronic Tongue to Study the Effect of the Use of Pieces of Wood and Micro-Oxygenation in the Aging of Red Wine. M. Gay, C. Apetrei, I. Nevares, M. del Álamo, J. Zurro, N. Prieto, J.A. de Saja, M.L Rodríguez-Méndez. 55, 6782-6788. 2010.*). Por otra parte, en colaboración con la empresa belga Nomacorc®, dedicada a la fabricación de corchos poliméricos, se ha estudiado la influencia de la porosidad del tapón, con el cual se embotella el vino tinto, en las propiedades organolépticas del mismo, dando lugar a una publicación en la revista Food Chemistry (*Analysis of the Influence of the Type of Closure in the Organoleptic Characteristics of a red Wine by Using an Electronic Panel. N. Prieto, M. Gay, S.Vidal, O. Aagaard, J.A. de Saja and M.L. Rodríguez-Méndez. 129, 589-594. 2011*).

En segundo lugar, las novedades más importantes alcanzadas en la presente Tesis Doctoral están relacionadas con el desarrollo de nuevos sensores voltamétricos miniaturizados y nanoestructurados basados en bisftalocianinas de lantánidos como materiales electroactivos. En este sentido se han desarrollado sensores denominados screen printed (SPE) modificados con diferentes $LnPc_2$ como materiales electroactivos. Estos electrodos miniaturizados se han empleado para estudiar la evolución en el tiempo de la frescura del pescado mediante la detección de las aminas biógenas desprendidas durante la descomposición del producto. La red de sensores SPE se ha comparado con una red análoga formada por CPEs, demostrando que los nuevos sensores miniaturizados aportan información similar a los CPEs, siendo los primeros más adecuados para uso comercial. Este trabajo se ha publicado en la revista Electrochimica Acta (*Biogenic Amines and Fish Freshness Assessment Using a Multisensory System Based on Voltammetric Electrodes. Comparison Between CPE and Screen-Printed Electrodes. M.L. Rodríguez-Méndez, M. Gay, C. Apetrei, J.A. de Saja. 54, 7033-7041. 2009*).

Por otra parte, se han desarrollado sensores voltamétricos nanoestructurados basados en $LnPc_2$ preparados mediante deposición electroforética (EPD), técnica que permite la obtención de películas delgadas en tiempos muy cortos, ofreciendo gran versatilidad y bajo coste en comparación con la técnica de Langmuir-Blodgett (LB), con la que se consiguen sensores nanoestructurados con tiempos de preparación mucho más largos e instrumentación más cara y compleja. La caracterización estructural, morfológica y electroquímica de estos sensores EPD ha dado lugar a una publicación en la revista Langmuir (*Films of Lutetium Bisphthalocyanine Nanowires as Electrochemical Sensors. M. Gay Martín, M.L. Rodríguez-Méndez and J.A. de Saja. 26(24) 19217-19224. 2010.*). Dado el éxito alcanzado por los sensores electrodepositados, se ha propuesto por primera vez una lengua electrónica basada en unidades nanoestructuradas de diferentes $LnPc_2$ y aplicada al estudio de antioxidantes en disolución. Este trabajo está publicado en la revista Electrochimica Acta (*Multisensor System Based on Bisphthalocyanine Nanowires for the Detection of Antioxidants. M. Gay Martín, J.A. de Saja, R. Muñoz, M.L. Rodríguez-Méndez. 68, 88-94. 2012*.)

2.2. ARTÍCULO 1. "New insights into sensors based on radical bisphthalocyanines."

M.L. Rodríguez-Méndez, M. Gay, J.A. de Saja.

Journal of Porphyrin and Phthalocyanines 2009, 13, 1159-1167.

2.2.1. Motivación y objetivos.

El trabajo que se adjunta a continuación consiste en una revisión en la que se presentan las excepcionales características que poseen las bisftalocianinas de lantánidos para su empleo en la fabricación de sensores resistivos, ópticos y electroquímicos. Muchas de las ventajas respecto a otros derivados de ftalocianina están relacionadas con el electrón desapareado de la $LnPc_2$, el cual está deslocalizado entre los dos anillos de ftalocianina. Una de las propiedades más importantes de estos materiales es que son semiconductores intrínsecos, lo que constituye una ventaja para las medidas eléctricas. Las $LnPc_2$ presentan una electroquímica muy rica debido a que posee numerosos estados de oxidación accesibles. Además, los procesos redox están acompañados de un cambio de color debido a las propiedades electrocrómicas de estos materiales.

Las propiedades eléctricas, ópticas y electroquímicas son extremadamente sensibles a las condiciones del entorno en el cual se encuentra la $LnPc_2$, siendo dicha característica la base de su uso como sensores.

2.2.2. Resumen del Review.

En este artículo de presenta el estado del arte de los sensores basados en $LnPc_2$. Se presentan los principales resultados obtenidos como sensores resistivos, ópticos, electroquímicos y másicos.

i) Sensores resistivos: Las primeras investigaciones se basaron en el empleo de MPcs como materiales sensibles debido a que la conductividad de dichos materiales puede modularse mediante la adsorción/desorción de gases en la superficie de los mismos. Las MPcs presentan ciertas desventajas como materiales sensibles, tales como su baja conductividad y los largos tiempos de desorción necesarios para recuperar la resistencia original del material. Sin embargo, las $LnPc_2$ debido a la semiconductividad intrínseca que presentan facilitan la medida de la resistencia, además de la sensibilidad hacia los gases objeto de medida. Los cambios producidos en la conductividad de la $LnPc_2$ son proporcionales a la concentración del gas en contacto con el sensor.

   La naturaleza del metal central coordinado a los dos anillos de ftalocianina así como la presencia de diferentes sustituyentes en el anillo aromático pueden modificar las propiedades sensibles de las $LnPc_2$.

Las diferentes técnicas de preparación de este tipo de sensores permiten obtener películas delgadas con una estructura determinada. Por ejemplo, cuando el grosor del material es menor, el proceso de desorción del gas es más rápido. En este caso, la técnica de Langmuir-Blodgett desempeña un papel fundamental en la preparación de películas delgadas con un alto orden estructural y un control preciso del grosor de las mismas, favoreciendo así tiempos de desorción cortos. La alta sensibilidad obtenida con estos materiales sensibles permite detectar no solo gases fuertemente oxidantes y reductores, sino también compuestos orgánicos volátiles (VOCs), que son los principales responsables de los aromas. Estos resultados evidencian la potencial aplicación de películas delgadas basadas en $LnPc_2$ en la detección de aromas de comida y bebidas.

ii) Sensores ópticos: Es bien conocido que las propiedades ópticas (UV-Vis) de las MPcs pueden modificarse mediante la exposición del material a gases donadores o aceptores de electrones. Dichos cambios son la base de su uso como sensores ópticos. Los cambios en el espectro electrónico provocados por la presencia de gases en contacto con el material sensible son mucho más intensos en el caso de las $LnPc_2$. La exposición a gases tales como NO_x provoca un desplazamiento en la banda Q hacia longitudes de onda mayores, asociado a un cambio de color del material, de verde (forma neutra) a rojo (forma oxidada). En presencia de gases dadores de electrones, tales como NH_3, el desplazamiento de la banda Q se produce hacia menores longitudes de onda, y el cambio de color que se observa es del verde (forma neutra) al azul (forma reducida). Este tipo de sensores basados en $LnPc_2$ han demostrado su utilidad en aplicaciones prácticas tales como la detección de humo de tabaco, el cual es rico en amoniaco.

iii) Además, las bisftalocianinas de lantánidos poseen una característica adicional que hace mayor su interés como materiales sensibles. Es la presencia de una banda de transferencia de carga intramolecular en el infrarrojo cercano (NIR), a 1300-1500 nm, la cual es sensible a la presencia de determinados gases y es de un gran interés, ya que coincide con las longitudes de onda que se usan en fibras ópticas utilizadas en telecomunicaciones. Así, ha sido posible diseñar sensores de fibra óptica con estos materiales.

iv) Sensores electroquímicos: Se han desarrollado sensores electroquímicos basados en MPcs como materiales sensibles empleando diferentes técnicas electroquímicas: amperometría, potenciometría o medidas de impedancia. Nuestro Grupo de investigación ha sido pionero en el desarrollo de sensores voltamétricos modificados con $LnPc_2$.

En este sentido, las respuestas que se obtienen contienen gran cantidad de información; relacionada con los procesos redox del material modificador, especies electroactivas presentes en disolución e interacciones entre la superficie del sensor y la disolución en la cual está inmerso. La técnica de preparación de este tipo de sensores, LB o CPE, determina las características estructurales de los mismos y por consiguiente su respuesta electroquímica. Los trabajos pioneros recogidos en este Review han sido el punto de partida de las investigaciones llevadas a cabo en esta Tesis Doctoral.

v) Narices electrónicas y lenguas electrónicas: El enfoque más prometedor de los sensores no específicos es el empleo de los mismos en redes de sensores. En tales sistemas numerosos materiales sensibles con selectividad cruzada hacia gases o líquidos se acoplan a métodos de procesado de señales. Estos métodos incluyen técnicas no supervisadas como PCA, con el fin de evaluar la capacidad de discriminación de la red de sensores entre distintas muestras estudiadas con diferentes características.

vi) También se pueden emplear técnicas de clasificación, tales como LDA (Linear Discrimination Analysis) o ANN (Artificial Neural Networks). Estos instrumentos se denominan narices electrónicas, cuando se aplican a muestras gaseosas, y lenguas electrónicas, cuando se aplican al estudio de muestras líquidas complejas.

2.2.3. Conclusiones

Las propiedades tan particulares que poseen las bisftalocianinas de lantánidos hacen de ellas unos materiales excelentes para la fabricación de sensores químicos. La semiconductividad intrínseca de las $LnPc_2$ y de sus derivados facilita las medidas eléctricas en sensores resistivos. Los cambios de color que tienen lugar debido a su exposición a gases donadores y aceptores de electrones constituyen las bases de los sensores ópticos. Las variaciones en la banda asociada a la transferencia de carga intramolecular presente en el espectro NIR permite el desarrollo de sensores de fibra óptica. Por otra parte, la rica electroquímica que poseen las $LnPc_2$ es la base de los sensores voltamétricos. Estos sensores se han empleado en la detección de gases, VOCs y en gran variedad de compuestos de interés en la industria de la alimentación.

Las redes de sensores basadas en $LnPc_2$ se han desarrollado debido a la amplia variedad de derivados y posibilidad de modificación de los mismos mediante una simple introducción de sustituyentes en los anillos aromáticos, lo cual hace que la sensibilidad y selectividad de cada uno de ellos sea diferente.

Journal of Porphyrins and Phthalocyanines
J. Porphyrins Phthalocyanines 2009; **13**: 1159–1167
Published at http://www.worldscinet.com/jpp/

New insights into sensors based on radical bisphthalocyanines

María Luz Rodriguez-Méndez*[a◊], Mónica Gay[a] and J. Antonio de Saja[b]

[a] *Departamento de Química Inorgánica, E.T.S. Ingenieros Industriales, University of Valladolid, Paseo del Cauce s/n, Valladolid 47011, Spain*
[b] *Departamento de Física de la Materia Condensada, Faculty of Science, C/Doctor Mergelina s/n University of Valladolid, Valladolid 47011, Spain*

Received 29 September 2008
Accepted 30 October 2008

ABSTRACT: The unique semiconducting, optical and electrochemical properties of radical lanthanide bisphthalocyanines make them ideal materials for sensing applications. A variety of chemical sensors have been developed using rare-earth bisphthalocyanine thin films. In this paper, the characteristics of sensors based on bisphthalocyanines are reviewed. The advantages of these sensors with respect to sensors developed using other metallophthalocyanines are discussed. Resistive sensors based on bisphthalocyanines change their conductivity when exposed to a variety of pollutant gases and volatile organic compounds. Because bisphthalocyanines are intrinsic semiconductors, the conductivity of their thin films is higher than the conductivity of metallophthalocyanine thin films. This facilitates the electrical measurements and enhances the sensitivity of the sensors. Optical sensors have also been developed based on the rich optical properties shown by bisphthalocyanines. Films characterized by a bright green color change to red or to blue upon oxidation or reduction. The changes also affect the charge-transfer band associated to the free radical that bisphthalocyanines show in the near infrared region. This band coincides with telecommunication wavelengths, making possible the fabrication of fiber optic sensors where a phthalocyanine film is deposited at one of the ends of the fiber. Electrochemical sensors have been developed taking advantage of the unique electrochemical behavior associated to the one-electron oxidation and one-electron reduction of the phthalocyanine ring. These reversible processes are extremely sensitive to the nature of the electrolytic solution. This has made possible the development of voltammetric sensors able to produce particular signals when immersed in different liquids. In the last part of the paper, the fundamentals and performance characteristics of electronic noses and electronic tongues based on bisphthalocyanines are described. Such devices have been successfully exploited in quality control, classification, freshness evaluation and authenticity assessment of a variety of food, mainly wines and olive oils.

KEYWORDS: bisphthalocyanine, sensor, electronic nose, electronic tongue.

INTRODUCTION

The observation that physicochemical properties of metallophthalocyanines (MPc) are modulated by the adsorption and desorption of gases has led to significant efforts to use them as sensing material in chemical sensors. A variety of transduction methods have been used that include resistive, optical, electrochemical, impedance or mass sensors [1–7].

Sandwich-type rare-earth bisphthalocyanines ($LnPc_2$) are among the most interesting materials for sensing applications (Fig. 1). In the double-decker complexes a lanthanide ion is coordinated with two phthalocyanine rings [8]. The $LnPc_2$ molecules bear one unpaired electron that is delocalized over the two macrocycles. The free radical character and the strong π-π interactions between

---

[◊] SPP full member in good standing

*Correspondence to: María Luz Rodríguez-Méndez, email: mluz@eis.uva.es, tel: +34 983-423540

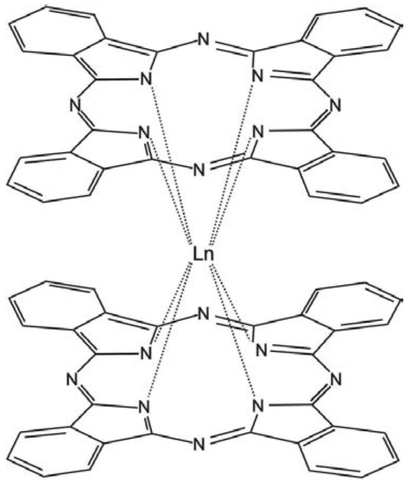


Fig. 1. Scheme of a rare-earth bisphthalocyanine

the two Pc units result in intriguing physicochemical properties. One of the most important properties of $LnPc_2$ is the high intrinsic conductivity ($\sigma = 10^{-6}$–10^{-3} $S.cm^{-1}$ at T = 300 K) while MPcs are insulators with conductivities ranging from 10^{-10} to 10^{-12} $S.cm^{-1}$ at 300 K [9–12]. This high intrinsic semiconductivity is an advantage for electric measurements. Rare-earth bisphthalocyanines have a particularly rich electrochemistry due to the accessibility of several oxidation states. Electrochemistry of $LnPc_2$ solutions in organic media revealed that the molecules can be reduced, or oxidized through many reversible one-electron steps [13–16]. These redox processes are usually accompanied by color changes, being green in the neutral state, reddish in the oxidized form and blue in the reduced form. The first oxidation and reduction potentials of monophthalocyanines are ≥1.5 V apart [17]. The accessibility of the oxidation states of $LnPc_2$ has made possible to study the electrochemistry of chemically modified electrodes (as thin films or carbon paste electrodes) immersed in aqueous solution. Changes in the oxidation state are usually accompanied by changes in the electronic absorption spectra and $LnPc_2$ are well-known as electrochromic materials [18–22].

The UV-vis spectra of MPcs and $LnPc_2$ are typically described by two main absorption bands that are assigned to $\pi \rightarrow \pi^*$ transitions: the Q band centered at about 640–690 nm which is responsible for the blue or green color of the compound, and the B band centered at about 320–350 nm [17–22]. $LnPc_2$ also show a band at 1400–1600 nm, related to the unpaired electron. The band corresponds to an intramolecular charge transfer: the radical monoion Pc ring acts as electron donor and the second Pc ring as an electron acceptor [23–26].

The electrical, optical and electrochemical properties of $LnPc_2$ are extremely sensitive to environmental conditions. The changes produced by the presence of other molecules can be monitored by different transduction methods [3, 4, 27, 28]. In addition, $LnPc_2$ can be deposited as thin films compatible with microelectronics. The methods used to prepare thin films include casting, dip coating, screen printing, spin coating, ultrahigh vacuum evaporation, the Langmuir-Blodgett (LB), the Langmuir-Shaeffer (LS) or the layer-by-layer technique (LbL), *etc.* [29–33]. Each method allows obtaining thin films with particular structures and properties. Special attention has been paid to the Langmuir-Blodgett technique that offers the possibility of controlling, in a very precise fashion, the organization in organic thin films [4].

In spite of their remarkable sensing behavior, bisphthalocyanines have not been so extensively studied as sensitive materials as the parent monophthalocyanine compounds. This is due to the difficulty of the synthesis and purification processes [17, 29]. Fortunately, an increasing number of groups are dedicating efforts to the synthesis of new bisphthalocyanine complexes [30–36]. In this paper, the sensing devices constructed using rare-earth bisphthalocyanines are reviewed (including sensors for electronic noses and electronic tongues) and their advantages discussed.

RESISTIVE GAS SENSORS

The first researches in gas sensors based on phthalocyanines were carried out using MPcs. It has been established that the electronic conductivity of MPc thin films can be modulated by the absorption and desorption of gases [1–4]. Resistive sensors based on MPcs have two advantages over conventional Metal Oxide Sensors (MOX). First, MPcs sensors can work at room temperature while MOX sensors need to be heated at *ca.* 300–350°C. The second advantage is the versatility of MPcs that allows tuning the selectivity of sensing materials so that the molecular chemical properties fit the gas-sensing application needs. The number of MPcs derivatives tested as gas sensors is increasing rapidly. MPcs have some disadvantages which are related to the low conductivity of MPc molecules and the long desorption times (typically several hours are needed to recover the original resistance). These have generated certain prevention towards phthalocyanines as sensitive materials for resistive sensors.

Sandwich-type bisphthalocyanines can overcome these important problems due to their high intrinsic conductivities that facilitate the experimental setup and increase the sensitivity. The exposure of $LnPc_2$ to oxidant gases such as NO_X causes drastic changes in the conductivity at room temperature [37–40]. The changes in the conductivity are intense and proportional to the concentration of gas in contact with the sensor. $LnPc_2$ based sensors can easily detect other gases such as halogens, HCl, H_2S, SO_2, NH_3 and a variety of Volatile Organic Compounds (VOCs) [41–45].

The nature of the central metal ion coordinated to the Pc rings and the presence of chemical groups linked to the peripheral benzene rings can modify the sensing properties of the phthalocyanines [37–47, 63]. The presence of electron-donating substituents such as *tert*butyl, phenyl, propyloxy, octyloxy, or crown ether groups decreases the conductivity of the films and modifies the sensitivity towards oxidant gases [32, 48–50]. Other interesting bisphthalocyanine derivatives have also been synthesized and tested as sensors. For instance, double-decker bisphthalocyanines bridged by a calixarene have shown interesting sensing properties [32].

Resistive sensors based on $LnPc_2$ have been prepared using different techniques. Each method allows obtaining thin films with a particular structure. It has been demonstrated that the structure influences the sensibility and the kinetics and the responses. Special attention has been paid to the Langmuir-Blodgett technique that offers the possibility of controlling, in a very precise fashion, the thickness and the organization in organic thin films [28, 41–47].

It is well-known that the recovery ratio of resistance is related to the penetration of gas molecules into the bulk phase of the sensitive layer [51]. Decreasing the film thickness makes the recovery process faster. The control on the thickness provided by the LB technique improves the recovery times. This approach can be used with $LnPc_2$ due to the high conductivities of these phthalocyanine derivatives. In contrast, it is highly difficult to measure accurately the conductivity of MPcs LB films. As the changes in conductivity observed in $LnPc_2$ sensors are quite intense, the desorption process can be shortened by exposing the sensors to the gas for only a few seconds instead of working under saturation conditions (Fig. 2).

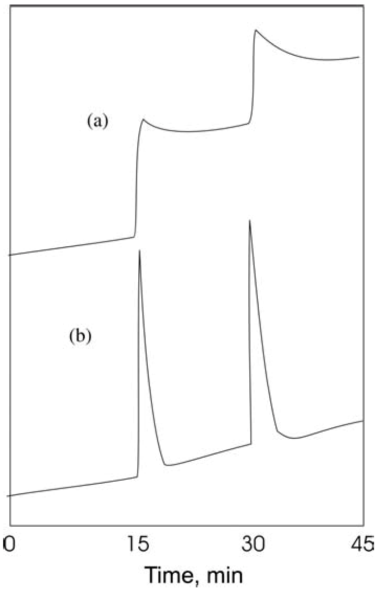


Fig. 2. Changes in conductivity of a $GdPc_2$ evaporated film (upper trace) and LB film (lower trace) exposed to NO_2

The above results have evidenced the potential application of $LnPc_2$ films in the detection of aromas of food and beverages [44, 52] or in environmental applications such as the detection of herbicides [53].

The fabrication of the first field-effect transistor based on phthalocyanines ($LuPc_2$ and ZnPc) was described 20 years ago [3, 55]. More recently, chemical field-effect transistor (ChemFET) was introduced as a new transducer for gas sensing, at first with a monophthalocyanine (NiPc) as active semiconductor [55, 76, 66–74], then by depositing an LB film of a $PrPc_2$ complex and octyloxy derivatives on the gate area of MOSFETs replacing the gate metal [52, 53]. Such LB film ChemFET gas sensors can detect NO_2 gas down to 5 ppm, whereas NiPc-based FETs have been used as ozone sensors in the ppb range [56].

OPTICAL SENSORS

The UV-vis spectra of thin films of MPcs can be modified by exposing the films to electron-donor or -acceptor gases. The sensors show a good sensitivity and selectivity depending on both the metal and the peripheral substituents [2–5]. The changes of electronic absorption spectra caused by gases are of special interest in $LnPc_2$ because the spectral modifications caused by gases are extraordinarily intense. For instance, exposure to NO_X causes a shift in the Q band to higher wavelengths that produces a change in the color of the films from green to red; the adsorption of NO_X is reversible, and the original spectrum can be recovered several hours later. The exposure to electron-donor gases such as ammonia produces a shift of the Q band to lower wavelengths and films change their color to blue (Fig. 3). Other gases such as SO_2, CO, Br_2 or Cl_2 have also been tested. The spectral changes are affected by the nature of the metallic ion and the peripheral substitutions. LB films of alkyl, phenyl, alcoxy and alkylthio-substituted bis(naphthalocyaninato) rare-earth complexes also show reversible changes in the UV-vis spectra when exposed to gases [19, 20, 32, 59–64]. Optical sensors based on bisphthalocyanines have found practical applications. For instance, LB films have been

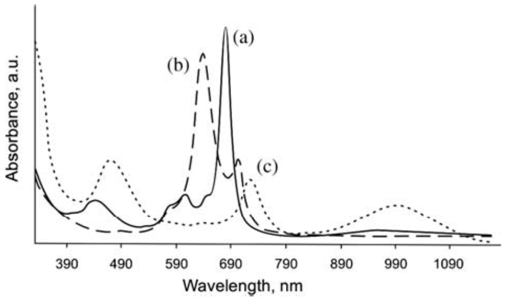


Fig. 3. UV-vis of a $LnPc_2$ (a) LB film (b) LB films exposed to NO_X (c) LB film exposed to NH_3

used to detect tobacco smoke, which is rich in ammonia (among other gases and particles) [66, 67].

$LnPc_2$ have an additional interest as optical gas sensors because the intramolecular charge transfer band in the near infrared region disappears upon the addition of hydrazine, HCl, or VOCs [26–28]. The reversible changes observed in the near-IR region occur at telecommunication wavelengths. This has made the fabrication of fiber-optic sensors possible where $LuPc_2$ Langmuir-Shaeffer films are deposited at one of the ends of the fibers; the other end is connected to an optical detector for measuring the reflected optical power that comes from the vapochromic material [68].

Spectroscopic studies have been carried out to elucidate the nature of the interaction gas-sensor. For instance, the interaction of NO_X causes changes in the vibrational modes related to the isoindole moiety [69].

MASS SENSORS

Phthalocyanines and porphyrins can be used to design sensors that analyze the increase in mass of a thin film using a Quartz Crystal Microbalance (QMC) or a Surface Acoustic Wave (SAW) device [2, 69]. However, few works have been carried out using bisphthalocyanine compounds. A composite of a $LnPc_2$ mixed with carbon nanotubes has been used as the sensing materials in quartz crystal microbalances and microcantilevers for the detection of amines [71]. Spin-coated films of a *tert*-butylcalix[4]arene bridged bis double-decker lutetium(III) phthalocyanine (Lu_2Pc_4) deposited onto a quartz crystal resonator has demonstrated to be sensitive to a variety of vapors [32].

ELECTROCHEMICAL SENSORS

Electrochemical sensors based on MPcs have been exploited as sensors for the assessment of many important inorganic, organic or biological compounds. A good review can be found in reference 13. Such devices have been developed using a range of electrochemical techniques. Amperometric sensors are based on the oxidation or reduction of electroactive compounds at the working electrode, when a constant potential is applied. The modification of the electrode surface with phthalocyanines can provide a range of electrodes with different selectivity. In electrocatalytic amperometric sensors, electrodes modified with certain phthalocyanines (mainly CoPc and its derivatives) have the ability to catalyze the oxidation or reduction of solved compounds by lowering the potential required for the catalyzed redox systems [13]. Amperometric enzyme sensors combining the specificity of enzymatic reaction using a phthalocyanine as a mediator have been developed [72, 73]. In ion-selective electrodes, potentiometric response of a metallophthalocyanine-doped membrane electrode is based on the coordination of the anion analyzed as an axial ligand to the central metal of the phthalocyanine molecule [13, 74]. Electrochemical impedance has been used to test the response of MPc films towards certain gases and liquids [75–77].

$LnPc_2$ have been used to develop only amperommetric and voltammetric sensors. Voltammograms of lanthanide (III) phthalocyanine thin films show two monoelectronic, fully reversible, oxidation and reduction processes. The redox transformations are accompanied by ion movements between the solution and the bulk material necessary for preserving the macroscopic electroneutrality of the electrode. For this reason, the peak positions and their sharpness depend on the nature and the concentration of the ions solved in the test solution and the electrochemical responses can be used to detect ions present in the solution [78]. This is illustrated in Fig. 4 where the electrochemical response of a $LuPc_2$ electrode immersed in KCl, $MgCl_2$, HCl and NH_3 is shown. The spectroelectrochemical response of Langmuir-Blodgett films of bisphthalocyanines bridged with calixarenes in the presence of different counterions has also been studied [22].

The technique used to prepare the electrodes influences the kinetics of the diffusion of ions. Electrodes prepared

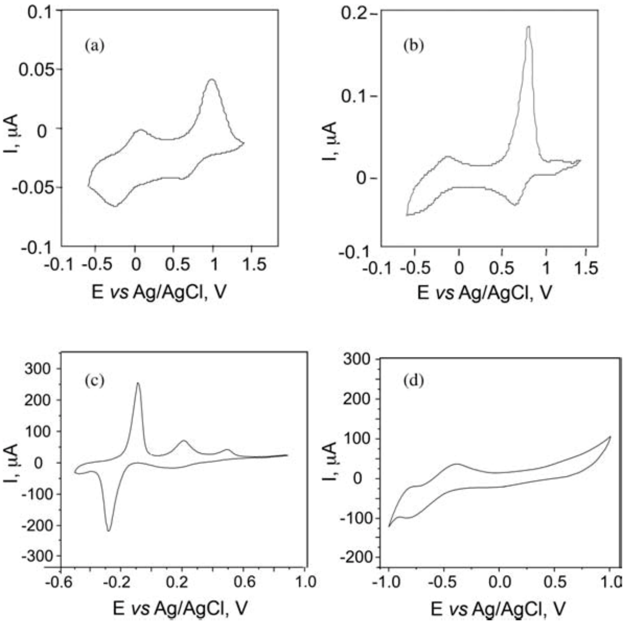


Fig. 4. Cyclic voltammograms of a $LuPc_2$ sensor exposed to 0.1 M (a) KCl (b) $MgCl_2$ (c) HCl and (d) NH_3

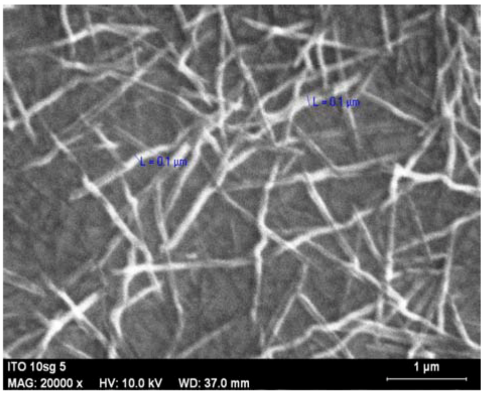


Fig. 5. Nanostructured films of $GdPc_2$ deposited under electrophoretic conditions onto ITO glass

using the Langmuir-Blodgett technique or by the carbon paste technique (mixing the bisphthalocyanine with carbon and a conglomerate) have been successfully used for the detection of ions and of a variety of electroactive substances. The kinetics is faster and the reversibility is improved when using Langmuir-Blodgett or Langmuir-Shaeffer films. However, carbon paste electrodes are easy to fabricate and their lifetime is longer [78].

Recently, nanostructured films have been prepared by means of electrophoretic techniques (Fig. 5). The electrochemical properties of the films have been evaluated towards solutions containing ammonia and a range of biogenic amines such as trimethyl amine and dopamine. It has been demonstrated that nanostructured films with a high surface/volume ratio can give rise to sensors with improved properties [79].

Electrodes modified with $LuPc_2$ dissolved in a thin film of nitrobenzene covering a graphite electrode have also been tested as voltammetric sensors for the detection of ions. In such electrodes, the $LnPc_2$ acts as a mediator for the electron transfer between the solid electrode and a substrate in the aqueous phase. The monoelectronic, fully reversible, oxidation and reduction processes, can be utilized for examination of anion and cation transfers between different phases [79, 80]. Similar studies have been carried out using bis(tetra-*t*-butylphthalocyaninato) (tetra-*t*-butylphthalocyaninato and hexadecachlorophthalocyaninato) derivatives [78]. The transfer of ions in microparticles of lutetium (bis-*tert*-butylphthalocyaninato) complex immobilized on the surface of paraffin-impregnated graphite electrode has also been studied [82].

Voltammetric sensors chemically modified with lanthanide bisphthalocyanines have also been used to analyze solutions containing electroactive substances. In this case, voltammograms show peaks of two different origins: peaks associated to the oxidation-reduction of the analytes present in the solution, and the transient responses associated to the electrode material. But the important issue is that the interactions that occur between the electrode and the solution can improve extraordinarily the selectivity of the electrodes. These interactions include: (i) the oxidant or reducing character of the solution can modify the oxidation potential of the electrode material, (ii) the electrocatalytic activity of the electrode material can facilitate the oxidation of the compounds dissolved in the test solution, and (iii) the response of the electrode material is related to the ability of the sensors to allow the diffusion of the counterions between the solution and the bulk material, an influx necessary to preserve the macroscopic electroneutrality of the electrode [78, 83]. All these redox processes and interactions give rise to rich voltammograms with a high degree of selectivity.

Finally, an innovative sensor based on the electrochromic properties of spin-coated films of a bisphthalocyanine derivative has been used for the assessment of nicotinamide adenine dinucleotide hydride (NADH) in water solutions [84].

ELECTRONIC NOSES AND ELECTRONIC TONGUES

The most promising approach to finding applications for non-specific chemical sensors is the use of arrays of sensors. In such systems several sensitive materials which exhibit cross selectivity to various gases or liquids are coupled with signal processing methods. These methods include non-supervised techniques such as Principal Component Analysis (PCA). Using PCA it is possible to discriminate between samples with different characteristics. Classification techniques can also be used that address the problem of identifying an unknown sample and assigning it to a certain set of previously learned categorized samples. Typical classification models used in electronic tongues are Linear Discrimination Analysis (LDA) and Artificial Neural Networks (ANN).

These instruments are known as electronic noses (when they are applied to the analysis of gases) [85] and electronic tongues (when they are applied to the analysis of complex liquids) [74, 86–88].

Thin films of different metalloporphyrins have been used as sensing materials in arrays of QMC sensors to discriminate a variety of gases [89] and wines [90, 91]. Porphyrins and metallophthalocyanines have also been investigated as the sensing layer in electronic optical noses to detect VOCs of interest in food analysis [92, 93]. In spite of the potential advantages of $LnPc_2$, up to now arrays of mass sensors have not been designed. Phthalocyanines and porphyrins have not been extensively used as sensitive materials in resistive sensors for electronic noses due to their low conductivity. In contrast, the high conductivity of lanthanide bisphthalocyanines facilitates the electrical measurements in array of sensors. An array of rare-earth bisphthalocyanine sensors coupled with a data treatment software has been used to discriminate among VOCs belonging to the main families of

odorant molecules (alcohols, carbonyls and esters) usually present in foods and beverages [44]. This system has been able to discriminate among olive oils of different qualities [52].

Electrochemical sensors (potentiometric, impedimetric or voltammetric) are the most widely used sensing units in electronic tongues [94–97].

Arrays of potentiometric sensors based on metallophthalocyanines and porphyrins solved in polymeric membranes and glassy carbon electrodes modified with porphyrins have demonstrated to be efficient ionophores to detect a variety of ions [98, 99]. A porphyrin-based electronic tongue has been applied to the detection of alcohol in beverages and to the analysis of wines [100]. Arrays of impedimetric electrodes covered with various organic materials (including conducting polymers, perylenes, phthalocyanines, carbon nanotubes or lignine) have been used to detect simple substances such as phenothiazine [101] and to analyze complex solutions such as coffee [102].

Bisphthalocyanines have also been used to construct arrays of electrochemical sensors. In such arrays square wave voltammetry and cyclic voltammetry have been used as measurement method. The principle behind voltammetric electrodes chemically modified with electroactive materials was presented in previous paragraphs. When a voltage is applied, peaks associated to the oxidation-reduction of the analytes present in the test solution can be observed. In addition, transient responses caused by redox processes associated to the phthalocyanine deposited onto the electrode material are also observed. Interactions between the solution and the bisphthalocyanine give rise to rich voltammograms with a high degree of selectivity.

An array formed by voltammetric electrodes modified with bisphthalocyanines has been used to discriminate between model solutions of basic tastes (citric acid to produce sourness, saltiness produced by NaCl or KCl, $MgCl_2$ or quinine to produce bitterness, sweetness produced by sucrose or glucose, and umami produced by monosodium glutamate [78]). The system has also been able to detect model solutions of bitterness [103]. Substances under study included $MgCl_2$, quinine, and four phenolic compounds extracted from olive oils which are mainly responsible for their bitterness. Antioxidants usually present in foods (citric acid, vanillin or pyrogallol) have been discriminated using an array formed by bisphthalocyanines and heteroleptic derivatives [104]. The content of antioxidants has also permitted the use of the electronic tongue to distinguish between seed oils and olive oils [105].

In spite of the difficulty of using arrays of sensors for the analysis of extremely complex liquids such as red wines, electronic tongues based on voltammetry has been able to discriminate red wines with different organoleptic characteristics [83,106]. The capabilities of the array of voltammetric sensors has been improved by extending the array to other families of sensitive materials (bisphthalocyanines, perylenes and conducting polymers). Using this hybrid system, it has been possible to discriminate between wines elaborated with different varieties of grapes [107] to detect adulterations [108] or to follow the aging of red wines using different methods [109]. These electronic tongues are not only able to discriminate but also the results have been correlated with the chemical parameters measured in wines and in olive oils. Good correlations have been found with the polyphenol contents and with the parameters related with the acidity [110, 111].

It has been demonstrated that the simultaneous utilization of electronic noses and electronic tongues can increase the amount of information extracted from a certain sample. The combination of sensors operating in liquid (electronic tongue) and in the head space (electronic nose) based on metalloporphyrins has been successfully used in clinical applications and in food analysis [88,112]. A combination of an electronic nose (based on MOX sensors) and an electronic tongue (based on bisphthalocyanines) has been used to discriminate wines with different organoleptic characteristics [113].

CONCLUSIONS AND FUTURE WORK

The particular properties of $LnPc_2$ make them one of the most interesting organic molecules in the fabrication of chemical sensors. The high conductivity of $LnPc_2$ and their derivatives facilitates the electrical measurements in resistive sensors. The color changes that occur after exposure to oxidant and reducing gases are the basis of optical sensors. The unique spectral variations in the NIR region at telecommunication wavelengths have permitted the development of fiber optic sensors. Finally, the rich electrochemistry of $LnPc_2$ is the basis of voltammetric sensors with a high degree of cross-selectivity.

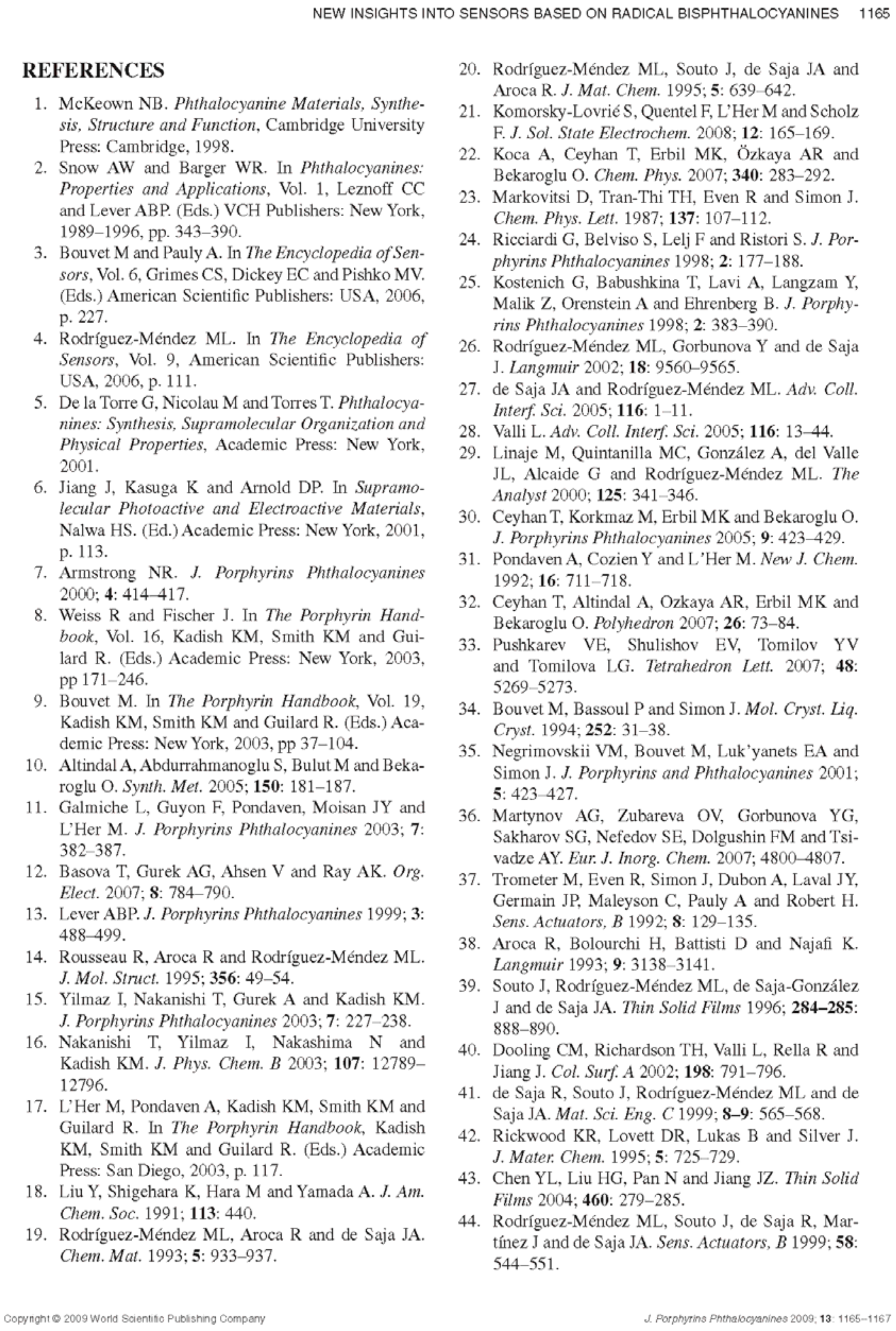
Such sensors have been used to detect a variety of gases, VOCs and a variety of compounds of interest in the food industry and in environmental applications, among others.

Finally, arrays of sensors based on bisphthalocyanines have been developed because there is a wide variety of different bisphthalocyanine molecules with large ranges of sensitivity available. Such systems have demonstrated enormous potential due to their advantageous performance characteristics.

The number of bisphthalocyanine derivatives tested as gas or liquid sensors is increasing rapidly. The possibility of having new molecules with purposely designed properties will provide a way of improving the selectivity of the sensors.

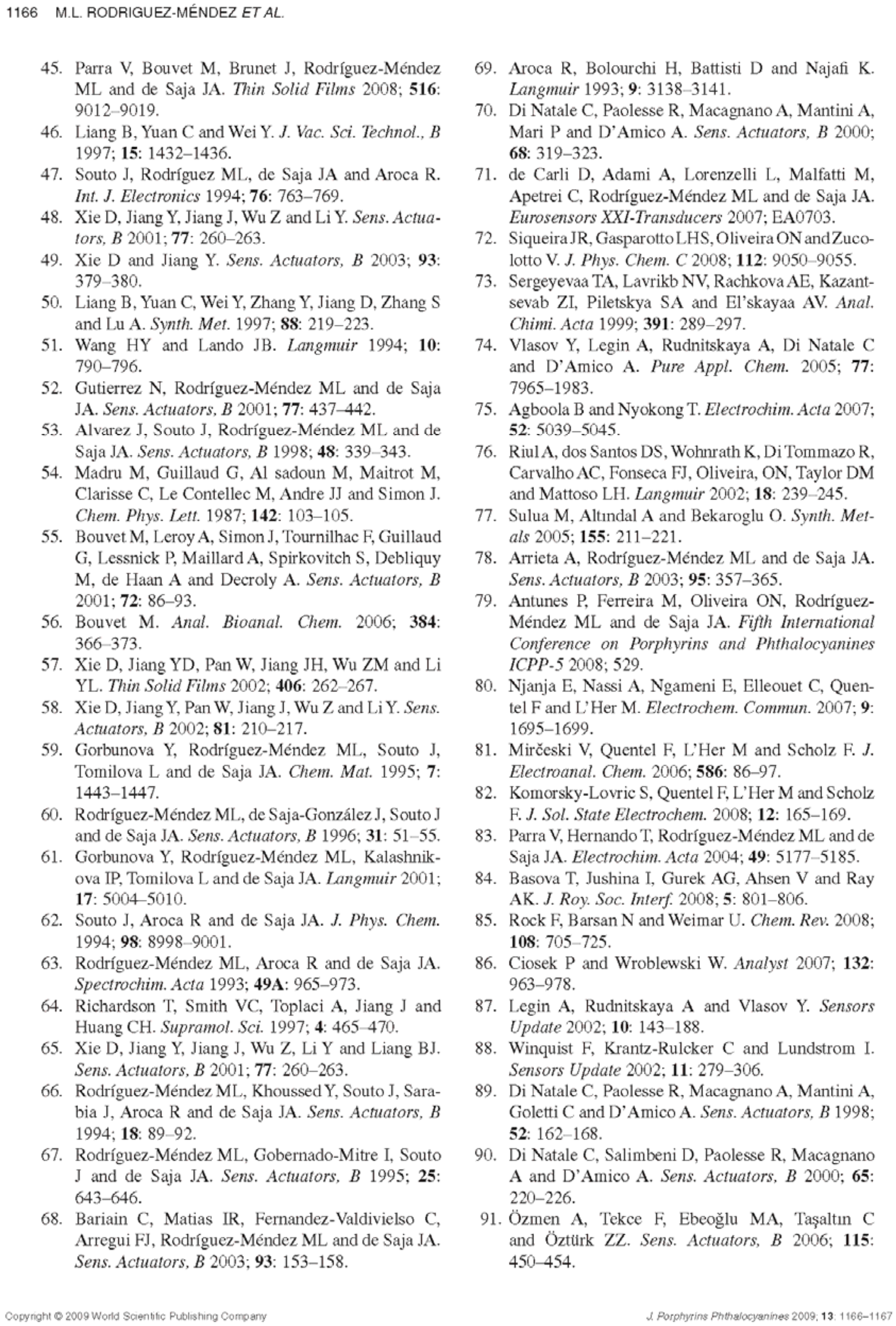
Acknowledgements

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

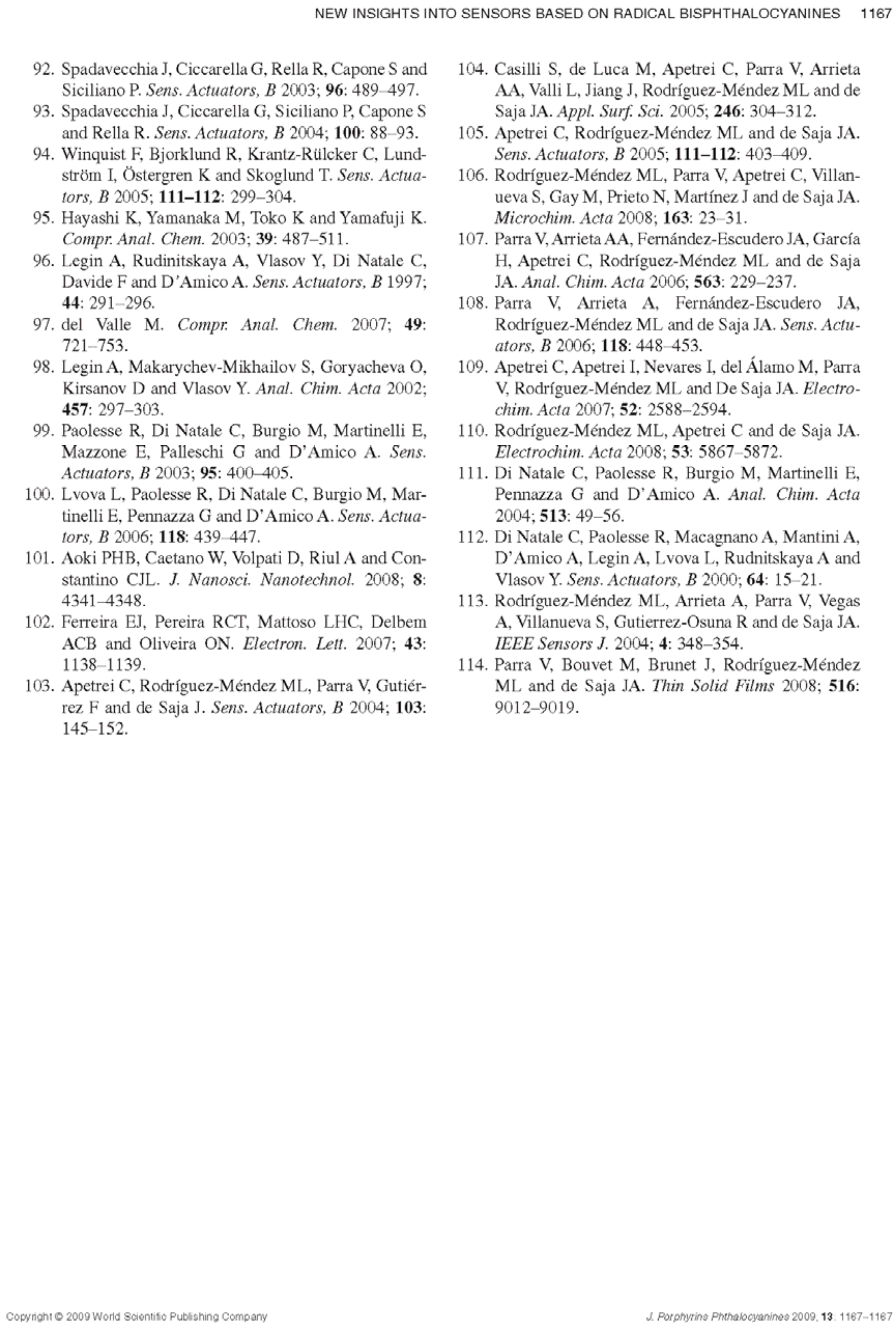


REFERENCES

1. McKeown NB. *Phthalocyanine Materials, Synthesis, Structure and Function*, Cambridge University Press: Cambridge, 1998.
2. Snow AW and Barger WR. In *Phthalocyanines: Properties and Applications*, Vol. 1, Leznoff CC and Lever ABP. (Eds.) VCH Publishers: New York, 1989–1996, pp. 343–390.
3. Bouvet M and Pauly A. In *The Encyclopedia of Sensors*, Vol. 6, Grimes CS, Dickey EC and Pishko MV. (Eds.) American Scientific Publishers: USA, 2006, p. 227.
4. Rodríguez-Méndez ML. In *The Encyclopedia of Sensors*, Vol. 9, American Scientific Publishers: USA, 2006, p. 111.
5. De la Torre G, Nicolau M and Torres T. *Phthalocyanines: Synthesis, Supramolecular Organization and Physical Properties*, Academic Press: New York, 2001.
6. Jiang J, Kasuga K and Arnold DP. In *Supramolecular Photoactive and Electroactive Materials*, Nalwa HS. (Ed.) Academic Press: New York, 2001, p. 113.
7. Armstrong NR. *J. Porphyrins Phthalocyanines* 2000; **4**: 414–417.
8. Weiss R and Fischer J. In *The Porphyrin Handbook*, Vol. 16, Kadish KM, Smith KM and Guilard R. (Eds.) Academic Press: New York, 2003, pp 171–246.
9. Bouvet M. In *The Porphyrin Handbook*, Vol. 19, Kadish KM, Smith KM and Guilard R. (Eds.) Academic Press: New York, 2003, pp 37–104.
10. Altindal A, Abdurrahmanoglu S, Bulut M and Bekaroglu O. *Synth. Met.* 2005; **150**: 181–187.
11. Galmiche L, Guyon F, Pondaven, Moisan JY and L'Her M. *J. Porphyrins Phthalocyanines* 2003; **7**: 382–387.
12. Basova T, Gurek AG, Ahsen V and Ray AK. *Org. Elect.* 2007; **8**: 784–790.
13. Lever ABP. *J. Porphyrins Phthalocyanines* 1999; **3**: 488–499.
14. Rousseau R, Aroca R and Rodríguez-Méndez ML. *J. Mol. Struct.* 1995; **356**: 49–54.
15. Yilmaz I, Nakanishi T, Gurek A and Kadish KM. *J. Porphyrins Phthalocyanines* 2003; **7**: 227–238.
16. Nakanishi T, Yilmaz I, Nakashima N and Kadish KM. *J. Phys. Chem. B* 2003; **107**: 12789–12796.
17. L'Her M, Pondaven A, Kadish KM, Smith KM and Guilard R. In *The Porphyrin Handbook*, Kadish KM, Smith KM and Guilard R. (Eds.) Academic Press: San Diego, 2003, p. 117.
18. Liu Y, Shigehara K, Hara M and Yamada A. *J. Am. Chem. Soc.* 1991; **113**: 440.
19. Rodríguez-Méndez ML, Aroca R and de Saja JA. *Chem. Mat.* 1993; **5**: 933–937.
20. Rodríguez-Méndez ML, Souto J, de Saja JA and Aroca R. *J. Mat. Chem.* 1995; **5**: 639–642.
21. Komorsky-Lovrić S, Quentel F, L'Her M and Scholz F. *J. Sol. State Electrochem.* 2008; **12**: 165–169.
22. Koca A, Ceyhan T, Erbil MK, Özkaya AR and Bekaroglu O. *Chem. Phys.* 2007; **340**: 283–292.
23. Markovitsi D, Tran-Thi TH, Even R and Simon J. *Chem. Phys. Lett.* 1987; **137**: 107–112.
24. Ricciardi G, Belviso S, Lelj F and Ristori S. *J. Porphyrins Phthalocyanines* 1998; **2**: 177–188.
25. Kostenich G, Babushkina T, Lavi A, Langzam Y, Malik Z, Orenstein A and Ehrenberg B. *J. Porphyrins Phthalocyanines* 1998; **2**: 383–390.
26. Rodríguez-Méndez ML, Gorbunova Y and de Saja J. *Langmuir* 2002; **18**: 9560–9565.
27. de Saja JA and Rodríguez-Méndez ML. *Adv. Coll. Interf. Sci.* 2005; **116**: 1–11.
28. Valli L. *Adv. Coll. Interf. Sci.* 2005; **116**: 13–44.
29. Linaje M, Quintanilla MC, González A, del Valle JL, Alcaide G and Rodríguez-Méndez ML. *The Analyst* 2000; **125**: 341–346.
30. Ceyhan T, Korkmaz M, Erbil MK and Bekaroglu O. *J. Porphyrins Phthalocyanines* 2005; **9**: 423–429.
31. Pondaven A, Cozien Y and L'Her M. *New J. Chem.* 1992; **16**: 711–718.
32. Ceyhan T, Altindal A, Ozkaya AR, Erbil MK and Bekaroglu O. *Polyhedron* 2007; **26**: 73–84.
33. Pushkarev VE, Shulishov EV, Tomilov YV and Tomilova LG. *Tetrahedron Lett.* 2007; **48**: 5269–5273.
34. Bouvet M, Bassoul P and Simon J. *Mol. Cryst. Liq. Cryst.* 1994; **252**: 31–38.
35. Negrimovskii VM, Bouvet M, Luk'yanets EA and Simon J. *J. Porphyrins and Phthalocyanines* 2001; **5**: 423–427.
36. Martynov AG, Zubareva OV, Gorbunova YG, Sakharov SG, Nefedov SE, Dolgushin FM and Tsivadze AY. *Eur. J. Inorg. Chem.* 2007; 4800–4807.
37. Trometer M, Even R, Simon J, Dubon A, Laval JY, Germain JP, Maleyson C, Pauly A and Robert H. *Sens. Actuators, B* 1992; **8**: 129–135.
38. Aroca R, Bolourchi H, Battisti D and Najafi K. *Langmuir* 1993; **9**: 3138–3141.
39. Souto J, Rodríguez-Méndez ML, de Saja-González J and de Saja JA. *Thin Solid Films* 1996; **284–285**: 888–890.
40. Dooling CM, Richardson TH, Valli L, Rella R and Jiang J. *Col. Surf. A* 2002; **198**: 791–796.
41. de Saja R, Souto J, Rodríguez-Méndez ML and de Saja JA. *Mat. Sci. Eng. C* 1999; **8–9**: 565–568.
42. Rickwood KR, Lovett DR, Lukas B and Silver J. *J. Mater. Chem.* 1995; **5**: 725–729.
43. Chen YL, Liu HG, Pan N and Jiang JZ. *Thin Solid Films* 2004; **460**: 279–285.
44. Rodríguez-Méndez ML, Souto J, de Saja R, Martínez J and de Saja JA. *Sens. Actuators, B* 1999; **58**: 544–551.



45. Parra V, Bouvet M, Brunet J, Rodríguez-Méndez ML and de Saja JA. *Thin Solid Films* 2008; **516**: 9012–9019.
46. Liang B, Yuan C and Wei Y. *J. Vac. Sci. Technol., B* 1997; **15**: 1432–1436.
47. Souto J, Rodríguez ML, de Saja JA and Aroca R. *Int. J. Electronics* 1994; **76**: 763–769.
48. Xie D, Jiang Y, Jiang J, Wu Z and Li Y. *Sens. Actuators, B* 2001; **77**: 260–263.
49. Xie D and Jiang Y. *Sens. Actuators, B* 2003; **93**: 379–380.
50. Liang B, Yuan C, Wei Y, Zhang Y, Jiang D, Zhang S and Lu A. *Synth. Met.* 1997; **88**: 219–223.
51. Wang HY and Lando JB. *Langmuir* 1994; **10**: 790–796.
52. Gutierrez N, Rodríguez-Méndez ML and de Saja JA. *Sens. Actuators, B* 2001; **77**: 437–442.
53. Alvarez J, Souto J, Rodríguez-Méndez ML and de Saja JA. *Sens. Actuators, B* 1998; **48**: 339–343.
54. Madru M, Guillaud G, Al sadoun M, Maitrot M, Clarisse C, Le Contellec M, Andre JJ and Simon J. *Chem. Phys. Lett.* 1987; **142**: 103–105.
55. Bouvet M, Leroy A, Simon J, Tournilhac F, Guillaud G, Lessnick P, Maillard A, Spirkovitch S, Debliquy M, de Haan A and Decroly A. *Sens. Actuators, B* 2001; **72**: 86–93.
56. Bouvet M. *Anal. Bioanal. Chem.* 2006; **384**: 366–373.
57. Xie D, Jiang YD, Pan W, Jiang JH, Wu ZM and Li YL. *Thin Solid Films* 2002; **406**: 262–267.
58. Xie D, Jiang Y, Pan W, Jiang J, Wu Z and Li Y. *Sens. Actuators, B* 2002; **81**: 210–217.
59. Gorbunova Y, Rodríguez-Méndez ML, Souto J, Tomilova L and de Saja JA. *Chem. Mat.* 1995; **7**: 1443–1447.
60. Rodríguez-Méndez ML, de Saja-González J, Souto J and de Saja JA. *Sens. Actuators, B* 1996; **31**: 51–55.
61. Gorbunova Y, Rodríguez-Méndez ML, Kalashnikova IP, Tomilova L and de Saja JA. *Langmuir* 2001; **17**: 5004–5010.
62. Souto J, Aroca R and de Saja JA. *J. Phys. Chem.* 1994; **98**: 8998–9001.
63. Rodríguez-Méndez ML, Aroca R and de Saja JA. *Spectrochim. Acta* 1993; **49A**: 965–973.
64. Richardson T, Smith VC, Toplaci A, Jiang J and Huang CH. *Supramol. Sci.* 1997; **4**: 465–470.
65. Xie D, Jiang Y, Jiang J, Wu Z, Li Y and Liang BJ. *Sens. Actuators, B* 2001; **77**: 260–263.
66. Rodríguez-Méndez ML, Khoussed Y, Souto J, Sarabia J, Aroca R and de Saja JA. *Sens. Actuators, B* 1994; **18**: 89–92.
67. Rodríguez-Méndez ML, Gobernado-Mitre I, Souto J and de Saja JA. *Sens. Actuators, B* 1995; **25**: 643–646.
68. Bariain C, Matias IR, Fernandez-Valdivielso C, Arregui FJ, Rodríguez-Méndez ML and de Saja JA. *Sens. Actuators, B* 2003; **93**: 153–158.
69. Aroca R, Bolourchi H, Battisti D and Najafi K. *Langmuir* 1993; **9**: 3138–3141.
70. Di Natale C, Paolesse R, Macagnano A, Mantini A, Mari P and D'Amico A. *Sens. Actuators, B* 2000; **68**: 319–323.
71. de Carli D, Adami A, Lorenzelli L, Malfatti M, Apetrei C, Rodríguez-Méndez ML and de Saja JA. *Eurosensors XXI-Transducers* 2007; EA0703.
72. Siqueira JR, Gasparotto LHS, Oliveira ON and Zucolotto V. *J. Phys. Chem. C* 2008; **112**: 9050–9055.
73. Sergeyevaa TA, Lavrikb NV, Rachkova AE, Kazantsevab ZI, Piletskya SA and El'skayaa AV. *Anal. Chimi. Acta* 1999; **391**: 289–297.
74. Vlasov Y, Legin A, Rudnitskaya A, Di Natale C and D'Amico A. *Pure Appl. Chem.* 2005; **77**: 7965–1983.
75. Agboola B and Nyokong T. *Electrochim. Acta* 2007; **52**: 5039–5045.
76. Riul A, dos Santos DS, Wohnrath K, Di Tommazo R, Carvalho AC, Fonseca FJ, Oliveira, ON, Taylor DM and Mattoso LH. *Langmuir* 2002; **18**: 239–245.
77. Sulua M, Altındal A and Bekaroglu O. *Synth. Metals* 2005; **155**: 211–221.
78. Arrieta A, Rodríguez-Méndez ML and de Saja JA. *Sens. Actuators, B* 2003; **95**: 357–365.
79. Antunes P, Ferreira M, Oliveira ON, Rodríguez-Méndez ML and de Saja JA. *Fifth International Conference on Porphyrins and Phthalocyanines ICPP-5* 2008; 529.
80. Njanja E, Nassi A, Ngameni E, Elleouet C, Quentel F and L'Her M. *Electrochem. Commun.* 2007; **9**: 1695–1699.
81. Mirčeski V, Quentel F, L'Her M and Scholz F. *J. Electroanal. Chem.* 2006; **586**: 86–97.
82. Komorsky-Lovric S, Quentel F, L'Her M and Scholz F. *J. Sol. State Electrochem.* 2008; **12**: 165–169.
83. Parra V, Hernando T, Rodríguez-Méndez ML and de Saja JA. *Electrochim. Acta* 2004; **49**: 5177–5185.
84. Basova T, Jushina I, Gurek AG, Ahsen V and Ray AK. *J. Roy. Soc. Interf.* 2008; **5**: 801–806.
85. Rock F, Barsan N and Weimar U. *Chem. Rev.* 2008; **108**: 705–725.
86. Ciosek P and Wroblewski W. *Analyst* 2007; **132**: 963–978.
87. Legin A, Rudnitskaya A and Vlasov Y. *Sensors Update* 2002; **10**: 143–188.
88. Winquist F, Krantz-Rulcker C and Lundstrom I. *Sensors Update* 2002; **11**: 279–306.
89. Di Natale C, Paolesse R, Macagnano A, Mantini A, Goletti C and D'Amico A. *Sens. Actuators, B* 1998; **52**: 162–168.
90. Di Natale C, Salimbeni D, Paolesse R, Macagnano A and D'Amico A. *Sens. Actuators, B* 2000; **65**: 220–226.
91. Özmen A, Tekce F, Ebeoğlu MA, Taşaltın C and Öztürk ZZ. *Sens. Actuators, B* 2006; **115**: 450–454.



92. Spadavecchia J, Ciccarella G, Rella R, Capone S and Siciliano P. *Sens. Actuators, B* 2003; **96**: 489–497.
93. Spadavecchia J, Ciccarella G, Siciliano P, Capone S and Rella R. *Sens. Actuators, B* 2004; **100**: 88–93.
94. Winquist F, Bjorklund R, Krantz-Rülcker C, Lundström I, Östergren K and Skoglund T. *Sens. Actuators, B* 2005; **111–112**: 299–304.
95. Hayashi K, Yamanaka M, Toko K and Yamafuji K. *Compr. Anal. Chem.* 2003; **39**: 487–511.
96. Legin A, Rudinitskaya A, Vlasov Y, Di Natale C, Davide F and D'Amico A. *Sens. Actuators, B* 1997; **44**: 291–296.
97. del Valle M. *Compr. Anal. Chem.* 2007; **49**: 721–753.
98. Legin A, Makarychev-Mikhailov S, Goryacheva O, Kirsanov D and Vlasov Y. *Anal. Chim. Acta* 2002; **457**: 297–303.
99. Paolesse R, Di Natale C, Burgio M, Martinelli E, Mazzone E, Palleschi G and D'Amico A. *Sens. Actuators, B* 2003; **95**: 400–405.
100. Lvova L, Paolesse R, Di Natale C, Burgio M, Martinelli E, Pennazza G and D'Amico A. *Sens. Actuators, B* 2006; **118**: 439–447.
101. Aoki PHB, Caetano W, Volpati D, Riul A and Constantino CJL. *J. Nanosci. Nanotechnol.* 2008; **8**: 4341–4348.
102. Ferreira EJ, Pereira RCT, Mattoso LHC, Delbem ACB and Oliveira ON. *Electron. Lett.* 2007; **43**: 1138–1139.
103. Apetrei C, Rodríguez-Méndez ML, Parra V, Gutiérrez F and de Saja J. *Sens. Actuators, B* 2004; **103**: 145–152.
104. Casilli S, de Luca M, Apetrei C, Parra V, Arrieta AA, Valli L, Jiang J, Rodríguez-Méndez ML and de Saja JA. *Appl. Surf. Sci.* 2005; **246**: 304–312.
105. Apetrei C, Rodríguez-Méndez ML and de Saja JA. *Sens. Actuators, B* 2005; **111–112**: 403–409.
106. Rodríguez-Méndez ML, Parra V, Apetrei C, Villanueva S, Gay M, Prieto N, Martínez J and de Saja JA. *Microchim. Acta* 2008; **163**: 23–31.
107. Parra V, Arrieta AA, Fernández-Escudero JA, García H, Apetrei C, Rodríguez-Méndez ML and de Saja JA. *Anal. Chim. Acta* 2006; **563**: 229–237.
108. Parra V, Arrieta A, Fernández-Escudero JA, Rodríguez-Méndez ML and de Saja JA. *Sens. Actuators, B* 2006; **118**: 448–453.
109. Apetrei C, Apetrei I, Nevares I, del Álamo M, Parra V, Rodríguez-Méndez ML and De Saja JA. *Electrochim. Acta* 2007; **52**: 2588–2594.
110. Rodríguez-Méndez ML, Apetrei C and de Saja JA. *Electrochim. Acta* 2008; **53**: 5867–5872.
111. Di Natale C, Paolesse R, Burgio M, Martinelli E, Pennazza G and D'Amico A. *Anal. Chim. Acta* 2004; **513**: 49–56.
112. Di Natale C, Paolesse R, Macagnano A, Mantini A, D'Amico A, Legin A, Lvova L, Rudnitskaya A and Vlasov Y. *Sens. Actuators, B* 2000; **64**: 15–21.
113. Rodríguez-Méndez ML, Arrieta A, Parra V, Vegas A, Villanueva S, Gutierrez-Osuna R and de Saja JA. *IEEE Sensors J.* 2004; **4**: 348–354.
114. Parra V, Bouvet M, Brunet J, Rodríguez-Méndez ML and de Saja JA. *Thin Solid Films* 2008; **516**: 9012–9019.

2.3. ARTÍCULO 2. “Electronic tongue based on voltammetric electrodes modified with materials showing complementary electroactive properties. Applications.”

M.L. Rodríguez-Méndez, V. Parra, C. Apetrei, S. Villanueva, M. Gay, N. Prieto, J. Martínez, J.A. de Saja.

Microchimica Acta 2008. 163, 23-31.

2.3.1. Motivación y objetivos.

El trabajo que se adjunta a continuación pretende dar una visión general de la investigación que se realiza en el Grupo de Sensores de la UVa. La novedad introducida por nuestro Grupo ha consistido en desarrollar una lengua electrónica voltamétrica basada en sensores modificados con compuestos electroactivos. En este caso, como red de sensores se ha elegido una compuesta por bisftalocianinas y perilenos, dada su complementariedad electroquímica. La red de sensores se ha expuesto a electrolitos sencillos, con el fin de explorar las características electroquímicas de los materiales sensibles en diferentes disoluciones acuosas. Además, se ha evaluado la capacidad de discriminación en cuanto a los sabores básicos; salado, ácido, amargo, dulce y umami. Por último, se han estudiado vinos blancos elaborados con distinto tipo de uvas y la capacidad del sistema electrónico para su discriminación.

2.3.2. Resumen de los resultados obtenidos.

i) La red de sensores estudiada se compone de CPEs modificados con bisftalocianinas de tres lantánidos diferentes ($LuPc_2$, $GdPc_2$, $PrPc_2$) y perilenos (tres bisperilenobisimido) como materiales modificadores. La elección de estos materiales se basa en la complementariedad que muestran ambas familias en cuanto a sus propiedades electroquímicas. La ventaja que presentan los electrodos modificados en comparación con los que no lo están (electrodos metálicos, por ejemplo) es que proporcionan señales muy complejas que contienen gran cantidad de información, relacionada con el material sensible, la disolución en la cual está inmerso y con las interacciones entre ambos. De esta manera, se ha empleado esta lengua electrónica en el estudio de diferentes electrolitos simples; KCl, HCl, NH_3 y $KClO_4$, con el objetivo de estudiar la influencia de los electrolitos iónicos en la respuesta voltamétrica de las biftalocianinas y perilenos.

ii) La respuesta de los electrodos modificados con bisftalocianinas frente a disoluciones simples como KCl se caracteriza por dos picos redox asociados a la oxidación $[Pc^{2-}Ln^{3+}Pc^{\cdot-}]^0 \leftrightarrow [Pc^{\cdot-}Ln^{3+}Pc^{\cdot-}]^+$ y reducción $[Pc^{2-}Ln^{3+}Pc^{\cdot-}]^0 \leftrightarrow [Pc^{2-}Ln^{3+}Pc^{2-}]^-$ del anillo de ftalocianina. Los potenciales asociados a dichos procesos dependen del metal central. De esta manera, los potenciales de oxidación son menores a medida que decrece el radio iónico del metal, observándose la siguiente secuencia E_{ox} $LuPc_2$ (0.68 V) < E_{ox} $GdPc_2$ (0.85 V) < E_{ox} $PrPc_2$ (1.0 V). La respuesta voltamétrica está condicionada por los iones presentes en disolución. La naturaleza de los iones afecta a los potenciales de los procesos redox debido a la difusión de los mismos hacia el interior del electrodo, la cual necesaria para mantener la electroneutralidad del sensor. La señal electroquímica en medio ácido (HCl) difiere significativamente de la obtenida en medio neutro (KCl).

Debido a la protonación de la $LnPc_2$ en medio ácido, aparece un nuevo pico de oxidación redox relacionado con el pH ácido. En presencia de NH_3 se dificulta la oxidación del anillo, además los picos relacionados con la forma reducida de la ftalocianina presentan una intensidad mucho menor que en el caso de emplear HCl como electrolito, sugiriendo así que el proceso de reducción se favorece en presencia de protones en el medio en el cual está sumergido el sensor. En $KClO_4$, el perfil voltamétrico es similar al obtenido en KCl con la diferencia de que en este caso, debido al carácter oxidante del anión ClO_4^-, se observa un desplazamiento de los picos de oxidación del anillo de ftalocianina hacia valores menores, facilitando así la oxidación.

iii) En el caso de emplear perilenos como modificadores, los procesos redox observados en KCl están asociados a la formación del anión y dianión del anillo de perileno. Estos procesos son el resultado de la alta afinidad electrónica que presentan dichos compuestos y la posibilidad de deslocalización de los electrones por el sistema aromático conjugado. En determinados derivados puede observarse además una oxidación irreversible del compuesto a altos valores de potencial.

Al igual que en el caso de las bisftalocianinas, debido a que los procesos redox de la molécula de perileno están acompañados de una difusión de los iones presentes en disolución hacia el interior del electrodo, su perfil voltamétrico depende en gran medida por las características de la disolución en la cual esté inmerso.

iv) Se ha estudiado la respuesta de la red frente a KCl (sabor salado), $MgCl_2$ e hidrocloruro de quinina (sabor amargo), ácido cítrico (sabor ácido), sacarosa (sabor dulce), ácido glutámico (sabor umami) y ácido tánico (sensación de astringencia). La red de sensores ha mostrado respuestas voltamétricas asociadas a las características del sabor estudiado. En el caso de los ácidos cítrico, glutámico y tánico, las ondas voltamétricas están influenciadas por el carácter ácido de la disolución, obteniéndose perfiles similares a los observados en HCl. En presencia de $MgCl_2$ como electrolito, la respuesta electroquímica de los sensores es similar a la observada en el caso de KCl como consecuencia de que los iones responsables de mantener la electroneutralidad del material sensible son los aniones Cl^-. A partir de las señales voltamétricas generadas por la red de sensores se ha llevado a cabo un estudio de Análisis de Componentes Principales (PCA).

Este estudio ha demostrado la capacidad de discriminación de la red frente a sabores básicos. Los clusters correspondientes al estudio de disoluciones ácidas (ácido cítrico, ácido tánico, ácido glutámico y quinina) aparecen en la misma región del gráfico PCA, y cercanos entre sí, ya que todos ellos otorgan un carácter ácido al medio líquido, responsable del comportamiento electroquímico tan diferente en comparación con disoluciones de pH neutro. En el caso del KCl y $MgCl_2$, sus clusters aparecen próximos y separados del resto. En este caso, debe señalarse que, aunque el primero está asociado al sabor salado y el segundo al sabor amargo, los sensores responden a compuestos químicos, no a percepciones humanas. Por ello, dado que ambos electrolitos inducen respuestas voltamétricas similares en los materiales sensibles, sus clusters aparecen cercanos. El cluster de la sacarosa aparece separado del resto de los sabores, esta evidente diferencia puede estar relacionada con su carácter reductor.

La capacidad de discriminación demostrada por la red de sensores formada por bisftalocianinas y perilenos es claramente superior a la estudiada anteriormente por nuestro Grupo de investigación, en la cual se empleaba una red formada únicamente por ftalocianinas. La superior discriminación obtenida con bisftalocianinas y perilenos se debe a la complementariedad electroquímica de ambos materiales. La oxidación del anillo de ftalocianina es sensible a ácidos y antioxidantes, mientras que los perilenos permiten observar claramente los procesos que tienen lugar a valores de potencial positivos.

v) En el presente trabajo se ha evaluado la posibilidad de emplear la red de sensores CPEs en el estudio de vinos blancos elaborados con distintos tipos de uva. En todos los casos, además de observarse los procesos redox asociados al material modificador, bisftalocianina o perileno, se observan los picos asociados a los compuestos electroactivos presentes en el vino, los polifenoles. El PCA demuestra que la red de sensores empleada es capaz de distinguir entre vinos blancos elaborados a partir de diferentes tipos de uva; Malvasía, Viura, Turruntés, Garnacha, Tempranillo (Logroño) y Tempranillo (Agoncillo).

vi) El círculo de correlación muestra la diferente información aportada por cada una de las variables introducidas en el PCA. En el caso de las bisftalocianas, las variables asociadas con cada uno de los sensores están influenciadas por el metal central. En el caso de los perilenos, se observa cómo sus variables aparecen en una región del círculo diferente, debido a la diferente información aportada por los mismos en comparación con las bisftalocianinas.

2.3.3. Conclusiones

Se ha desarrollado una red de sensores CPEs basados en dos familias de compuestos; bisftalocianinas y perilenos. Ambas familias presentan una reactividad química diferente, que proporciona información complementaria.

La variedad de respuestas observadas en el estudio de sabores básicos se debe a los procesos redox asociados a la oxidación/reducción de los materiales sensibles, bisftalocianinas y perilenos, a la oxidación/reducción de las especies en disolución susceptibles de sufrir procesos redox, a la difusión de los iones presentes en disolución hacia la superficie del electrodo para mantener la electroneutralidad y a la interacción entre el material sensible y la disolución.

La capacidad de discriminación de la red de sensores hacia sabores básicos es superior a la red formada únicamente por ftalocianinas o perilenos. Además, dicha red ha demostrado la capacidad de distinguir entre vinos blancos elaborados con distintos tipos de uva.

El círculo de correlación ha demostrado que la diferente información aportada por las variables asociadas a las bisftalcianinas y perilenos, quedando patente su complementariedad.

Microchim Acta (2008) 163: 23–31
DOI 10.1007/s00604-007-0907-8
Printed in The Netherlands

Microchimica Acta

Original Paper

Electronic tongue based on voltammetric electrodes modified with materials showing complementary electroactive properties. Applications

M. L. Rodríguez-Méndez[1], V. Parra[1], C. Apetrei[1], S. Villanueva[2], M. Gay[1], N. Prieto[2], J. Martinez[3], J. A. de Saja[2]

[1] Department of Inorganic Chemistry, E.T.S. Ingenieros Industriales, Valladolid, Spain
[2] Department of Condensed Matter Physics, University of Valladolid, Valladolid, Spain
[3] CIDA, Carretera de Logroño-Mendavia, Logroño, Spain

Received 13 July 2007; Accepted 12 November 2007; Published online 21 January 2008

Abstract. An update of the work carried out by our group in the field of voltammetric sensors is presented. A simplified array formed by carbon paste electrodes (CPE) modified with three rare-earth bisphthalocyanines and three perylenes has been constructed. Chemical modifiers have been selected because of their complementary redox activity. Using square wave voltammetry, the array has provided a rich variety of voltammetric responses towards acids, bases, ionic solutions and antioxidants such as tannic acid or citric acid. This cross-selectivity is due to diffusion of ions inside the electrode to the sensitivity of the materials to protons and to the presence of oxidizing or reducing agents (i.e. perchlorate or tannins). The array of electrodes has been used to analyse white wines elaborated with different varieties of grapes. Principal component analysis conducted using Kernel functions demonstrated that the capability of discriminating white wines is similar to that observed in red wines. Loading plots confirm that the information provided by sensors modified with phthalocyanines and perylenes is highly complementary.

Keywords: Electronic tongue; multi-sensor system; phthalocyanine; bisphthalocyanine; perylene; wine

In recent years, efforts have been carried out to develop multisensor systems for the recognition and discrimination of complex liquids (the so-called "electronic tongues") [1–6]. Most of the works in the field have been carried out using potentiometric devices. In particular, lipid/polymer membrane sensor arrays have been used to discriminate between various beverages such as coffees, juices and beers [1, 7, 8]. The discrimination of beverages has also been attempted by using conventional ion selective electrodes or ISFETS combined with electrodes based on chalcogenide vitreous materials [9–11] and with electrodes based on conducting polymers [12] or porphyrines [13, 14]. A disposable all-solid-state planar-type potentiometric electronic tongue has been developed with the carbon paste electrode array screen-printed on a polymeric substrate [15]. Impedance spectroscopy has also been used as transduction method for the analysis of a variety of liquids [16].

Voltammetric sensors -used as working electrodes in a standard three-electrode configuration- have also been widely studied [2, 17]. Their main advantage is that it is possible to obtain sensors with different sensitivity by chemical modification [17, 18]. In previous

---

Correspondence: M. L. Rodríguez-Méndez, Department of Inorganic Chemistry, E.T.S. Ingenieros Industriales, P° del Cauce s/n, 47011 Valladolid, Spain, e-mail: mluz@eis.uva.es

works, our group has developed voltammetric electrodes chemically modified with electroactive materials. In these works, a variety of sensitive molecules, including phthalocyanines, conducting polymers, ferrocene or perylenes have been tested. The chemical modification has allowed obtaining a variety of electrodes with significant cross-selectivity [5, 19]. Electrodes based on these materials have been used separately or combined in different ways to test a range of samples. For instance, electrodes modified with phthalocyanines or conducting polymers have been used separately to discriminate model solutions of basic tastes. Arrays of phthalocyanines, conducting polymers and perylenes have successfully discriminated complex solutions such as red wines [20–24]. Results indicated that to obtain optimal results, polymers need to operate under different working conditions (working range and wave form) than phthalocyanines, or perylenes. Such differences make difficult to register voltammograms of polymeric electrodes simultaneously with phthalocyanines or perylenes.

In this work, an update of the work carried out by our group in the field of voltammetric electrodes is presented. Based in the previous experience of the group, a simplified sensor array has been constructed. It consists in six electrodes modified with phthalocyanines and perylenes that have been selected because their complementary electroactive properties. Molecules used in the array included three rare earth bisphthalocyanines ($LnPc_2$), where Ln = Lu, Gd and Pr. Their electrochemical behaviour is dominated by the oxidation of the phthalocyanine ring and is clearly influenced by the central metal atom [25]. Three perylene derivatives have also been selected because their electron affinity, that facilitates their electrochemical reduction [26]. Besides the complementary reactivity, electrodes based on bisphthalocyanines and perylenes can be prepared using similar techniques and they show stable signals under square wave voltammetry (SWV). SWV has an exceptional interest for electroanalytical usage, because its ability to discriminate double layer charging current. In the case of sensor arrays, SWV is advantageous because the time consumed by analysis is lower than for cyclic voltammetry (CV). Moreover, this voltammetric technique favours the data treatment, providing single curves instead of the bi-valuated curves provided by CV. Such curves can be treated by a windowing slicing method using kernel functions that capture the information along the global response [23].

The discriminating capability of the array has been evaluated towards simple solutions, including basic tastes and antioxidants. In addition, the performance of this simplified array has been tested towards six white wines elaborated using six different Spanish grape varieties. This series of wines has permitted to evaluate whether the array of sensors is able to discriminate among organoleptic characteristics induced by the variety of grape in white wines.

Experimental

Model solutions of basic tastes

The artificial taste solutions were obtained by solving substances responsible for a certain taste (0.1 mol · L ^{1}) in ultrapure water (MilliQ). Citric acid (for acid), KCl (for salty), sucrose (for sweetness), $MgCl_2$ and quinine-hydrochloride (for bitterness) and glutamic acid (for "umami") were chosen as models of basic tastes. In order to evaluate the perception of the of astringency, a solution of tannic acid was added to the study. All chemicals were of reagent grade and used as supplied (Aldrich Chemical Ltd.).

Wine samples

Wine samples were provided by the *Centro de Investigacion y Desarrollo Agrario CIDA* (La Rioja. Spain). This centre characterised the wines chemically and also by a human panel test following the regulations CEE 2676/90 and of the "Office Internationale de la Vigne et du Vin" (OIV) [27].

Samples under study were six Spanish white wines elaborated using some common Spanish varieties of white grapes (Table 1). It has to be noticed that two of the wines included in the study were elaborated using the same variety of grape (Tempranillo) that were growth in different regions. Vinification parameteres (vintage, vinification and ageing method) were the same for all the samples studied. For comparison purposes, a red wine elaborated with the variety Tempranillo (red variety) was included in the study.

Sensor preparation

Three rare-earth bisphthalocyanine molecules ($LnPc_2$) with different central metals were synthesized and purified in their neutral radical state following previously published procedures [28, 29]. The bisphthalocyanines under study were: praseodymium(III) bisphthalocyaninate($PrPc_2$), gadolinium(III) bisphthalocyaninate($GdPc_2$) and Lutetium(III) bisphthalocyaninate($LuPc_2$). In addition, two perylene diimide derivatives: N,N′-bis(neopentyl)-3,4,9,10-perylenebis(dicarboximide) (denoted as BNPTCD), N,N′-bis(piperidinemethyl)-3,4,9,10-perylenebis(dicarboximide) (PTCDIPp), and one imide the:

Table 1. Wines elaborated with different grape variety

	Grape variety	Origin	Vintage
BL1	Viura	Logroño	2003
BL2	Garnacha Blanca	Logroño	2003
BL3	Malvasía	Logroño	2003
BL4	Turruntés	Logroño	2003
BL5	Tempranillo Blanco	Agoncillo	2003
BL6	Tempranillo Blanco	Logroño	2003

N-octyl-3,4,9,10-perylenebis(dicarboximide) (OPTCD) were included in the study. Perylenes were synthesised and characterised according to the procedure described in Ref. [30] and references therein.

Carbon paste electrodes (CPEs) were prepared as previously reported [19] by mixing graphite powder (Ultracarbon, Ultra F purity) and the corresponding bisphthalocyanine or perylene (15% w/w).

Electrochemical measurements

The electrochemistry was carried out in an EG & G PARC 263A potentiostat/galvanostat (M270 Software) using a conventional three-electrode cell. The reference electrode was an Ag|AgCl/KCl sat. and the counter electrode was a platinum wire. CPEs were used as working electrodes. Cyclic voltammograms (CV) were registered from −1.0 up to 1.3 V at a scan rate of 0.1 V · sec^{-1}, and starting at 0 V. Square wave voltammetry (SWV) was performed at the same potential range by using a frequency (*f*) of 15 Hz, an amplitude (E_{sw}) of 0.1 V and a step high (ΔE_s) of 0.07 V.

Electroded were conditioned by running cyclic voltammograms in aqueous 0.1 mol · L^{-1} KCl until a stable voltammetric response was attained. Then electrodes were immersed in the sample under studied and five voltammograms were run to stabilise the signal. Finally, SWV voltammograms were recorded. Electrodes were cleaned after measuring each wine sample. For this purpose electrodes were immersed in 0.1 mol · L^{-1} KCl and CV were run until the original signal was recovered.

Statistic analysis

A non-supervised multivariate method such as principal component analysis (PCA) was used for the analysis of the signals obtained from the solutions of basic tastes and white wines. For this purpose, 20 mL of the sample were placed in 25 mL flask. Seven replicates of each sample were prepared. In order to improve the robustness of the experiments, the samples were measured in a random order.

The voltammetric curves were mathematically pre-processed and used as data source for statistical analysis. A windowed slicing method was used to reduce the number of data per sample [23, 31, 32]. Using this method, SWV curves were multiplied by 10 smooth, bell shaped windowing functions (called kernels), and integrated with respect to potential. The idea behind this compression technique was to capture the information along the global response, to obtain 10 values that were used as the input variable for statistical analysis.

Results

Electrochemical characterisation of CPE electrodes in different electrolytes

Voltammograms obtained when LnPc$_2$ CPE electrodes are immersed in 0.1 mol · L^{-1} KCl show

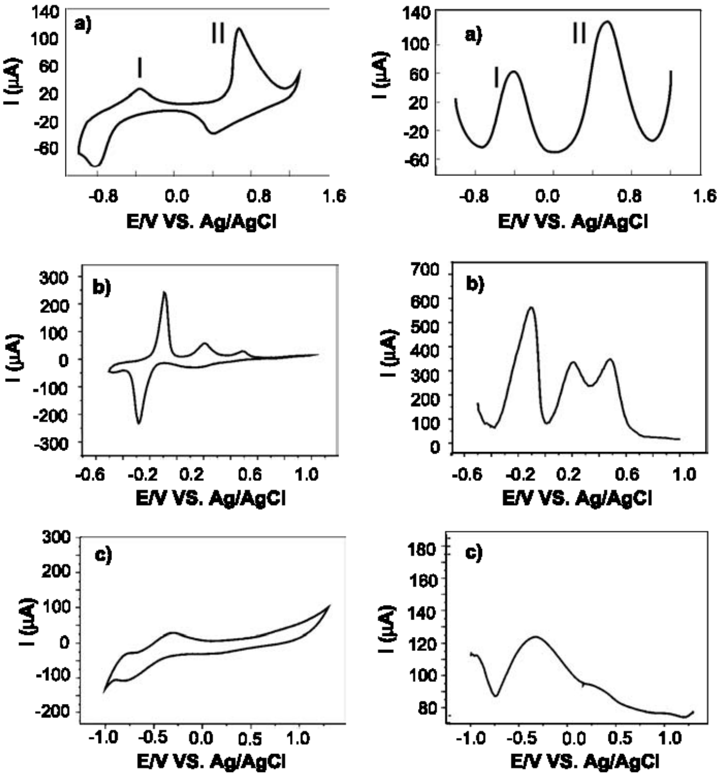


Fig. 1. CV voltammograms (*left*) and SWV responses (*right*) of a LuPc$_2$-CPE based sensor immersed in 0.1 mol · L^{-1} (a) KCl, (b) HCl, and (c) NH$_4$OH

two redox peaks corresponding to the reduction of the neutral form of the phthalocyanine molecule $[Pc^{2-}Ln^{3+}Pc^{2-}]^- \leftrightarrow [Pc^{2-}Ln^{3+}Pc^{\bullet -}]^0$ (peak I) and to the oxidation of the neutral form of the phthalocyanine $[Pc^{2-}Ln^{3+}Pc^{\bullet -}]^0 \leftrightarrow [Pc^{\bullet -}Ln^{3+}Pc^{\bullet -}]^+$ (peak II) [19, 28]. The potential at which ring oxidation occurs (peak II) is related to the nature of the central metal atom. Oxidation takes place at lower potentials as the ionic radius decreases (1.0 V for $PrPc_2$; 0.85 V for $GdPc_2$ and 0.68 V for $LuPc_2$ in SWV) (Fig. 1). In addition, the relative intensity of the peak associated to the oxidation increases when advancing in the lanthanide series.

The charge, size and chemical properties of ions present in the electrolytic solution have an important influence on the peak positions and their sharpness. This is due to the diffusion of ions inside the sensitive material that occur during oxidation or reduction of the electrode.

Figure 1 shows the electrochemical response of a $LuPc_2$ CPE electrode immersed in $0.1\,mol \cdot L^{-1}$ HCl and in $0.1\,mol \cdot L^{-1}$ NH_4OH. Voltammograms registered in HCl, show two consecutive oxidation peaks. The quasi reversible peak at $E_{1/2} = 0.19\,V$ is related to the formation of the monocationic $LnPc_2{}^+$; the second irreversible peak ($E = 0.5\,V$) is associated to the formation of the dicationic species $LnPc_2{}^{2+}$. These results are in good agreement with the expected chemical behaviour of lanthanide phthalocyanines which can be easily protonated in acidic media giving rise to a cationic form. The redox pair associated to the reduction of the phthalocyanine ring increases its intensity and shifts to less negative potentials ($E_{1/2} = -0.1\,V$ instead of the $E_{1/2} = -0.57\,V$ observed in KCl).

In the presence of NH_4OH, oxidation is not longer observed in the voltage range analysed, confirming that reduction is more difficult in the absence of protons.

Voltammograms registered in $0.1\,mol \cdot L^{-1}$ $KClO_4$ or $0.1\,mol \cdot L^{-1}$ $LiClO_4$, are similar to those observed in KCl. However, the oxidant character of the $ClO_4{}^-$ anion provokes a shift of the peaks to lower potentials (0.9.0 V for $PrPc_2$; 0.67 V for $GdPc_2$ and 0.57 V for $LuPc_2$ in SWV).

CV and SWV of CPEs modified with perylene imide or diimides have also been registered. Figure 2a shows examples of SWV responses of two perylene derivatives. When immersed in KCl, the OPTCD imide derivative shows a broad peak at −0.7 V that is accompanied by a shoulder at −0.5 V. These redox processes are ascribed to the formation of the anion and the dianion of the perylene molecule, respectively. Additionally, an oxidation process due to the formation of the perylene cation from the neutral perylene is observed at about 1.0 V. Noticeable differences are observed in the behaviour of the diimide derivative. This is due to the particular electronic level configuration of the perylene, which is symmetrically bisubstituted by methylpyperidino groups.

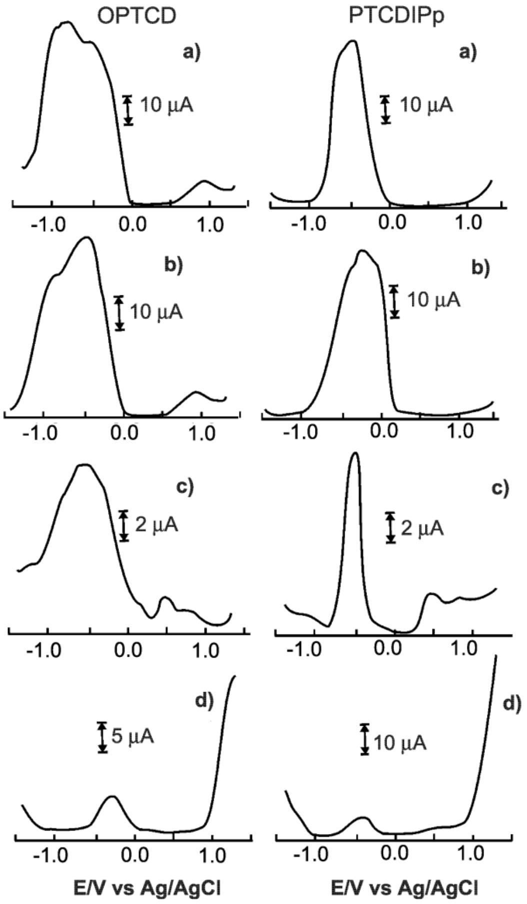


Fig. 2. SWV responses for OPTCD (*left*) and PTCDIPp (*right*) based-CPEs immersed in $0.1\,mol \cdot L^{-1}$ (a) KCl, (b) $KClO_4$, (c) tannic acid, (d) quinine-hydrochloride

Also in perylene based electrodes, redox transformations are accompanied by ion movements between the solution and the bulk material. This diffusion is necessary to preserve the macroscopic electroneutrality of the electrode. As an example, Fig. 2b illustrates the electrochemical behaviour of perylenes in the presence of an oxidant anion such as $ClO_4{}^-$. The main

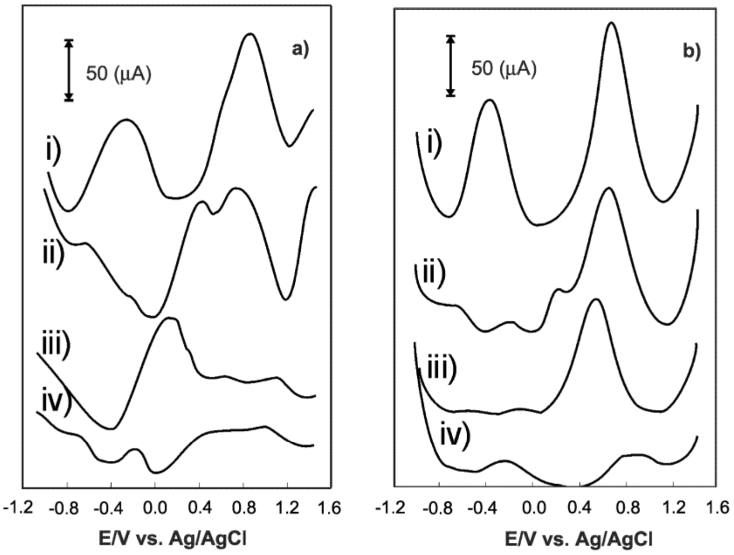


Fig. 3. SWV voltammograms of a (a) $GdPc_2$ electrode and (b) a $LuPc_2$ electrode, immersed in $0.1\,mol \cdot L^{-1}$ i) KCl, ii) citric acid, iii) quinine-hydrochloride and iv) glutamic acid

changes are related to the relative intensities of the peaks produced by the formation of the monoanion and the dianion.

Electrochemical responses towards model solutions of basic tastes

Cyclic voltammograms of sensors based on bisphthalocyanine CPEs immersed in solutions of basic tastes have already been published by our group [19]. In this work, the study has been extended to perylenes and square wave voltammetry has been used instead of cyclic voltammetry. In addition, other model solutions of basic tastes have been incorporated to the analysis: KCl (model of saltiness), $MgCl_2$ quinine-hydrochloride (models of bitterness), sucrose (model of sweetness), citric acid (model of sourness), glutamic acid (model for umami) and tannic acid (model of astringency).

Figure 3 illustrates the responses of $LnPc_2$ CPE electrodes towards model solutions of basic tastes. As observed in the figure the responses of citric acid, quinine and glutamic acid differ significantly from those shown in Fig. 1 for KCl. The SWV of $LnPc_2$ towards citric acid shows well defined waves at ca. 0.7 V (corresponding to oxidation of Pc ring) and ca. -0.5 V (corresponding to reduction of Pc ring). In good agreement with results obtained in HCl, the oxidation of the phthalocyanine ring is facilitated in the presence of protons. In addition, a third peak associated to the oxidation of the citric acid appears at 0.24 V. SWV in tannic acid (not presented in the figure), also show an additional small peak at ca. 0.5 V associated to the oxidation of the organic acid. This peak is difficult to observe because it overlaps with the wave associated to the oxidation of the phthalocyanine ring. When using glutamic acid, sucrose or quinine as electrolytes, broad and complex characteristic peaks can be observed in the range 0.55–0.9 V. The curve obtained when using $MgCl_2$ as electrolyte (not shown in the figure) is similar to that obtained for KCl however, the relative intensity of the peak associated to the reduction of the Pc ring is clearly decreased. The similarities in the one electron oxidation peaks obtained by using KCl and $MgCl_2$ as electrolytes can be explained taken into the account that in both cases, chloride anions are incorporated into the film layer to compensate the generated positive charges. In contrast, diffusion of divalent magnesium Mg^{2+} to compensate negative charges formed during reduction is more difficult than diffusion of monovalent K^+.

Figure 2 show examples of SWV responses of two perylene imide derivatives running in different model solutions associated to basic tastes. When sensors are immersed in ascorbic or tannic acid (Fig. 2c), important changes and shifts occur in the peak associated to the perylene at negative potentials. In addition a new peak associated to the organic acid can be observed at ca. 0.5 V. SWV registered in quinine-hydrochloride

(Fig. 3d) or glutamic acid solutions, show very weak peaks.

Experiments have been carried out to evaluate the stability of the sensors. It has to be pointed out that electrodes need a conditioning step that consist in performing 5–10 cyclic voltammograms to establish an equilibrium with the electrolytic medium. Square wave voltammograms recorded after that conditioning step were highly reproducible. The position of the peaks was accurately maintained after 10 cycles, and only small variations in the intensity of some peaks (%RSD <2%) were observed when increasing the number of replicas (Fig. 4).

Curves were pre-processed by using a data reduction technique based on predefined response "bell-shaped-windowing" or "kernels" [23, 31]. These functions were used as the input data source for statistical analysis. For variable reduction and separation into classes, principal component analysis (PCA) was used. Figure 5 shows the scores plot of the two first principal components. As observed in the figure, the array was able to discriminate the model solutions of basic tastes. The clusters corresponding to acid solutions (citric acid, tannic acid, glutamic acid and quinine-hydrochloride) appear close together in the upper part of the diagramme. The ionic KCl and $MgCl_2$ appear far apart from the clusters corresponding to the organic solutions. The cluster corresponding to sucrose is also well separated from the rest. This difference can be the consequence of its reducing character.

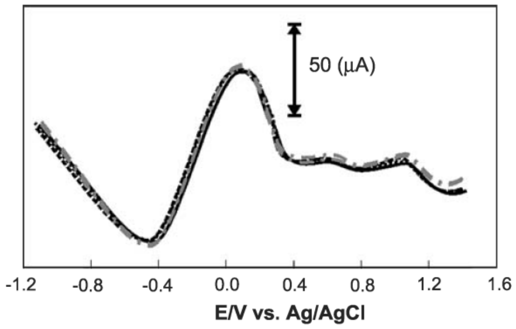


Fig. 4. SWV voltammograms (10 replicates) of a $GdPc_2$ based-CPE immersed in 0.1 $mol \cdot L^{-1}$ aqueous solution of glutamic acid

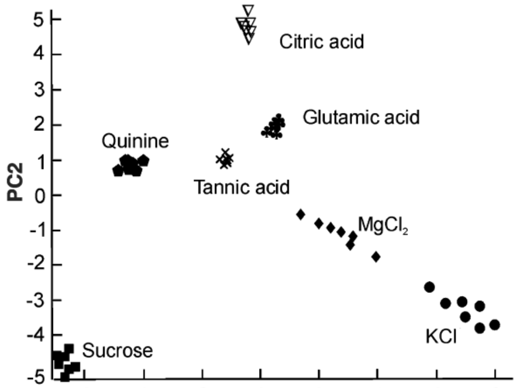


Fig. 5. PCA score plot of model basic taste solutions

It is important to notice that the array of sensors respond to chemical compounds not to human feelings of taste. This is the reason why $MgCl_2$ and quinine (both responsible of bitter taste) appear far one from each other in the diagram. In addition $MgCl_2$ and KCl appear close because the similar chemical nature of both ionic compounds.

The capability of discrimination demonstrated by the array formed by bisphthalocyanines and perylenes is clearly superior to that previously reported using an array formed only by phthalocyanines [19]. This is due to the complementary reactivity presented by phthalocyanines and perylenes: the oxidation of the phthalocyanine ring is extremely sensitive to acids and to antioxidants, while perylenes allow a clear observation of redox processes that occur at positive potentials. This complementary reactivity leads to an important degree of cross-selectivity necessary in multisensor systems.

Analysis white wines elaborated with different varieties of grapes

The response of lanthanide phthalocyanine and perylene based sensors towards red wines has already been studied by our group [20–24]. In this work, the possibility of using voltammetric sensors to analyse white wines has been explored. Voltammograms provided by white wines are similar to those obtained for red wines. In all cases, voltammograms show the redox peaks associated to the bisphthalocyanine or perylene molecule. In addition, a peak at ca. 0.3 V, which is associated to the polyphenolic content of wines is also observed.

In spite of the similarities with red wines, important differences exist in SVW obtained from red and white

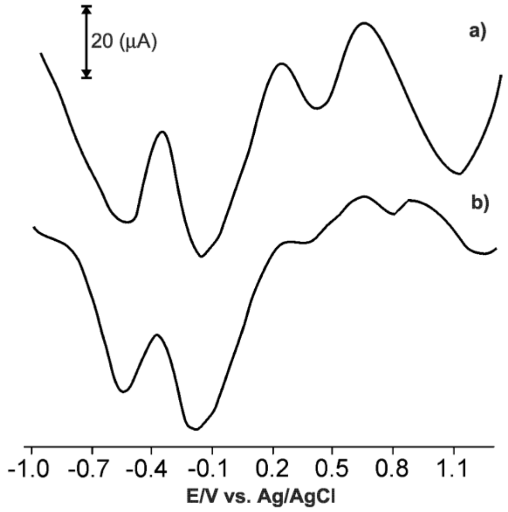


Fig. 6. SWV voltammograms of a $LuPc_2$ electrode immersed in (a) white wine from the variety tempranillo white and (b) a red wine from the variety tempranillo red

wines. Figure 6 shows the SWV obtained using a $LuPc_2$ electrode immersed in a red wine of the variety tempranill (red variety) o and a white whine obtained from grapes of the variety tempranillo (white variety). In the case of phthalocyanines, main differences are related to the peak associated to the oxidation of the phthalocyanine ring (peak II). This peak splits into two peaks in the case of red wines and appears as a simple peak in the case of white wines. In voltammograms registered using perylenes, differences are related to the increase in intensity of the peak associated to the two one-electron reduction of perylene.

The array of electrodes has been used to analyse white wines elaborated with different varieties of grapes. Obviously, sensors produce similar but distinguishable electrochemical responses when exposed to different wines. The main variations rely on changes of the relative intensity and shifts in the positions of peaks from one wine to another. In addition, the central metal atom coordinated to the phthalocyanine ring also influences the voltammograms: the intensity of peak at -0.4 V decreases drastically its intensity when going from the end to the beginning of the rare earth series ($Lu > Gd > Pr$), being almost negligible in SWV registered from $PrPc_2$ CPEs. Moreover, the potentials corresponding to the peak associated to the oxidation of the neutral radical bisphthalocyanine form at ca. 0.7 V, shifts to lower values when advancing in the lanthanide series. This tendency is also observed for the peak related to polyphenols (0.45 V for $PrPc_2$, 0.20 V for $GdPc_2$ and 0.16 V for $LuPc_2$), confirming the important role of the sensing material in the redox behaviour of the system.

In order to evaluate the repeatability of the CPE sensors immersed in wines, samples were measured ten consecutive times. In all cases, voltammograms resulted highly reproducible and only slight variations on the intensity on some peaks were observed (%RSD no larger than 15% after 10 consecutive measurements). This value is larger than the observed is simple solutions and is due to the complexity of wines, where solid particles suspended in the sample can disturb the measurements.

With the aim of evaluating the discriminating capability of the sensor array towards white wines, sensors based on phthalocyanine and perylene were repeatedly exposed (seven repetitions) to six white wines elaborated using different Spanish varieties of grapes. SWV curves were used to extract the input variables (kernels) necessary to conduct principal component analysis (PCA). Figure 7 shows the results of the PCA as three-dimensional score plot. The first three components explain the 42, 21 and 16% of the captured information. As observed in the figure the clusters corresponding to the wines are well separated from each other indicating that samples can be clearly discriminated. The relative positions of the clusters are related to the characteristics of the wines. Forinstance, the clusters corresponding to samples BL5 and BL6 are located on the upper part of the diagramme. The proximity of the clusters is in good agreement with the similar characteristics of wines elaborated with the same variety of grape (Tempranillo). In turn, samples BL1 and BL4 (Viura and Turruntés) with

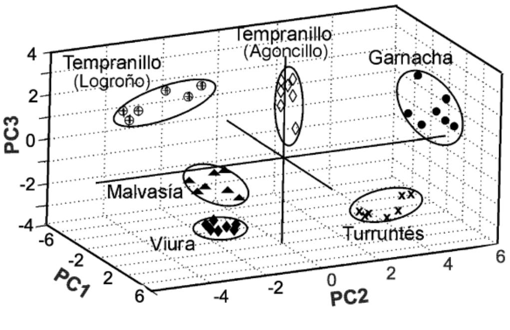


Fig. 7. PCA score plot resultant of the electronic tongue data (% explained variance: PC1 = 42%; PC2 = 21%; PC3 = 16%)

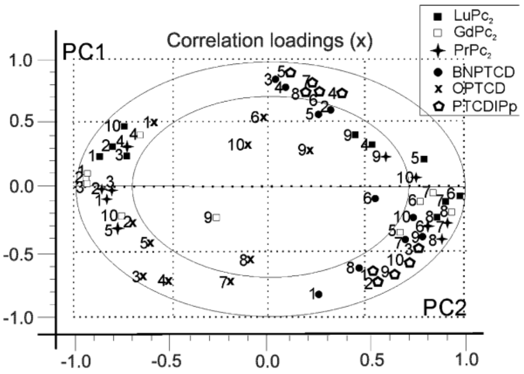


Fig. 8. Contribution of the variables (kernels) on PC1 and PC2

marked organoleptic and chemical differences appear on the lower part of the figure.

Figure 8 shows the contribution of the variables (kernels) on PC1 and PC2. Most of the kernel functions obtained from the SVW have shown a high contribution to the two principal components PC1 and PC2 (>75%). The different location of loadings associated to different sensors is the consequence of the complementary information brought by each variable. For instance, kernels $LnPc_2$-1, $LnPc_2$-2 and $LnPc_2$-3 appear on the left side of the diagram whereas, $LnPc_2$-5, $LnPc_2$-7 and $LnPc_2$-8 are located on the right side of the correlation circle. The contribution of kernels calculated from $GdPc_2$ and $PrPc_2$ sensor show a similar behaviour, but the influence of the central metal atom supplements the information of the array. Most of the kernels obtained from perylenes are located in the upper or the lower side of the circle. This contribution complements the information provided by phthalocyanine sensors and improves the performance of the array.

Conclusions

A simplified array formed by voltammetric sensors based on three rare-earth bisphthalocyanines and three perylenes, has been developed. Both families of materials have a different chemical reactivity that has been used to obtain sensors bringing complementary information.

The variety of responses observed when the array is immersed in model solutions of basic tastes is due to (a) redox processes associated to the oxidation-reduction of phthalocyanine or perylene; (b) oxidation/reduction of molecules with redox activity present in the solution (i.e. citric acid or tannic acid); (c) diffusion of the counterions between the solution and the bulk material and (d) other interaction phthalocyanine/perylene with the -solution (i.e. antioxidant activity of compounds present in the solution).

The capability of discrimination towards basic tastes shown by this simplified array is superior to that observed in arrays formed only by phthalocyanines or perylenes. The array has also demonstrated its capability to discriminate among white wines elaborated with different varieties of grapes. The correlation circle has demonstrated the high contribution of the variables provided by the sensor array. The different location of loadings associated to different sensors is the consequence of the complementary information brought by each family of compounds.

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

References

1. Toko K, Murata T, Matsumo T, Kikkawa Y, Yamafugi K (1992) Taste map of beer by a multichannel taste sensor. Sens Mater 4: 145
2. Krantz-Rulcker C, Stenberg M, Winquist F, Lundstrom I (2001) Electronic tongues for environmental monitoring based on sensors arrays and pattern recognition: a review. Anal Chim Acta 426: 217
3. Legin A, Rudnitskaya A, Vlasov Y (2002) Electronic tongues: sensors systems and applications. Sensors Update, Wiley-VCH 11: 279
4. Bratov A, Dominguez C (2005) Chemical multi-sensor array of liquids. Monolithic integration using microelectronic technology In: Morrison D, Milanovich F, Ivnitski D, Austin T R (eds) Defence against bioterror: detection technologies. Implementation strategies and commercial apportunities, Spinger, Berlin, p 273
5. de Saja J A, Rodriguez-Mendez M L (2005) Sensors based on double-decker rare earth phthalocyanines. Adv Colloid Interface Sci 1–3: 1
6. Gallardo J, Alegret S, del Valle M A (2004) Flow-injection electronic tongue based on potentiometric sensors for determination of nitrate in the presence of chloride. Sens Actuators 101: 72
7. Di Natale C, Davide F A M, D'Amico A, Sberveglieri G, Nelli P, Flagia G, Perego C (1995) Complex chemical pattern recognition with sensor array: the discrimination of vintage years of wine. Sens Actuators 24–25: 801
8. Toko K (1998) Electronic sensing of tastes. Electroanalysis 10: 657
9. Di Natale C, Davide F A M, D'Amico A, Sberveglieri G, Nelli P, Faglia G, Perego C (1996) Complex chemical pattern recognition with sensor array: the discrimination of vintage years of wine. Sens Actuators 33: 83
10. Legin A, Rudinitskaya A, Vlasov Y, Di Natale C, Davide F, D'Amico A (1997) Tasting of beverages using an electronic

tongue based on potentiometric sensor array. Sens Actuators 44: 291

11. Jimenez C, Moreno L, De Haro C, Muñoz F X, Florido A, Rivas P, Fernández A M, Martín P L, Brasov A, Domínguez C (2003) Development of a multiparametric system based on solid-state microsensors for monitoring a nuclear waste repository. Sens Actuators 91: 103
12. Ju M J, Hayashi K, Toko K (2001) Analysis of tastes of amino acids using surface-polarity controlled sensors. Sens Materials 13: 169
13. Lvova L, Paolesse R, Di Natale C, Burgio M, Martinelli E, Pennazza G, D'Amico A (2006) Detection of alcohols in beverages: an application of porphyrin-based Electronic tongue. Sens Actuators B 118: 439
14. Di Natale C, Paolesse R, Burgio M, Burgio M, Martinelli E, Pennazza G, D'Amico A (2004) Application of metalloporphyrins-based gas and liquid sensor arrays to the analysis of red wine. Anal Chim Acta 513: 49
15. Ermolenko Y, Yoshinobu T, Mourzina Y, Furuichi K, Levichev S, Schoning M J, Vlasov Y, Iwasaki H (2003) The double K^+/Ca^{2+} sensor based on laser scanned silicon transducer (LSST) for multi-component analysis. Talanta 59:785
16. Riul A, de Sousa H C, Malmegrim R R, dos Santos D S, Carvalho A C P L F, Fonseca F J, Oliveira O N, Mattoso L H C (2004) Wine classification by taste sensors made from ultra-thin films and using neural networks. Sens Actuators B 98: 77
17. Winquist F, Rydberg E, Holmin S, Krantz-Rulcker C, Stenberg M, Lundstrom I (2002) Flow injection analysis applied to a voltammetric electronic tongue. Anal Chim Acta 471: 159
18. Schreyer S K, Mikkelsen S R (2000) Chemometric analysis of square wave voltammograms for classification on quantitation of untreated beverage samples. Sens Actuators 71: 147
19. Arrieta A, Rodríguez-Méndez M L, de Saja J A (2003) Langmuir-Blodgett film and carbon paste electrodes based on phthalocyanines as sensing units for taste. Sens Actuators 95: 357
20. Parra V, Arrieta A, Fernández-Escudero J A, Rodríguez-Méndez M L, de Saja J A (2006) Electronic tongue based on chemically modified electrodes and voltammetry for the detection of adulterations in wines. Sens Actuators 118: 448
21. Arrieta A, Apetrei C, Rodriguez-Mendez M L, et al (2004) Voltammetric sensor array based on conducting polymer-modified electrodes for the discrimination of liquids. Electrochimica Acta 49: 4543
22. Rodríguez-Méndez M L, Arrieta A, Parra V, Bernal A, Vegas A, Villanueva S, Gutiérrez-Osuna R, de Saja J A (2004) Fusion of three sensory modalities for multimodal characterization of red wines. IEEE Sensor J 4: 348
23. Parra V, Arrieta A, Fernández-Escudero J A, García H, Apetrei C, Rodríguez-Méndez M L, de Saja J A (2006) E-tongue based on an hybrid array of voltammetric sensors based on phthalocyanines, perylene derivatives and conducting polymers: discrimination capability towards red wines elaborated with different variety of grapes. Sens Actuators 115: 54
24. Apetrei C, Apetrei I, Nevares I, del Alamo M, Parra V, Rodríguez-Méndez M L, De Saja J A (2007) Using an e-tongue based on voltammetric electrodes to discriminate among red wines aged in oak barrels or aged using alternative methods. Correlation between electrochemical signals and analytical parameters. Electrochim Acta 52: 2588
25. Rousseau R, Aroca R, Rodríguez-Méndez M L (1995) Extended Hückel molecular orbital model for lanthanide diphthalocyanine complexes. J Mol Struct 356: 49
26. Parra V, del Caño T, Rodríguez-Méndez M L, de Saja J A, Aroca R F (2005) Electrochemical characterization of two perylenetetracarboxylic diimides: Langmuir-Blodgett films of carbon paste electrodes. Chem Mat 16: 358
27. OIV (1990) Recueil des méthodes internationales d'analyse des vines et des mouts, OIV, Paris
28. Liu Y, Shikehara K, Yamada A (1989) Purification of lutetium diphthalocyanine and electrochromism of its Langmuir-Blodgett films. Thin Solid Films 179: 303
29. Linaje M, Quintanilla M C, González A, del Valle J L, Alcaide G, Rodríguez-Méndez M L (2000) Optimization of the síntesis of lutetium bisphthalocyanine using the Taguchi methodology. Analyst 125: 341
30. Del Caño T, Duff J, Aroca R (2002) Molecular spectra and molecular organization in thin solid films of bis (neopentyl-imido)perylene. Appl Spectrosc 56: 744
31. Gutierrez-Osuna R, Nagle H T (1999) A method for evaluating data-preprocesing tecniques for odor classification with an array of gas sensor. IEEE Sensor J 29: 626
32. Scott S M, James D, Ali Z (2006) Data analysis for electronic nose systems. Microchim Acta 156: 183

2.4. ARTÍCULO 3. “Application of an electronic tongue to study the effect of the use of pieces of wood and micro-oxygenation in the aging of red wine”

M. Gay, C. Apetrei, I. Nevares, M. del Álamo, J. Zurro, N. Prieto, J.A. de Saja, M.L. Rodríguez-Méndez.

Electrochimica Acta 2010. 55, 6782-6788.

2.4.1 Motivación y objetivos.

El envejecimiento del vino tinto en barricas de roble seguido de una reducción en botella es la práctica de elaboración habitual de vinos de vinos de calidad. No obstante, en los últimos años han surgido métodos alternativos que sustituyen el efecto de las barricas con la incorporación de pequeños trozos de madera de roble tostado, denominados chips o staves, en depósitos de acero inoxidable, lo cual lleva consigo una sustancial reducción de los costes de producción.

En el presente trabajo se ha estudiado el efecto del empleo de métodos alternativos en el envejecimiento de vinos tintos mediante una lengua electrónica basada en CPEs.

Como novedad con respecto a anteriores trabajos realizados por nuestro Grupo, las muestras de vino estudiadas han sido microoxigenadas, con el fin de simular la difusión de oxígeno que se produce a través de los poros de las barricas de roble. Por tanto, nos encontramos ante muestras de vino de elevada complejidad debido a la gran similitud de las mismas con las preparadas mediante métodos tradicionales.

El envejecimiento de vinos tintos empleando chips y staves se ha comparado con el que se consigue de la forma tradicional a través de un panel de cata humano, análisis químicos y nuestra lengua electrónica. Los resultados aportados por la red de sensores se han correlacionado con los datos generados por los catadores y los análisis de los vinos realizados en el laboratorio con el fin de evaluar la validez de las medidas obtenidas por nuestro sistema electrónico.

2.4.2 Resumen de los resultados obtenidos.

i) Los vinos experimentales estudiados han sido elaborados en el Campus de Palencia, donde se han realizado los análisis químicos y la evaluación mediante el panel de cata humano. Las muestras se han elaborado a partir de la variedad de uva tempranillo. Una parte del vino se ha introducido en un tanque de acero inoxidable en ausencia de madera, dicho vino constituye el vino control. Un segundo grupo de muestras se ha preparado mediante el envejecimiento de vino en tanques de acero inoxidable a los cuales se han añadido chips (muestras C1-C4) y staves (S1-S4).

ii) Finalmente, el tercer grupo de muestras lo forman vinos envejecidos en barricas de roble (B1-B4). En todos los casos se han empleado dos tipos de maders, roble americano y roble francés. En el caso de los métodos alternativos, con el fin de emplear la misma superficie de madera en contacto con el vino se ha añadido la cantidad necesaria de chips y staves para igualar la superficie en contacto con las muestras en la barrica de roble. Además, dichas muestras de vino han sido microoxigenadas, con el objetivo de simular los procesos de difusión de oxígeno existentes en las barricas a través de sus poros. Se ha realizado un estudio de las muestras de vino a lo largo del tiempo; después de 1 mes (T1), 3 meses (T2), 5 meses (T3) y 6 meses (T4) en contacto con la madera. Posteriormente, los vinos se embotellaron y se siguió su evolución en la botella a los 5 meses (T5), 11 meses (T6) y 14 meses (T7).

iii) La lengua electrónica empleada para abordar dicho estudio está compuesta por sensores CPEs basados en bisftalocianinas ($LuPc_2$, $GdPc_2$) y monoftalocianinas (CoPc). Además se han incluido en la red un CPE sin modificar y un electrodo de platino. El conjunto de sensores se ha empleado en la monitorización del envejecimiento del vino mediante el método tradicional y el alternativo. Los voltamogramas obtenidos cuando los sensores se sumergen en el vino presentan los picos asociados al material sensible (en el caso de los sensores modificados) y los relacionados con los polifenoles del vino. Además, la curva voltamétrica se ve afectada por las interacciones entre el sensor y el vino. Dependiendo de las características estructurales de los polifenoles, los picos de oxidación de los mismos aparecen a distintos valores de potencial. Así, los fenoles con grupos hidroxilo en posición orto (ácido cafeico, ácido galico, ácido tánico, catequina) presentan potenciales de oxidación menores que aquellos con un único grupo –OH (resveratrol, ácido cumárico, ácido vanílico) en su estructura aromática. Los distintos perfiles voltamétricos obtenidos con la red de sensores para cada vino en particular hacen posible el seguimiento de la evolución de las muestras.

iv) Se han realizado correlaciones entre los datos obtenidos con la lengua electrónica y los datos aportados por los análisis químicos de los vinos bajo estudio. Como parámetros seleccionados en la aplicación del modelo de correlación PLS-1 se han elegido los que presentan una mayor repercusión en las propiedades gustativas de los vinos. En este sentido, se han considerado los taninos, glicerol, grado alcohólico, extracto seco, acidez total, acidez volátil, ácido tartárico y azúcares reductores.

El modelo PLS-1 muestra en general buenos coeficientes de correlación con todos los parámetros seleccionados (excepto en el caso de la acidez total y volátil), especialmente con los antocianos, los cuales son compuestos polifenolicos responsables del color del vino y de los procesos de polimerización y condensación de los taninos. Estas reacciones dan lugar a cambios en las estructuras fenolicas, lo cual determina la astringencia de los vinos. Dado el importante papel que desempeñan los polifenoles en el envejecimiento de los vinos y dado que su electroactividad se determina mediante sensores electroquímicos, se ha establecido un modelo PLS-1 para correlacionar la matriz de datos procedente de la lengua electrónica con los polifenoles totales, polifenoles poco polimerizados y polifenoles altamente polimerizados. En este caso, se han obtenido excelentes correlaciones para todos ellos, obteniendo altos coeficientes de correlación, pendientes cercanas a 1 y ordenadas en el origen próximas a 0.

v) Los datos aportados por el sistema electrónico se han correlacionado con un panel de cata humano. La lengua electrónica es un sistema que proporciona una medida global de las muestras bajo estudio de forma objetiva y sin fatiga, mientras que el panel de cata humano se basa en percepciones humanas. Los parámetros evaluados por los catadores han sido el volumen en boca, acidez, intensidad tánica, tanino dulce, tanino verde, astringencia y persistencia. Las mejores correlaciones obtenidas han sido con la intensidad tánica, tanino verde y astringencia, lo que corrobora las correlaciones encontradas con los análisis químicos.

De esta manera se confirma que la lengua electrónica es un sistema complementario al panel de cata humano a la hora de evaluar las muestras de vino bajo estudio. Dicho resultado es de máximo interés en el campo de las lenguas electrónicas.

vi) Con el objetivo de analizar la capacidad de la lengua electrónica para detectar el método de envejecimiento empleado en la elaboración de los vinos tintos, se han realizado PCA de las muestras en diferentes periodos. Se observa que, en el primer mes de envejecimiento del vino en contacto con madera, el gráfico de PCA muestra cómo el vino control aparece separado del resto de las muestras que han estado en contacto con la madera. Los clusters correspondientes a los vinos envejecidos en barricas y empleando chips y staves, aparecen discriminados parcialmente del resto, probablemente debido al envejecimiento acelerado inducido por las piezas de madera. Cuando el envejecimiento continúa, las diferencias entre los vinos se hacen menos pronunciadas, y después de los 3 meses (T2) en contacto con madera las muestras no pueden ser discriminadas unas de otras.

vii) Después del embotellamiento del vino (T5) las diferencias químicas entre los vinos se hacen más evidentes y éstos pueden ser discriminados de acuerdo a su método de envejecimiento por la lengua electrónica. En este caso, en el PCA se observan además subclusters asociados con el diferente tipo de roble empleado en el envejecimiento; americano o francés. Estos resultados concuerdan con los aportados por los análisis químicos de las muestras, los cuales revelan que las muestras sólo pueden distinguirse unas de otras en la etapa inicial y final del envejecimiento de los vinos.

viii) Con el objetivo de evaluar la capacidad del sistema electrónico en la predicción del tipo de sistema de envejecimiento empleado, se ha construido un modelo PLS-DA aplicado al estudio de los vinos en diferentes etapas de elaboración. El gráfico PLS-DA aplicado a los periodos de muestreo T1, T2 y T5 muestra la misma posición relativa de los clusters observados en el PCA, confirmando dichas observaciones. Uno de los principales intereses de la discriminación entre vinos envejecidos mediante el sistema tradicional y los alternativos es detectar el uso de las piezas de madera en el producto final. Por este motivo, la evaluación de la capacidad de discriminación de la red de sensores entre los diferentes tipos de envejecimiento se ha realizado a partir de la construcción de un modelo formado únicamente por las muestras de vinos embotellados (T5-T7). Con el modelo PLS-DA construido para tal fin se han obtenido altos valores de correlación para los 3 sistemas; barricas, chips y staves, pendientes cercanas a la unidad y ordenadas en el origen próximas a cero. Además, los errores residuales obtenidos han sido muy bajos, demostrando la posibilidad de abordar la detección de métodos alternativos en el envejecimiento de vinos tintos mediante el empleo de nuestra lengua electrónica basada en CPEs modificados con bisftalocianinas.

2.4.3 Conclusiones

El empleo de sistemas alternativos en el envejecimiento de vinos tintos, tales como chips y staves, puede detectarse con una lengua electrónica basada en CPEs modificados con bisftalocianinas como materiales sensibles en los primeros meses de envejecimiento y en el producto final embotellado.

En la presente investigación, las muestras de vino estudiadas tenían como dificultad añadida con respecto a trabajos anteriores realizados por nuestro Grupo de investigación la microoxigenación de los vinos envejecidos con chips y staves con el fin de simular la difusión de oxígeno que tiene lugar a través de los poros de la barrica de roble y así, intentar igualar al máximo las características organolépticas de los vinos elaborados con métodos alternativos y tradicionales. La red de sensores ha sido capaz de abordar dichas muestras tan complejas de forma exitosa.

La capacidad de discriminación del sistema electrónico entre los dos tipos de sistemas se basa en los cambios que se producen en la composición polifenólica durante el envejecimiento de los vinos. La red de sensores ha demostrado ser además sensible al origen de la madera, apareciendo subclusters asociados a roble americano y roble francés, respectivamente.

Los modelos de predicción han demostrado que la lengua electrónica es capaz de detectar el empleo de sistemas alternativos en los vinos embotellados. Además, se han encontrado buenas correlaciones entre la matriz de datos aportada por el sistema electrónico y los parámetros químicos de las muestras estudiadas, especialmente con los polifenoles. También se ha encontrado una concordancia entre los datos electroquímicos obtenidos con los sensores y los resultados proporcionados por un panel de cata humano, especialmente con la astringencia. Estas excelentes correlaciones indican que la red de sensores presentada puede emplearse como herramienta analítica para predecir el contenido de polifenoles en vinos tintos.

La lengua electrónica puede ser considerada como un método adecuado y complementario para la detección del uso de métodos alternativos en el envejecimiento de vinos tintos.

Electrochimica Acta 55 (2010) 6782–6788

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Application of an electronic tongue to study the effect of the use of pieces of wood and micro-oxygenation in the aging of red wine

M. Gay[a], C. Apetrei[b], I. Nevares[c], M. del Alamo[d], J. Zurro[e], N. Prieto[f], J.A. De Saja[f], M.L. Rodríguez-Méndez[a,*]

[a] Department of Inorganic Chemistry, E.T.S. Ingenieros Industriales, University of Valladolid, Paseo del Cauce s/n, 47011 Valladolid, Spain
[b] Department of Chemistry, Faculty of Sciences, European Excellence Research Centre for Environmental Problems, "Dunărea de Jos" University of Galati, Romania
[c] Department of Agricultural Engineering, E.T.S.I.A., University of Valladolid, Spain
[d] Department of Analytical Chemistry, E.T.S.I.A., University of Valladolid, Spain
[e] Infirmary School Palencia, University of Valladolid, Spain
[f] Department of Condensed Matter Physics, Faculty of Sciences, University of Valladolid, Spain

ARTICLE INFO

Article history:
Received 24 March 2010
Received in revised form 24 May 2010
Accepted 29 May 2010
Available online 8 June 2010

Keywords:
Electronic tongue
Wine
Polyphenol
Chips
Phthalocyanine

ABSTRACT

The ageing of red wines matured in oak barrels and wines treated soaking pieces of wood of different sizes (chips or staves) in micro-oxygenated stainless steel tanks has been monitored periodically using an electronic tongue, chemical analysis and a panel of experts. The use of micro-oxygenation in stainless steel tanks, lets get wines with characteristics similar to wines aged in oak barrels. However, differences in the phenolic content and in particular in the anthocyanin levels are observed during the first steps of ageing and in the final product.

In the early stages of ageing, Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) calculated from the electronic tongue outputs have permitted the discrimination between wines aged with traditional and alternative methods due to the faster rate of ageing caused by chips or staves. After 5 months of ageing, the use of alternative ageing methods cannot be longer detected. However, when the ageing continues in a reducing atmosphere (bottled wines), the electronic tongue has demonstrated a good capability to discriminate and classify bottled wines previously aged in oak barrels from those previously treated with oak chips and oak staves. The effect of the size of the pieces and of the type of wood can also be detected by the e-tongue. Using Partial Least Squares (PLS-1) good correlations have been found between the electrochemical signals provided by the array of sensors and the polyphenolic content parameters. Good correlations have also been established with the scores given by the panel of experts, in particular with the astringency.

1. Introduction

Recently, alternative ageing methods have been developed that can simplify the traditional maturing of wines in oak barrels [1]. One of these techniques consists in adding small oak wood pieces (chips or staves) to wines kept in stainless steel tanks. A gentle oxidation of tanks is necessary to simulate the micro-oxydation that occurs in barrels due to the diffusion of oxygen trough the barrel pores [2–4]. The oxygen dissolved, promotes the oxidation of certain chemical compounds, resulting in wines with a high degree of similarity with wines aged in oak barrels. For this reason, there is a need to develop methods able to detect the use of such alternative ageing methods.

The phenolic composition of wines is strongly influenced by the experimental conditions of the ageing. It is well known that the final chemical and organoleptic characteristics of wines depend on the type of wood used (origin, drying and toasting of the wood), contact time with wine, temperature etc. In the case of treatment with pieces of wood additional parameters such as the size or the conditions of the micro-oxygenation have also an important influence in the phenolic spectrum of the final product [5–9]. For this reason, the phenolic composition can help to characterise and detect wine ageing styles.

A promising approach to analyse wines consists in the use of electronic tongues [10–18]. Such systems are formed by an array of sensors where several sensing units, which exhibit different responses to various compounds, are coupled with signal processing methods based on pattern recognition or artificial neural networks.

Our group has developed an electronic tongue dedicated to the analysis of red wines. It consists in an array of voltamperometric sensors chemically modified with electroactive substances (phthalocyanines and perylenes). These materials are sensitive to

---

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

0013-4686/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.electacta.2010.05.090

several components present in wines including species affecting the pH and species with redox reactivity [19–21]. A data treatment has also been developed to process the voltammetric signals. The e-tongue has been able to discriminate wines of different grape variety or among wines aged in different types of oak barrels [14,16,22]. In addition, in a previous work, our group demonstrated the possibility of using an electronic tongue to discriminate between bottled wines that were aged in barrels or treated with oak chips [23]. In this early work, micro-oxygenation was not used during the treatment with oak chips and wines were analysed only after bottling.

The objective of this work is to establish the capability of our electronic tongue to monitor the ageing of a red wine aged in traditional oak barrels of different origins (French or American) and the same wine aged in stainless steel tanks where pieces of wood of different sizes and origins have been added. In the case of stainless steel tanks, artificial micro-oxygenation has been used in tanks in order to simulate as much as possible the diffusion of oxygen that occurs trough the barrel pores. It is important to highlight that in this work the wines have been monitored from the beginning of the ageing in contact with wood (6 months) and continued in wines bottled during 14 months. The high number of samples allowed us enhancing the quality of the mathematical models and looking at the effect of ageing on the intermediate products.

The capability to discriminate between traditional and alternative methods has been evaluated using Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA). The wines have also been monitored using standard chemical analysis and by a human panel.

Prediction models to calculate chemical parameters from data registered with the electronic tongue have been constructed by means of Partial Least Squares (PLS-1). Special attention has been paid to correlations with polyphenolic compounds which are key components of the ageing process.

2. Experimental

2.1. Wine samples under study

The 13 wine samples prepared are listed in Table 1. Grapes of the variety *Tempranillo* coming from the D.O. Ribera de Duero (Spain) were used in the study. After fermentation, the wine obtained was aged following different methodologies. One part of the wine was introduced in a stainless steel tank of 100 L of capacity with no contact with wood. This wine was used as a control. A second set of samples was prepared by maturing the wine in 100 L stainless steel tanks where pieces of oak wood were added: eight wines were obtained by adding wood samples of different sizes (chips or staves) and of two different origins (American and French) (samples C1–C4 and S1–S4). Finally, a third set of samples was obtained by ageing the wine in oak barrels (225 L of capacity) using American and French oak wood (samples B1–B4).

Table 2
Sampling schedule including ageing stage, duration of each step and total elapsed time.

Sampling	Stage of ageing	Duration of ageing step, months	Total elapsed time, months
T1	Contact with wood	1	1
T2	Contact with wood	2	3
T3	Contact with wood	2	5
T4	Contact with wood	1	6
T5	Bottle	5	11
T6	Bottle	6	17
T7	Bottle	3	20

In order to use a similar surface of wood in contact with wine (in both barrels and tanks where pieces of wood were added), the surface/volume ratio of the 225 L barrels was calculated (surface of the barrels was 2.04 m^2). Thus, 600 g of chips were added to each tank to obtain a similar surface/volume ratio. Similar calculations were carried out for staves.

In order to monitor the ageing the 13 wines under study were analysed periodically: after 1 month (T1), 3 months (T2), 5 months (T3) and after 6 months (T4) of contact with wood. Then, wines aged by different methods were bottled and analysed periodically after 5 (T5), 11 (T6) and 14 (T7) months of ageing in bottle. The sampling schedule is summarised in Table 2.

2.2. Chemical analysis

The chemical analysis included titratable acidity (TA as $g L^{-1}$ tartaric acid), volatile acidity (VA as $g L^{-1}$ acetic acid), tartaric acid (T as $g L^{-1}$ of tartaric acid), dry extract (DE as $g L^{-1}$), tannins (TAN as $g L^{-1}$), alcoholic degree (AD as %), glycerol (G as $g L^{-1}$), reducing sugars (S, as $g L^{-1}$). These parameters were analysed following the international methods [24].

In addition, parameters related with the phenolic content were also analysed: phenolic compounds (TP as $g L^{-1}$ gallic acid), low-polymerised phenolics (LPP as $g L^{-1}$ gallic acid), high-polymerised phenolics (HPP as $g L^{-1}$ gallic acid), analysed following Folin Ciocalteau method [25], anthocyanins (ACY as $g L^{-1}$ of malvidin-3-glucoside) analysed according to the Paronetto method [26], catechins (CAT as $g L^{-1}$ of (+)-catechin), analysed following Ribereau Gayon and Stonstreet method [27,28].

2.3. Electronic tongue

Electrochemical measurements were carried out using a three-electrode cell. The reference electrode was an $Ag|AgCl/KCl_{sat}$ and the counter electrode was a platinum wire. Chemically modified

Table 1
Wine samples under study.

Sample	Ageing vessel	Oak type	Type of wood	Micro-oxygenation
C1	Stainless steel (100 L)	American	Chips	Yes
C2	Stainless steel (100 L)	American	Chips	Yes
C3	Stainless steel (100 L)	French	Chips	Yes
C4	Stainless steel (100 L)	French	Chips	Yes
S1	Stainless steel (100 L)	American	Staves	Yes
S2	Stainless steel (100 L)	American	Staves	Yes
S3	Stainless steel (100 L)	French	Staves	Yes
S4	Stainless steel (100 L)	French	Staves	Yes
B1	Oak barrel (225 L)	American	Barrel	No
B2	Oak barrel (225 L)	American	Barrel	No
B3	Oak barrel (225 L)	French	Barrel	No
B4	Oak barrel (225 L)	French	Barrel	No
Control	Stainless steel (100 L)	–	–	No

carbon paste electrodes (CPE) sensors were used as the working electrodes. Three phthalocyanines (lutetium and gadolinium bisphthalocyanines and cobalt phthalocyanine) where used as chemical modifiers for the CPEs. In addition, one unmodified carbon paste electrode and one platinum electrode were included in the array. The electrochemical experiments were carried out following a previously published method [19–23]. Cyclic voltammograms (CV) were recorded from −1.0 to +1.3 V at a scan rate of 0.1 V s^{-1}. CV were used to analyse the oxidation potentials of the wines under study. For the electronic tongue measurements, Square Wave Voltammetry (SWV) was performed at a potential scan ranging from −1.0 to 1.3 V, using f = 15 Hz; E_{sw} = 90 mV; ΔE_s = 7 mV (except in the case of CoPc, ΔE_s = 5 mV). The electrochemical experiments were performed at a controlled temperature of 25 °C.

2.4. Human panel

A human panel was formed by a group of 10 professors in oenology with a large experience in wine taste analysis. Wines were presented in random order to the panellists. The wine tasting took place in an air-conditioned room (21 °C) with isolated booths. Judges assessed the taste using a tasting evaluation sheet that included eight sensory descriptors (acidity, tannic intensity, sweet tannin, green tannin, astringency and persistence). The different terms were evaluated in a scale from 1 to 5 (1, null, very weak; 2, weak; 3, medium; 4, strong; 5, very strong). The average of the scores given by the panellists was calculated (RSD 0.4). All the sensory evaluations were carried out under Spanish Standardisation Rules (UNE) [29].

2.5. Data analysis

All samples were measured seven times with each sensor. The analysis was carried out using as input data source pre-processed voltammograms obtained by the adaptation of a data reduction technique based on predefined response “bell-shaped-windowing” curves called “kernels” [22,23]. Using this method, the SWV curve (i vs. E) is multiplied by a number of 10 smooth, bell-shaped-windowing functions, and integrated with respect to potential. The idea behind this pre-processing technique is to capture the information throughout the global response to obtain 10 parameters per curve (Fig. 1).

Principal Component Analysis (a non-supervised technique) and Partial Least Squares Discriminant Analysis (PLS-DA) were used as discrimination and classification tools. PLS-1 regressions were used for estimating the correlations between electrochemical data and the polyphenolic content or the data of the human panel. 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).

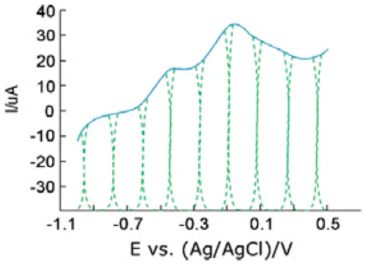


Fig. 1. Square wave voltammogram and kernels which allow obtaining 10 parameters per curve.

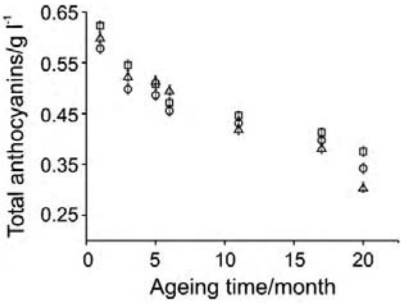


Fig. 2. Variation of anthocyanins content during ageing of wines in (△) oak barrel; (o) oak staves; (□) oak chips.

3. Results and discussion

3.1. Monitoring the ageing of wines using chemical parameters

Wines aged in oak barrels and wines treated with oak chips/staves were analysed periodically by chemical methods. The general trends observed in the variations of the chemical parameters were similar for wines aged using both methods. However, several important differences could be observed in the case of polyphenolic compounds especially regarding the anthocyanin levels (Fig. 2). During the contact with wood, the decrease of the anthocyanin concentration was faster that during ageing in bottle [6]. After 1 year in bottle, the anthocyanin levels observed in wines aged using pieces of wood were higher than those observed in wines aged in barrels. Wines treated with staves showed intermediate values between chips and barrels. This result is in good agreement with previously published results [5,30,31] and confirms the importance of the size of the pieces of wood.

3.2. Ageing monitoring by means of the electronic tongue

The array of voltammetric electrodes was used to analyse the wines. As it has already been reported, complex voltammograms were obtained [22,23] (Fig. 3). The peak position and their intensities bring information about the chemical composition of the wines [14–22]. Of particular interest are the two peaks associated to polyphenols. The first peak is a reversible peak with an intense anodic wave at ca. 0.13–0.47 V. This peak can be associated to phenolic and flavonoid acids which possess easily oxidable orto-diphenol groups. Such compounds include caffeic acid, gallic acid, tannic acid, catechin, etc. In addition other compounds such as ascorbic acid can also be oxidised in this region [31]. A second broad and also quasi-reversible peak associated to polyphenols can be observed at ca. 0.9–1.0 V and corresponds to polyphenols that do not possess orto-diphenol groups (i.e. t-resveratrol, cumaric acid, vanillic acid, etc.).

Due to the variability in the nature and the concentration of polyphenols present in different wines, voltammograms registered from different wines differ one from another. In addition, changes can also be detected during maturing of wines. Ageing in contact with wood, causes an increase of the intensity of the peaks at 0.13–0.47 V associated to the presence of polyphenols. The intensity decreases during ageing in bottle.

In addition, during ageing in contact with wood (from T1 to T4), the peak potential of polyphenols at ca. 0.2 V shifts to lower values (Fig. 4). This fact can be associated to the modification of the chemical nature of polyphenols that facilitate their oxidation. After bottling, wines change to a reducing atmosphere. Under these

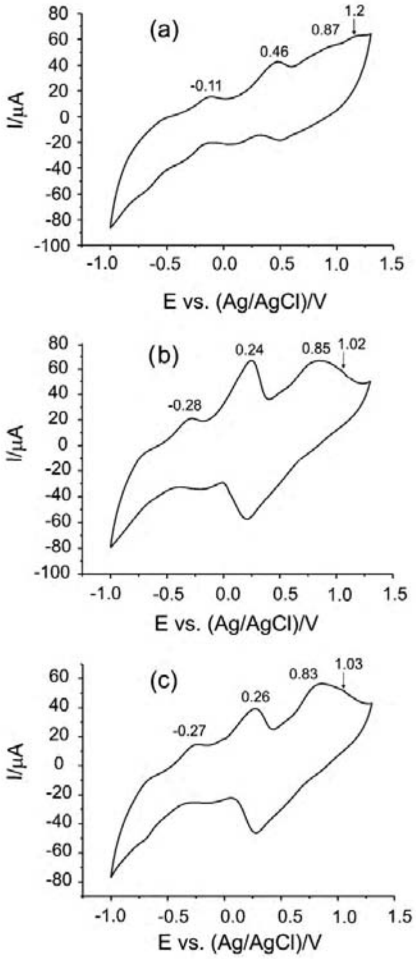


Fig. 3. Cyclic voltammetry of a LuPc$_2$ CPE electrode immersed in (a) C1 (T1); (b) C1 (T3); (c) C1 (T6). Scan rate 0.1 V s^{-1}.

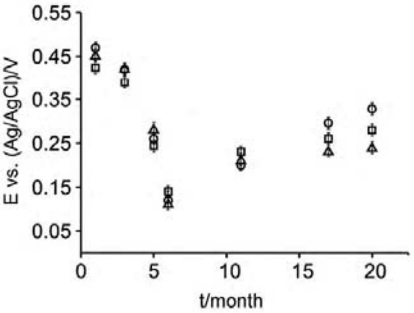


Fig. 4. Variation of the peak potential associated to polyphenols during the ageing of wines (average value) (△) oak barrel; (o) oak staves; (□) oak chips.

Table 3
Correlations with chemical parameters. Results of PLS-1 in calibration and validation.

Parameter	Slope	Offset	Correlation	RMSE
Tannins				
Calibration	0.863	0.311	0.929	0.222
Prediction	0.851	0.916	0.916	0.240
Glycerol				
Calibration	0.790	1.908	0.889	0.142
Prediction	0.763	2.156	0.760	0.158
Alcoholic degree				
Calibration	0.824	2.429	0.908	0.166
Prediction	0.814	2.715	0.886	0.184
Dry extract				
Calibration	0.842	4.620	0.917	0.230
Prediction	0.826	5.068	0.897	0.256
Titratable acidity				
Calibration	0.763	1.156	0.873	0.064
Prediction	0.728	1.326	0.851	0.069
Volatile acidity				
Calibration	0.694	0.162	0.833	0.042
Prediction	0.662	0.179	0.784	0.047
Tartaric acid				
Calibration	0.900	0.137	0.948	0.058
Prediction	0.875	0.171	0.928	0.068
Reducing sugars				
Calibration	0.864	0.190	0.928	0.144
Prediction	0.846	0.215	0.918	0.122

conditions, the oxidation of polyphenols is more difficult and the oxidation potential increases slowly from T5 to T7. The differences observed between wines aged in oak wood and those treated with oak chips or staves are small and become more important in the last sampling steps (bottled wines). The changes in intensity and the position of the peaks associated to polyphenols can explain the capability of the electronic tongue to monitor the ageing of wines.

3.3. Correlations between electronic tongue and chemical analysis

Partial Least Squares (PLS-1) regressions were performed to model the relationships between the electrochemical signals provided by the array of sensors and the chemical parameters measured in wines. In order to establish a robust model, a test validation was carried out using 84 of the 91 samples (chips, staves and barrels) analysed in the seven the sampling periods (T1–T7) as the training set. In order to validate the model, seven samples (one of each sampling period) were used as the test set. The samples of the validation test selected were all aged using American chips.

In a first approach, the chemicals parameters selected for this study included parameters with a well-known influence in the gustative properties of wines: tannins, glycerol, alcoholic degree, dry extract, total acidity, volatile acidity, tartaric acid and reducing sugars.

Results are shown in Table 3. The best correlations were found for tartaric acid with regression coefficients of 0.948 in calibration and 0.928 in validation. Additionally, low values of RMSE (root mean square error) in calibration (0.058) and prediction (0.068) were accomplished.

Correlations with parameters related with acidity (total acidity and volatile acidity) showed lower correlation coefficients.

Anthocyanins are among the most important phenolic compounds which are responsible of the colour and participate in the

Table 4
Correlations with polyphenols content. Results of PLS-1 in calibration and validation.

Parameter	Slope	Offset	Correlation	RMSE
Total Polyphenols				
Calibration	0.868	0.284	0.932	0.056
Prediction	0.854	0.315	0.921	0.060
Low-polymerised polyphenols				
Calibration	0.902	0.132	0.949	0.055
Prediction	0.893	0.144	0.938	0.061
High-polymerised polyphenols				
Calibration	0.880	0.097	0.938	0.076
Prediction	0.871	0.104	0.931	0.080
Catechins				
Calibration	0.866	0.144	0.930	0.091
Prediction	0.859	0.119	0.921	0.077
Anthocyanins				
Calibration	0.914	0.042	0.956	0.024
Prediction	0.908	0.044	0.950	0.022

Table 5
Correlation with human panel. Results of PLS-1 in calibration and validation.

Parameter	Slope	Offset	Correlation	RMSE
Volume				
Calibration	0.700	1.724	0.836	0.180
Prediction	0.670	1.894	0.804	0.195
Acidity				
Calibration	0.736	1.290	0.858	0.129
Prediction	0.710	1.418	0.819	0.145
Tannic intensity				
Calibration	0.663	2.038	0.814	0.254
Prediction	0.643	2.157	0.787	0.269
Sweet tannin				
Calibration	0.628	0.856	0.792	0.434
Prediction	0.604	0.910	0.763	0.460
Green tannin				
Calibration	0.865	0.436	0.930	0.252
Prediction	0.850	0.488	0.916	0.276
Astringency				
Calibration	0.816	0.811	0.903	0.345
Prediction	0.798	0.895	0.875	0.390
Persistency				
Calibration	0.593	2.289	0.770	0.215
Prediction	0.547	2.549	0.713	0.237

polymerisation and condensation of tannins. These reactions give rise to changes in the phenolic structure that in turn influences the astringency of wines. Due to the important role of polyphenols in the ageing of wines, a PLS-1 model was established in order to find the correlation between the responses of the electronic tongue and total polyphenols (TP), low-polymerised polyphenols (LPP), high-polymerised polyphenols (HPP), catechins (CAT) and anthocyanins (ACY). Also in this case, all the samples (chips, staves and barrels) analysed in the seven of the sampling periods (T1–T7) were included in the study.

As observed in Table 4, excellent correlations were found for all the polyphenols analysed. Root mean square error (RMSE) in calibration and validation are expressed in the original units of the variable (values measured chemically are in the range: PT, 2.1–2.3 $\mathrm{g\,L^{-1}}$; LPP, 1.1–1.5 $\mathrm{g\,L^{-1}}$; HPP, 0.6–1.2 $\mathrm{g\,L^{-1}}$; CAT, 0.7–0.8 $\mathrm{g\,L^{-1}}$ and ACY, 0.4–0.5 $\mathrm{g\,L^{-1}}$). RMSEs residual errors obtained for calibration and prediction are lower than 15% for all phenolic compounds under study.

3.4. Correlations between electronic tongue and human panel

Nowadays it is clear that electronic tongues measure the chemical composition of the tested samples instead of human perceptions. However, as the chemical composition is related with tastes and flavours, attempt has been made to find correlations between the results obtained with the electronic tongue and the scores given by a human panel in the gustative phase using PLS-1. Parameters scored included volume, acidity, tannic intensity, sweet tannin, green tannin, astringency and persistency. In good agreement with the results found in the previous paragraphs, the best correlations were found with the tannic intensity and with the green tannin which in turn explain the high correlations also found with the astringency (Table 5).

3.5. Discrimination and classification of wines by means of the electronic tongue

One of the main objectives of this work was to establish the capability of our electronic tongue to monitor the ageing of wines and its capability to detect the particular characteristics induced by traditional and alternative ageing methods.

Wines aged in oak barrels and wines treated with pieces of wood (chips and staves) were analysed periodically using the electronic tongue.

The changes in the chemical composition that occur during maturing of wines, could be detected by the array of sensors facilitating the monitoring of the ageing. Fig. 5 shows the PCA scores plot of the responses of the array of voltammetric sensors immersed in the wines under study. The first three principal components captured the 51%, 11% and 8% of the variance respectively.

Wines aged during 1 and 2 months (T1 and T2) appear on the left side of the diagram at positive PC1 values, far apart from the other samples (T3–T7) which are located in the right part of the figure at negative PC1 values. Bearing in mind that the array of sensors is sensitive to the antioxidant capability of wines (and the oxygen dissolved plays an important role in this capability), the clear discrimination of wines collected in T1 and T2 can be related with the fast rate of oxygen consumption that takes place during the first months of ageing.

During the oxidative ageing in contact with wood, clusters corresponding to samplings T1–T4 shift from positive to negative first Principal Component (*X* axis). During maturing in bottle (reductive atmosphere), a displacement of clusters associated to samplings T5–T7 in the opposite sense is observed.

In some cases subclusters inside each sampling period are observed. This can be clearly noticed in samplings T5–T7 and is related to the use of different ageing methods. In order to evaluate the possibility to discriminate between wines aged in oak barrel and wines treated with pieces of wood, the samples collected at each sampling period where analysed separately using PCA.

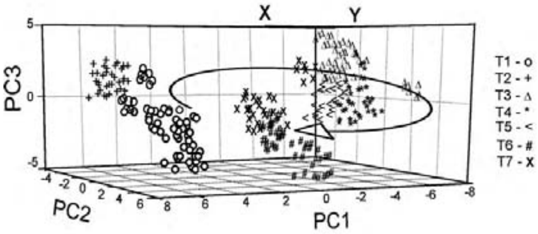


Fig. 5. Score plot of the PCAs of the array of voltammetric sensors exposed to the wines under study. T1 (o), T2 (+), T3 (Δ), T4 (*), T5 (<), T6 (#) and T7 (x) correspond to samples collected at different elapsed times.

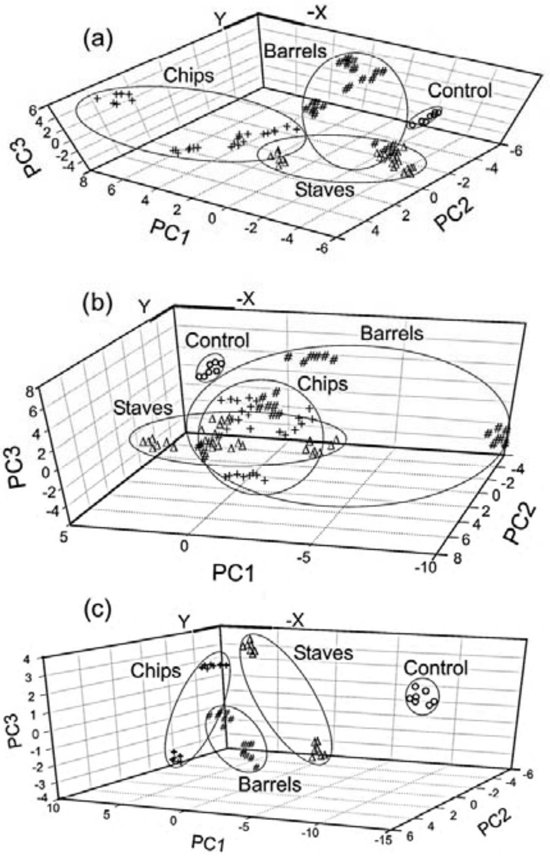


Fig. 6. PCA scores plot of wines sampled at (a) T1, (b) T4 and (c) T6. Symbols refer to: (o) control wine; (+) wines treated with chips; (△) wines treated with staves and (#) wines aged in barrels.

Fig. 6a shows the result of the PCA using samples collected at T1. The wine control (non contact with wood) appears far apart from the wines aged in contact with wood. Clusters corresponding to wines aged in barrels, and using chips and staves appear partially discriminated, probably because pieces of wood induce an accelerated ageing. When the ageing continued, the differences between wines decreased progressively (Fig. 6b), and after 3 months of ageing (sampling T4) wines could not be longer distinguished.

Then when wines were bottled (T5), differences increased progressively and wines elaborated following different methods could be distinguished using the array of sensors (Fig. 6c). Here again, subclusters are observed that correspond to the use of different types of wood (American or French) during the ageing.

The results shown in Figs. 5 and 6 obtained using the electronic tongue are in good agreement with the results found by chemical analysis because PCA detect patterns in the data measured in wines aged by different methods only at the beginning of the ageing and in the final stages.

In order to evaluate the capability of the system to predict the type of method used for ageing, prediction models based on PLS-DA were constructed. Fig. 7 shows the result of the PLS-DA calculated for wines bottled at T1, T2 and T7. The relative location of the samples retains the general structure of the PCA score plots shown in Fig. 5, confirming the previous observations.

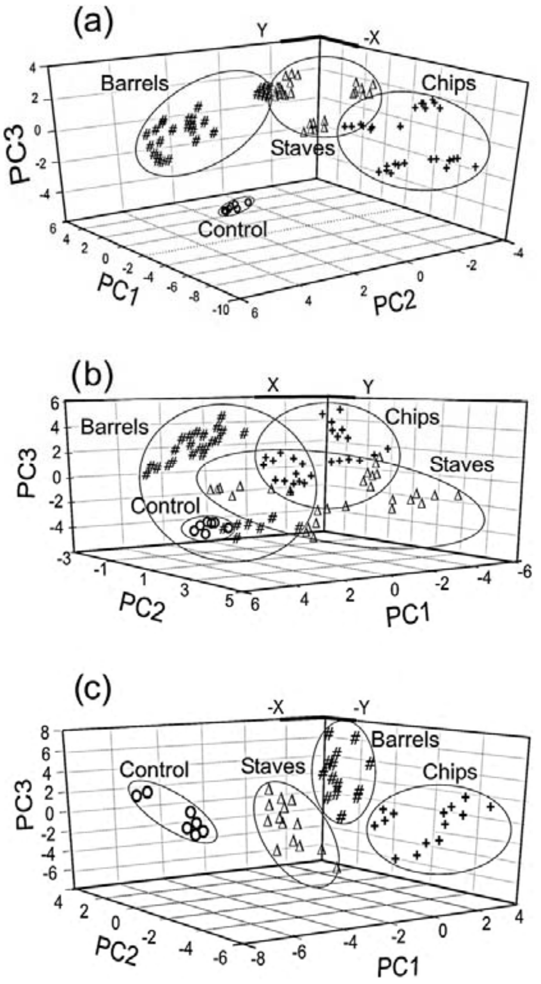


Fig. 7. PLS-DA scores plot of wines sampled at (a) T1, (b) T2 and (c) T5. Symbols refer to: (o) control wine; (+) wines treated with chips; (△) wines treated with staves and (#) wines aged in barrels.

One of the main interests of the discrimination between wines aged in oak barrel and wines treated with pieces of wood is to detect the use of such alternative practices in the final product. When a wine is consumed it may have suffered variable periods of bottling. For this reason, the capability of the system to predict the ageing method was evaluated constructing a model formed by samples T5–T7 where different bottling periods were contemplated. Table 6 collects the quantitative data derived from the PLS-DA classification model. As observed, both the calibration and validation values involved a good-quality modelling performance (slope near 1, offset near 0 and large correlation between sensors and categorised variables) In addition, almost negligible RMSEC and RMSEP values were accomplished. Sensitivity and specificity were found >0.880 in all cases.

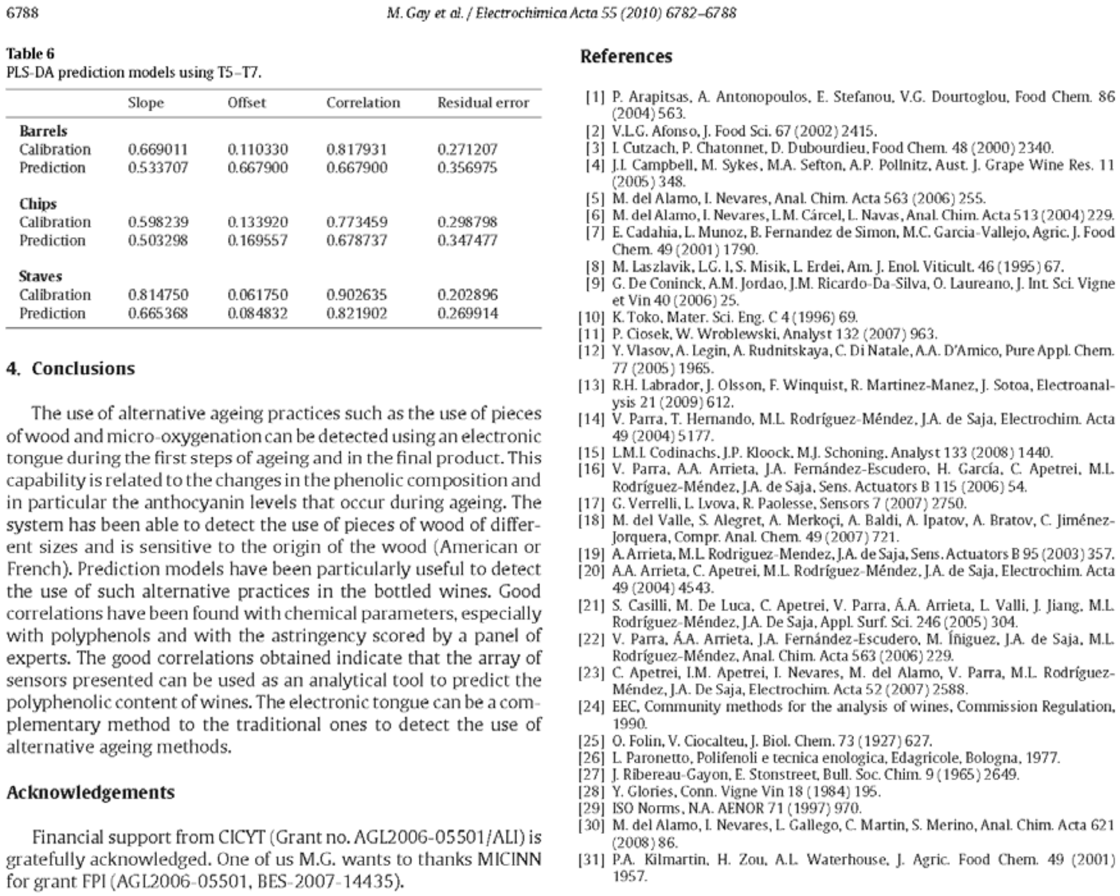


Table 6
PLS-DA prediction models using T5–T7.

	Slope	Offset	Correlation	Residual error
Barrels				
Calibration	0.669011	0.110330	0.817931	0.271207
Prediction	0.533707	0.667900	0.667900	0.356975
Chips				
Calibration	0.598239	0.133920	0.773459	0.298798
Prediction	0.503298	0.169557	0.678737	0.347477
Staves				
Calibration	0.814750	0.061750	0.902635	0.202896
Prediction	0.665368	0.084832	0.821902	0.269914

4. Conclusions

The use of alternative ageing practices such as the use of pieces of wood and micro-oxygenation can be detected using an electronic tongue during the first steps of ageing and in the final product. This capability is related to the changes in the phenolic composition and in particular the anthocyanin levels that occur during ageing. The system has been able to detect the use of pieces of wood of different sizes and is sensitive to the origin of the wood (American or French). Prediction models have been particularly useful to detect the use of such alternative practices in the bottled wines. Good correlations have been found with chemical parameters, especially with polyphenols and with the astringency scored by a panel of experts. The good correlations obtained indicate that the array of sensors presented can be used as an analytical tool to predict the polyphenolic content of wines. The electronic tongue can be a complementary method to the traditional ones to detect the use of alternative ageing methods.

Acknowledgements

Financial support from CICYT (Grant no. AGL2006-05501/ALI) is gratefully acknowledged. One of us M.G. wants to thanks MICINN for grant FPI (AGL2006-05501, BES-2007-14435).

References

[1] P. Arapitsas, A. Antonopoulos, E. Stefanou, V.G. Dourtoglou, Food Chem. 86 (2004) 563.
[2] V.L.G. Afonso, J. Food Sci. 67 (2002) 2415.
[3] I. Cutzach, P. Chatonnet, D. Dubourdieu, Food Chem. 48 (2000) 2340.
[4] J.I. Campbell, M. Sykes, M.A. Sefton, A.P. Pollnitz, Aust. J. Grape Wine Res. 11 (2005) 348.
[5] M. del Alamo, I. Nevares, Anal. Chim. Acta 563 (2006) 255.
[6] M. del Alamo, I. Nevares, L.M. Cárcel, L. Navas, Anal. Chim. Acta 513 (2004) 229.
[7] E. Cadahia, L. Munoz, B. Fernandez de Simon, M.C. Garcia-Vallejo, Agric. J. Food Chem. 49 (2001) 1790.
[8] M. Laszlavik, L.G. I, S. Misik, L. Erdei, Am. J. Enol. Viticult. 46 (1995) 67.
[9] G. De Coninck, A.M. Jordao, J.M. Ricardo-Da-Silva, O. Laureano, J. Int. Sci. Vigne et Vin 40 (2006) 25.
[10] K. Toko, Mater. Sci. Eng. C 4 (1996) 69.
[11] P. Ciosek, W. Wroblewski, Analyst 132 (2007) 963.
[12] Y. Vlasov, A. Legin, A. Rudnitskaya, C. Di Natale, A.A. D'Amico, Pure Appl. Chem. 77 (2005) 1965.
[13] R.H. Labrador, J. Olsson, F. Winquist, R. Martinez-Manez, J. Sotoa, Electroanalysis 21 (2009) 612.
[14] V. Parra, T. Hernando, M.L. Rodríguez-Méndez, J.A. de Saja, Electrochim. Acta 49 (2004) 5177.
[15] L.M.I. Codinachs, J.P. Kloock, M.J. Schoning, Analyst 133 (2008) 1440.
[16] V. Parra, A.A. Arrieta, J.A. Fernández-Escudero, H. García, C. Apetrei, M.L. Rodríguez-Méndez, J.A. de Saja, Sens. Actuators B 115 (2006) 54.
[17] G. Verrelli, L. Lvova, R. Paolesse, Sensors 7 (2007) 2750.
[18] M. del Valle, S. Alegret, A. Merkoçi, A. Baldi, A. Ipatov, A. Bratov, C. Jiménez-Jorquera, Compr. Anal. Chem. 49 (2007) 721.
[19] A. Arrieta, M.L. Rodriguez-Mendez, J.A. de Saja, Sens. Actuators B 95 (2003) 357.
[20] A.A. Arrieta, C. Apetrei, M.L. Rodríguez-Méndez, J.A. de Saja, Electrochim. Acta 49 (2004) 4543.
[21] S. Casilli, M. De Luca, C. Apetrei, V. Parra, Á.A. Arrieta, L. Valli, J. Jiang, M.L. Rodríguez-Méndez, J.A. De Saja, Appl. Surf. Sci. 246 (2005) 304.
[22] V. Parra, Á.A. Arrieta, J.A. Fernández-Escudero, M. Íñiguez, J.A. de Saja, M.L. Rodríguez-Méndez, Anal. Chim. Acta 563 (2006) 229.
[23] C. Apetrei, I.M. Apetrei, I. Nevares, M. del Alamo, V. Parra, M.L. Rodríguez-Méndez, J.A. De Saja, Electrochim. Acta 52 (2007) 2588.
[24] EEC, Community methods for the analysis of wines, Commission Regulation, 1990.
[25] O. Folin, V. Ciocalteu, J. Biol. Chem. 73 (1927) 627.
[26] L. Paronetto, Polifenoli e tecnica enologica, Edagricole, Bologna, 1977.
[27] J. Ribereau-Gayon, E. Stonstreet, Bull. Soc. Chim. 9 (1965) 2649.
[28] Y. Glories, Conn. Vigne Vin 18 (1984) 195.
[29] ISO Norms, N.A. AENOR 71 (1997) 970.
[30] M. del Alamo, I. Nevares, L. Gallego, C. Martin, S. Merino, Anal. Chim. Acta 621 (2008) 86.
[31] P.A. Kilmartin, H. Zou, A.L. Waterhouse, J. Agric. Food Chem. 49 (2001) 1957.

2.5 ARTÍCULO 4. "Analysis of the influence of the type of closure in the organoleptic characteristics of a red wine by using an electronic panel."

N. Prieto, M. Gay, S. Vidal, O. Aagaard, J.A. de Saja, M.L. Rodríguez-Méndez.

Food Chemistry 2011. 129, 589-594.

2.5.1. Motivación y objetivos.

El trabajo que se presenta a continuación surge como colaboración con la empresa belga Nomacorc®, dedicada a la producción de tapones sintéticos para el embotellado de vinos. Nomacorc® persigue la fabricación de tapones poliméricos que permitan una difusión controlada de oxígeno a través de los mismos, de modo que se utilice el tapón adecuado para que las características organolépticas de los vinos sean óptimas.

El oxígeno desempeña un papel fundamental en las características organolépticas de un vino, tales como olor, aroma y sabor. La gestión del mismo a través del proceso global de producción es de vital importancia. Cuando el vino está embotellado, el oxígeno que difunde a través del tapón se puede controlar mediante corchos sintéticos con tamaños de poro determinados. En este sentido, Nomacorc® ha liderado un proyecto internacional en el que han colaborado diversos grupos expertos en campos complementarios cuyo objetivo ha sido estudiar el efecto del oxígeno en las características organolépticas del vino. Para ello expertos enólogos de la Universidad de Burdeos han elaborado vinos utilizando diferentes estrategias, con el fin de obtener vinos con diferente capacidad antioxidante (contenido polifenólico) y contenido de oxígeno (mediante microoxigenación). Estos vinos han sido embotellados utilizando tapones sintéticos con diferente grado de porosidad (*Nomacorc Light stoppers* y *Nomacorc Classic stoppers*), de tal manera que la difusión del oxígeno a través de los mismos es diferente. Los vinos así producidos han sido analizados en laboratorios de toda Europa. Nuestro Grupo de Investigación fue el encargado de analizar las características organolépticas de los vinos mediante un panel de cata electrónico formado por un ojo electrónico, una nariz electrónica y una lengua electrónica. Dado que la lengua electrónica es el sistema desarrollado y estudiado en la presente Tesis, es sobre el cual se hará mayor hincapié, comentando de forma superficial los resultados obtenidos por el ojo y nariz electrónica.

2.5.2. Resumen de los resultados obtenidos.

i) Las muestras de vino se han elaborado a partir de la variedad de uva Garnacha. Se han realizado dos tipos de estrujado, el tradicional (Trad) y otro mediante la técnica *Flash Release* (FR). De esta manera, el vino elaborado con el estrujado tradicional presenta bajo contenido en polifenoles, mientras que el segundo, debido a la técnica de estrujado empleada presenta un alto contenido polifenólico. Cada uno de estos dos vinos se ha dividido posteriormente en dos tanques, en uno de los cuales se realizó microoxigenación. De esta manera se han obtenido cuatro tipos de vinos diferentes: Trad, Trad + Mox, FR y FR + Mox. A continuación, cada tipo de vino se embotelló con cuatro tipos de tapones sintéticos: Light 21%, Classic 21%, Classic 4% y Classic 1%, cada uno con diferente capacidad de difusión de oxígeno. De esta manera se han obtenido 16 muestras de vino.

ii) A partir de los espectros UV-Vis de los vinos bajo estudio se han calculado las coordenadas CIELab (ojo electrónico), las cuales se han empleado como variables en el PCA. El ojo electrónico es capaz de discriminar las muestras de acuerdo al tapón empleado.

iii) En el caso de la nariz electrónica, se ha empleado un sistema basado en una red de sensores resistivos formados por óxidos metálicos semiconductores. Se ha observado que el sistema electrónico discrimina los vinos de acuerdo al tapón, observándose además tendencias discriminatorias en cuanto al contenido polifenolico.

iv) La lengua electrónica empleada está constituída por CPEs modificados con ftalocianias: CoPc y bisftalocianinas: $LuPc_2$, $LuPc_2^t$ y perilenos: BpPTCD, OcPTCDMI y BuPTCMI. Además, se han incluído en la red de sensores un CPE sin modificar y un electrodo de platino.

v) Las medidas electroquímicas realizadas con los CPEs muestran los picos asociados al material modificador y aquellos relacionados con los compuestos electroactivos presentes en el vino, los polifenoles. Dado el principio de detección de la técnica, es de esperar que la discriminación de las muestras por parte de la red de sensores sea en base al contenido polifenólico de las mismas.

vi) Además, en el gráfico de PCA se ha observado que los vinos que no han sido microoxigenados aparecen separados de los que sí lo han sido. Las tendencias observadas en cuanto a la microoxigenación se deben a que el oxígeno desempeña un papel decisivo en la astringencia, la cual afecta de manera notoria a las características gustativas del vino.

vii) Las curvas voltamétricas obtenidas se han pre-tratado empleando funciones kernel, que proporcionan 10 variables por cada sensor. Dado el elevado número de variables proporcionado por la lengua electrónica, en este caso 80 (10 variables por 8 sensores), en comparación con las aportadas por el ojo y la nariz electrónica, se ha realizado una selección de variables, con el fin de reducirlas y evitar que la lengua electrónica tuviera mayor peso en el tratamiento de datos del panel electrónico. Para ello, se han seleccionado las variables con mayor peso estadístico en el círculo de correlación, seleccionando 3 variables por cada sensor. De esta manera, el número de variables se ha reducido de 80 a 24, obteniéndose resultados similares.

viii) El modelo PLS-DA se ha aplicado para la predicción de las diferentes clases establecidas previamente: contenido polifenólico, microoxigenación y tapón. Los resultados demuestran que la capacidad de clasificación por parte de la lengua electrónica es satisfactoria en cuanto al contenido de polifenoles y a la microoxigenación, obteniendo altos valores de correlación y bajos errores residuales. Cuando se establece como clase el tapón, los valores de correlación obtenidos han sido mucho peores.

ix) Se han fusionado los tres sistemas electrónicos con el fin de evaluar si el panel electrónico, formado por el ojo, la nariz y la lengua electrónica, permite una mejor discriminación que en el caso de emplear los sistemas separadamente. De esta manera, las señales provenientes de los 2 sistemas se han combinado en una matriz de datos y se ha realizado un PCA para evaluar la capacidad de discriminación.

x) Los clusters observados en el PCA son pequeños, lo que permite una sencilla discriminación de las muestras. Esto demuestra que la fusión de las tres redes de sensores mejora la discriminación de los vinos. Además, el PCA permite una discriminación simultánea de acuerdo al tipo de tapón empleado en el embotellamiento, al contenido polifenólico y existencia o no de micooxigenación. Esta mejora en la capacidad de discriminación se confirma mediante el modelo PLS-DA aplicado al panel electrónico, en el cual se obtienen altos coeficientes de correlación para todas las clases y errores residuales bajos en todos los casos.

2.5.3. Conclusiones

El panel electrónico formado por un ojo, nariz y lengua electrónica se ha aplicado a un problema industrial, en colaboración con la empresa Nomacorc®, con el fin de evaluar si los distintos tapones empleados en el embotellamiento del vino afectan a las características organolépticas del mismo, así como la realización de microoxigenación o no previa al embotellamiento y el tipo de estrujamiento.

El ojo y nariz electrónicos son capaces de discriminar las muestras de acuerdo al tapón empleado, siendo la última capaz de discriminar entre los vinos microoxigenados de los que no lo están.

Por su parte, la lengua electrónica se ve afectada por la cantidad de polifenoles presentes y por la microoxigenación realizada previa al embotellamiento.

Además, con el fin de unificar en una única matriz de datos, las variables procedentes de los tres sistemas electrónicos, se ha realizado una reducción de variables, eligiendo las 3 variables de mayor peso para cada sensor, lo cual constituye una novedad en cuanto al tratamiento de datos.

La fusión del ojo, nariz y lengua electrónica mejora la discriminación proporcionada por los sistemas individuales, permitiendo distinguir simultáneamente las muestras de vino estudiadas de acuerdo al tipo de tapón empleado, realización o no de microoxigenación y contenido polifenólico.

Food Chemistry 129 (2011) 589–594

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Analysis of the influence of the type of closure in the organoleptic characteristics of a red wine by using an electronic panel

N. Prieto [a,b], M. Gay [a,c], S. Vidal [d], O. Aagaard [d], J.A. de Saja [b,c], M.L. Rodriguez-Mendez [b,c,*]

[a] *Department of Inorganic Chemistry, Escuela de Ingenierías Industriales, University of Valladolid, Paseo del Cauce, 59, 47011 Valladolid, Spain*
[b] *Department of Condensed Matter Physics, Faculty of Sciences, University of Valladolid, 47011 Valladolid, Spain*
[c] *Unidad Asociada Instituto de Estructura de la Materia, CSIC, Spain*
[d] *Nomacorc, ZI Les Plénesses, 7 Chemin de Xhénorie, 4890 Thimister Clermont, Belgium*

ARTICLE INFO

Article history:
Received 29 July 2009
Received in revised form 21 February 2011
Accepted 24 April 2011
Available online 29 April 2011

Keywords:
E-nose
E-tongue
E-eye
Electronic panel
Oxygen transmission rate
Closures
Micro-oxygenation

ABSTRACT

An electronic panel formed by an electronic nose, an electronic tongue and an electronic eye has been successfully used to evaluate the organoleptic characteristics of red wines vinified using different extraction techniques and micro-oxygenation methods and bottled using closures of different oxygen transmission rates (OTR).

The three systems have demonstrated a good capability of discrimination by means of Principal Component Analysis (PCA). Partial Least Squares Discriminant Analysis (PLS-DA) has permitted to establish prediction models based on the type of closure, the polyphenol content or the effect of micro-oxygenation. The best correlations found using the e-eye and the e-nose are related to the OTR of the closure. In contrast, the electronic tongue is more sensitive to the polyphenol content. The discrimination and prediction capabilities of the system are significantly improved when signals from each module are combined. The electronic panel can be a useful tool for the characterisation and control of oxygen and antioxidant capability of red wines.

1. Introduction

Oxygen plays a pivotal role in the evolution of wine by impacting its colour, aroma and mouth-feel properties. Managing oxygen in oenology remains critical as both excessive exposure and excessive protection lead to sensory defects known as oxidation and reduction. To efficiently manage it, oxygen must be controlled along the whole value chain from grape harvest to bottle storage. Very often oxygen introduction is uncontrolled as during pumping over and other operations of wine transfer and at bottling. New techniques were developed aiming better control of oxygen exposure. For example, a mild oxygenation process, referred to as micro-oxygenation, has been proposed to simulate the continuous low oxygen uptake taking place in barrels (Atanasova, Flucrand, Cheynier, & Moutounet, 2002). This technique has become common practice to improve the quality of red wines, enhance colour intensity and stabilize wine pigments. In bottle, oxygen exposure is usually low but can be quite variable depending on the type of closure. The wine industry can now also take advantages of engineered solutions to deliver known and reproducible amounts of oxygen into bottles through the closures, that can be manufactured with specific and controlled oxygen transfer rate (OTR).

Usually, the quality of wines is evaluated by a sensory panel composed of trained experts or by chemical analysis. In the last years electronic noses (Peris & Escuder-Gilabert, 2009; Rock, Barsan, & Weimar, 2008) and tongues (Ciosek & Wroblewski, 2007; Labrador, Olsson, Winquist, Martinez-Manez, & Sotoa, 2009; Parra, Hernando, Rodríguez-Méndez, & de Saja, 2004; Vlasov, Legin, Rudnitskaya, Di Natale, & D'Amico, 2005) have been developed to operate in an analogous manner as the human senses can perceive odours and tastes. They are based on the combination of non-selective chemical sensors endowed with sufficient cross-selectivities coupled with chemommetric methods.

Wines have been extensively analysed using arrays of resistive sensors as electronic noses combined with preconcentration techniques such as Solid Phase Microextraction (that eliminate water and ethanol) (Capone et al., 2000; Hahn et al., 2003; Lozano, Santos, & Horrillo, 2008; Penza & Cassano, 2004; Villanueva et al., 2006). In the case of electronic tongues, the use of arrays of electrochemical sensors is of particular interest due to the important role that the oxygen and the antioxidants play in the organoleptic characteristics and also in the health benefits of red wines (Moreno i Codinachs et al., 2008; Parra, Arrieta, Fernández-Escudero, Rodríguez-Méndez, & de Saja, 2006; Riul et al., 2003; Verrelli, Lvova, Paolesse, Di Natale, & D'Amico, 2007). Colour is an important part

---

[*] Corresponding author. Address: Department of Inorganic Chemistry, E. T. S. Ingenieros Industriales, University of Valladolid, Spain. Tel.: +34 983 423540; fax: +34 983 423310.
E-mail address: mluz@eis.uva.es (M.L. Rodriguez-Mendez).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.04.071

of the red wine quality. It is generally accepted that an increase in colour coincides with an improvement in phenol structure, an increase in aroma intensity and hence, an increase in wine quality. In the search for objective, easily quantifiable quality parameters, colour is therefore a logical option and has been investigated as a possible wine quality parameter for many years (Perez-Caballero, Ayala, Echavarri, & Negueruela, 2003).

Several attempts have been made to combine e-noses and e-tongues an even with e-eye (Cosio, Ballabio, Benedetti, & Gigliotti, 2007; Di Natale et al., 2004; Rodríguez-Méndez et al., 2004). It has been demonstrated that the simultaneous utilisation of electronic noses and electronic tongues can increase the amount of information extracted from a specific sample. In previous works, our group has developed an electronic panel purposely dedicated to the analysis of red wines (Rodríguez-Méndez et al., 2004). The electronic nose was based on an array of metal oxide sensors coupled to an SPME system. The chemical nature of the SPME fibre and the protocol of absorption and desorption of gases has been optimised to increase the amount of volatiles absorbed while minimising the absorption of ethanol and water (Villanueva, Rodríguez-Mendez, & de Saja, 2008). The electronic tongue developed by our group consisted of an array of voltammetric sensors chemically modified with electroactive substances. The sensing materials included phthalocyanines and perylenes that interact with components of wines namely with their antioxidant capability (Arrieta, Rodríguez-Méndez, & de Saja, 2003; Casilli et al., 2005). Thus, the electronic tongue has been able to discriminate wines of different qualities, grape variety or among wines aged in different types of oak barrels and oak chips (Apetrei et al., 2007; Parra et al., 2006; Rodríguez-Méndez et al., 2004). The system has been completed with colour measurement system based on CIELab coordinates calculated from spectrophotometric data (Rodríguez-Méndez et al., 2004).

The aim of this study was to monitor the influence of oxygen pick-up, before (micro-oxygenation, Mox) and after bottling (nano-oxygenation), on wine sensory evolution. The rate of nano-oxygenation was controlled by combining consistent OTR closures and different oxygen controlled storage conditions. Since oxygen consumption is known to be related to phenolic compounds (Singleton, 1987; Waterhouse & Laurie, 2006), the same experiments were performed on wines differing by their phenolic content, obtained from the same grapes either by Flash Release (FR, higher phenolic content) (Morel-Salmi, Souquet, Bes, Cheynier, & Moutounet, 2006) or traditional soaking.

The potential of the e-panel to discriminate among wines with different oxygen levels and antioxidant capabilities is evaluated by using Principal Component analysis. Partial Least Squares Discriminant Analysis (PLS-DA) is also used to investigate prediction models based on (a) the polyphenol content (b) the contribution of micro-oxygenation and (c) the closure OTR.

2. Materials and methods

2.1. Samples

Ten thousand kilograms of grapes from Vitis Vinifera var. Grenache (2007), grown at INRA Pech Rouge experimental Unit station (Gruissan, Southern France) were harvested on two plots at commercial maturity in September 2007. Grapes from the first plot (22°Brix, pH = 3.6) were used for traditional winemaking and those from the second plot (25°Brix, pH = 3.7) were used for Flash Release trial. The treatment by FR consisted in destemming and crushing the grapes, heating them at 95 °C during 6 min with biological vapour, and then submitting them to a strong vacuum (pressure closed to 60 hPa).

Two wines were prepared by traditional soaking (Trad) and Flash Release (FR) respectively. Each of these two wines was then divided in two batches submitted or not to micro-oxygenation (Mox/noMox), yielding four wines in total: FR, FR + Mox, Trad, Trad + Mox.

For both traditional soaking and Flash Release trial, the musts were distributed into two 50 hL fermentation stainless steel tanks equipped with temperature control, added with sulphite (5 g hL^{-1}) and with 500 g yeast (LBRouge, Lallemand, 10 g hL^{-1}), and fermented to dryness. The cap was punched down daily to ensure mixture of the marc with the fermenting liquid phase. A supplementation of nitrogen nutrition was done (20 g hL^{-1} DPA) after a decrease of 30 points of density. After 8 days of maceration, when alcoholic fermentation was finished (controlled by sugar analysis), the wines were racked (O_2 pickup: 0.3 ppm), transferred to 20 hL tanks and inoculated with lactic bacteria (Lalvin VP41, Lallemand) to start malolactic fermentation. At the end of the malolactic fermentation, the wines were racked into other 20 hL inox tank (O_2 pickup: 4 ppm) and sulphites were added (3 g hL^{-1}). Each of the wines was transferred from the 20 hL tank into four 2.7 hL tanks, adapted to perform Mox with a height of 3 m and a surface of wine of 0.09 m^2. Mox was performed with a 10-channels Oenodev system, at 5 mg O_2 L^{-1} $month^{-1}$ during 3 weeks. The noMox modalities were stored in the same cellar in similar tanks. The total oxygen quantities introduced (pick-up at racking and Mox) into these four wines (Tradmox, Trad, FRmox, FR) during winemaking were estimated in mg L^{-1} as follows: 8.66; 5.79; 9; 3.85, respectively. The wines were bottled in 375 mL glass bottles (Saint-Gobain bordelaise 39). Each of the four wines was divided in four batches in order to obtain four OTR conditions: one batch was closed with Nomacorc Light stoppers and stored in ambient air (21% oxygen). The three remaining batches were closed with Nomacorc Classic stoppers and stored respectively in ambient air (21% oxygen) and in stainless steel drums where oxygen levels were kept constant at either 4% oxygen or 1% oxygen. All the wines were stored in the same closed room at a constant temperature (23 °C). The OTR were calculated using Fibox 3 trace fibre optic oxygen metre (PreSens Precision Sensing GmbH, Regensburg, Germany) (Diéval, Veyret, Vidal, Aagaard, & Vidal, 2009) and were found to be 11.9, 8.0, 1.9 and 0.8 μL oxygen/bottle/day for Light 21%, Classic 21%, Classic 4% and Classic 1%, respectively. Wines included in the study are listed in Table 1.

2.2. Electronic eye

Colour measurements were made in a Shimadzu 2101 UV–Vis Spectrophotometer, using 1 mm path length glass cell (Rodríguez-Méndez et al., 2004). The CIELab coordinates (L^*, a^*, b^*, C, H, S) were calculated following the recommendations of the Comission Internationale of L'Eclairage for the CIE illuminant D_{65} and 10° standard observer conditions.

2.3. Electronic nose

The array of 15 gas sensors was constructed using inorganic metal oxide (MOX) sensing units that were selected according to the previous experience of our group (Villanueva et al., 2006). Sensors were mounted in a stainless steel test box with an internal volume of 75 mL. The test box was kept at a constant temperature (50 °C) throughout the experiments. Data collection was performed through a *PC-LPM-16* data acquisition card from National Instruments interfaced to a personal computer. Sensors were polarised using a constant voltage of 5 V provided by a *FAC-662B* programmable power supply. The scan rate used to measure the resistance was 0.5 s. Data were monitored in real time and the

Table 1
Wines prepared for this study.

	Crush	Microoxygenation	Type of closure
Grenache must	Trad low polyphenols (W and X)	Not micro-oxygenated (W)	Ligth 21% (W1)
			Classic 21%(W2)
			Classic 4% (W3)
			Classic 1% (W4)
		Micro-oxygenated (X)	Ligth 21% (X1)
			Classic 21% (X2)
			Classic 4% (X3)
			Classic 1% (X4)
	FR high polyphenols (Y and Z)	Micro-oxygenated (Y)	Ligth 21% (Y1)
			Classic 21% (Y2)
			Classic 4% (Y3)
			Classic 1% (Y4)
		Not micro-oxygenated (Z)	Ligth 21% (Z1)
			Classic 21% (Z2)
			Classic 4% (Z3)
			Classic 1% (Z4)

graphs could be followed using Visual Basic software from Microsoft.

The *SPME* (Solid Phase Microextraction) sampling method was used as the injection method. A volume of 3 mL of wine was placed in 10 mL vial that was kept at 40 °C during 15 min, with a stirring speed of 650 rpm. Then a bipolar fibre of polydimethylsiloxane coated with carbowax and divinylbenzene (*PDMS/CW/DVB, supelco*) was exposed to the headspace of the vial for 15 min. Then, the *SPME* fibre was placed in a heated injection port of a gas chromatograph at 250 °C. The volatile compounds were driven to the test chamber using constant gas flow of 150 mL min^{-1} (air 100 mL min^{-1}, nitrogen 50 mL min^{-1}).

2.4. Electronic tongue

An array formed by voltammetric carbon paste electrodes (CPEs) based on bisphthalocyanines and perylenes was constructed according to previously published procedures (Apetrei et al., 2007; Parra et al., 2006). Two bisphthalocyanines the lutetium bisphthalocyanine ($LuPc_2$) and the octatert-butyl substituted analogue ($LuPc^t_2$) and one monophthalocyanine, the cobalt metallophthalocyanine (CoPc) have been used as modifiers. In the case of the perylenes, the *N*,*N*-bis(methylpiperidine)-3,4,9,10-perylenebis(dicarboximide) (BpPTCD), the *N*-octyl-3,4,9,10-perylenebis(dicarboximide) (OcPTCMI) and the *N*-butyl-3,4,9,10-perylenebis(dicarboximide) (BuPTCMI) were included in the study. An unmodified carbon pasted electrode (CPE) and a platinum electrode were also included in the array of sensors.

The measures were carried out using three electrode cell: CPEs were used as working electrode, a platinum wire as the counter electrode and a Ag/AgCl as the reference electrode. Square wave voltammetry was performed at potential scan ranging from −1.0 to 1.3 V using f = 15 Hz, Esw = 0.1 V, ΔE = 0.007 V (except for PcCo, ΔE = 0.005 V).

2.5. Data processing

Each wine was measured with the three systems separately. In order to carry out statistical analysis, seven replicates were registered for each measurement.

Data analysis involved an initial pre-processing of the corresponding signals. The UV–Vis spectra were used to calculate CIELab parameters giving raise to six input values per sample. In the case of e-nose, the maximum values of the transient responses of each one of the 15 MOX sensors were selected as input variables. Voltammetric signals were pre-treated using a previously developed method, based on kernel functions where the voltammetric curves were divided in 10 bell-shaped curves (Parra et al., 2004). The kernel method allows the data number to be reduced without losing important information throughout the total response.

The selected variables were used as the input parameters for PCA pattern recognition analysis. The Partial Least Squares Discriminant Analysis (PLS-DA) method was used to establish prediction models based on the polyphenol content, the effect of micro-oxygenation and closure OTR. In order to establish the prediction model on the polyphenol content, samples were divided into two different classes: class 1 included Flash-Released wines (Y and Z) and class 2 included traditional soaking wines (W and X). In order to evaluate the influence of micro-oxygenation, wines were grouped in two sets: the first one included wines micro-oxygenated (wines X and Y) and wines not micro-oxygenated (wines W and Z). Finally the prediction model of the effects of the closure OTR was analysed by establishing two classes, one including high OTR (1 and 2) and wines bottled with low OTR closures (wines 3 and 4).

Validation was performed using cross-validation. The RMSEP (Root Mean Square Error of Prediction), slope and correlation coefficient of predicted versus measured correlation line were used to evaluate the efficiency of applied regression model.

Data fusion coming from the three instruments was carried out by constructing a data matrix containing the information provided by the three apparatus. Data treatment was performed with commercial Unscrambler (v. 9.1, 2004, CAMO PROCESS AS, Norway) and Matlab (V.7.2, MathWorks, USA) software.

3. Results and discussion

Wines were analysed using the three systems (electronic tongue, electronic nose and electronic eye) separately in order to study the capability of each system to analyse the organoleptic properties. Then, a data fusion procedure was followed in order to assess the performance of the whole system.

3.1. Analysis of colour: e-eye

UV–Vis spectra of wines were measured and the CIELab parameters were calculated.

Wines sealed with closures 1 and 2 showed distinct spectra. In contrast, wines bottled using closures 3 and 4 were more similar. The type of closure affected strongly the lightness (L^*) of wines; the highest L^* values corresponded to wines sealed using closures 3 and 4, and were ranged from 25.09 to 27.10. The lower L^* (darker) corresponded to wines sealed with closures 1 and 2 (values ranged from 21.10 to 24.59). Depending on oxygen permeating through the closures, wine polyphenolics undergo different reactions that will eventually end up with different types of pigments. The newly formed pigments will show new colour properties and thus intensity of colour of the wines will be modified accordingly.

CIELab coordinates were used as the input variable for PCA analysis. PCA scores plot is shown in Fig. 1. As observed in the figure, the First Principal Component (PC1), that explains 69% of the information, contrasted wines according to the type of closure. Wines 1 and 2 appear in the right side of the graph. Wines sealed with stoppers 3 and 4 were not clearly discriminated due to the similarity of the spectral features, and appear in the left side of the plot. This result is in accordance with the fact that the sealing conditions 3 and 4 are very similar (1.9 and 0.8 μL oxygen/bottle/day).

The Partial Least Squares Discriminant Analysis (PLS-DA) method was used to establish prediction models based on the polyphenol content, the effect of micro-oxygenation and the type of closure. Results shown in Table 2 demonstrate that CIELab coordinates are not influenced by the polyphenol content and the use of microoxygenation, for this reason, errors in the predictions are high. In contrast, the model established for predicting the groups according to the type of closure demonstrated a good-quality ability in discriminating and recognising wines based on different OTR.

3.2. Analysis of odours: e-nose

The wines under study were exposed to the array of MOX sensors. The peak height was used as the input variable for PCA analysis. Fig. 2 shows the scores plot of the three first principal components (PC1 = 68%; PC2 = 18% and PC3 = 6%). The wines analysed could be clearly discriminated from each other, exception made of wines W3 and W5, that also showed very similar spectra. Wines bottled with high OTR closures (closures 1 and 2) appear on the left side of the figure (negative *X*). In the region of positive *X*, wines bottled using low OTR closures could be found. Differences caused by micro-oxygenation or polyphenol content were less marked.

In order to evaluate the prediction capability of the system, PLS-DA was conducted. A quantitative evaluation of the regression models is given in Table 2. Calibration was carried out using the test set validation. The small number of latent variables (5) and the closeness of calibration and prediction error rates demonstrate that these results were statistically valid. The model showed good percentages of correct classifications. The results indicated that the classes of wines according to the closure OTR and the polyphenolic content may be reasonably considered as separate from each other. The results obtained can be discussed in light of oenological meaning. The two extraction techniques used to get different polyphenol content also played a role in extracting aromatic compounds (free and precursor forms). These two different aromatic potentials were then submitted to different oxygen regimes through different OTR. This has created different oxygen conditions that had modified the "bouquet" of the different wines. It is known that the same wine put under different closures can evolve differently from reduced to oxidised wine.

3.3. Analysis of taste: e-tongue

The response of the array of carbon paste electrodes (CPEs) towards wine samples was analysed. As stated before, curves were pre-treated using kernel functions in order to obtain 10 variables per sensor. This pre-treatment gives rise to a high number of input variables (8 sensors × 10 variables = 80 variables). For this reason, a selection of variables (three variables per sensor) was carried out using the PCA loading plot and selecting those variables with higher correlation loadings. Using this method the total number of variables was reduced to 24. These values were used as the input parameters for PCA pattern recognition analysis. Fig. 3 shows the PCA scores plot of measurements using the electronic tongue. A partial overlapping between the wines studied could be observed; however, a certain degree of discrimination was observed between wines with a high polyphenol content (left side of the plot) and wines with low polyphenol content (right side of the plot). In addition, non-micro-oxygenated wines tend to appear in the upper side of the plot and micro-oxygenated wines appear in the lower part of the plot. The percentage the explained variance was of a 35% for PC1, 18% for PC2 and 11% for PC3.

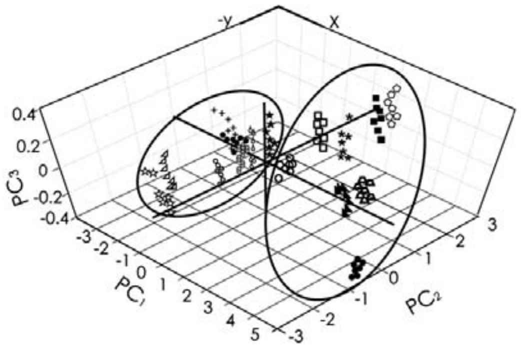


Fig. 1. PCA Scores plot of the CIELab coordinates. PC1, PC2 and PC3 bring the 68%, 31% and 1% of the variance respectively. The samples are marked with the following symbols: W1 (⌓), W2 (✶), W3 (+), W4 (✸), X1 (□), X2 (○), X3 (◿), X4 (☆), Y1 (●), Y2 (◣), Y3 (▯), Y4 (⬡), Z1 (⬠), Z2 (■), Z3 (★), Z4 (△).

PLS-DA models were developed for prediction of the different classes of wines: high and low polyphenol content, micro-oxygenated and not micro-oxygenated wines, and wines bottled with different types of closures. Full cross validation was used in the considered models. The discrimination results of calibration and validation sets are shown in Table 2. When grouped by polyphenolic content, the obtained prediction coefficient was 0.970801 (5 latent variables). The calculated coefficient of prediction was also high 0.933645 (6 latent variables), when classifying micro-oxygenated and not micro-oxygenated wines. In contrast, the capability of the system to classify wines according the closure OTR (6 latent variables) was clearly lower (0.877746) with a residual error of 0.240189.

These results are in good agreement with previously published results where the correlation between the sensors signals and the polyphenolic content of wines and with components with redox properties was demonstrated (Casilli et al., 2005; Parra et al., 2004). These results are also in phase with what was expected from this trial. Flash-Release is a technique known to extract a high amount of tannins. Astringency increases with tannin concentration, degree of polymerisation and galloylation (Vidal et al., 2003). Micro-oxygenation is used to stabilize colour but also to soften tannins (reduce astringency) notably through the formation of new polyphenols known as ethyl-bridged tannins (Atanasova et al., 2002). These wine tannins had been found to be bitter (Vidal et al., 2004). The mouth feel properties are thus depending on the extraction techniques but also to the oxygen treatment during winemaking. The amount of oxygen added through the closures also played a role in modelling wine mouth feel properties even if its effect was lower than those of extraction techniques and micro-oxygenation.

3.4. Sensor fusion: electronic panel

As demonstrated in previous sections each instrument provides a complementary piece of information that contributed to the discrimination of the studied wines. For instance, electronic nose and electronic eye responses are sensitive to the effects caused by the closure OTR. In contrast, the liquid sensory system can discriminate the flavour attributes which are influenced by the presence of compounds with redox activity and by the antioxidant character of wine (polyphenolic content and micro-oxygenation). Signals coming from the three systems were merged in a data matrix formed by six CIELab coordinates (colour), 15 variables extracted from the signals produced by the 16 MOX sensors (odour) and 24 data selected from the voltammetric curves provided by the electrochemical sensors (3 kernel values by sensor) were used as feature vectors for the multivariate analysis. The first principal components, shown in Fig. 4, capture 29%, 19% and 12% of the total variance, respectively. The PCA clusters were small, allowing an

Table 2
PLS-DA prediction models using the different systems.

	Slope		Offset		Correlation		Residual Error	
	Calibration	Prediction	Calibration	Prediction	Calibration	Prediction	Calibration	Prediction
Prediction models using CIELab coordinates								
Polyphenol[a]	0.402	0.383	0.300	0.310	0.634	0.605	0.387	0.398
Mox[a]	0.387	0.369	0.307	0.313	0.622	0.593	0.391	0.403
Closure[a]	0.844	0.843	0.078	0.077	0.919	0.914	0.197	0.204
Prediction models using e-nose								
Polyphenol[a]	0.732	0.702	0.134	0.150	0.855	0.826	0.260	0.282
Mox[a]	0.566	0.526	0.217	0.257	0.753	0.703	0.330	0.356
Closure[a]	0.740	0.720	0.127	0.135	0.860	0.840	0.255	0.272
Prediction models using e-tongue coordinates								
Polyphenol[a]	0.960	0.950	0.020	0.023	0.980	0.971	0.100	0.120
Mox[a]	0.914	0.888	0.043	0.933	0.956	0.933	0.147	0.180
Closure[a]	0.846	0.877	0.077	0.100	0.920	0.877	0.196	0.240
Prediction models using the panel test								
Polyphenol[a]	0.973	0.955	0.013	0.022	0.986	0.980	0.082	0.101
Mox[a]	0.923	0.902	0.039	0.050	0.961	0.943	0.139	0.167
Closure[a]	0.964	0.948	0.018	0.027	0.982	0.973	0.094	0.115

[a] Polyphenol = type of crush (Trad or FR that produces wines with high or low polyphenolic content); Mox = type of micro-oxigentation (Mox or notMox); Closure = type of closure (high or low porosity).

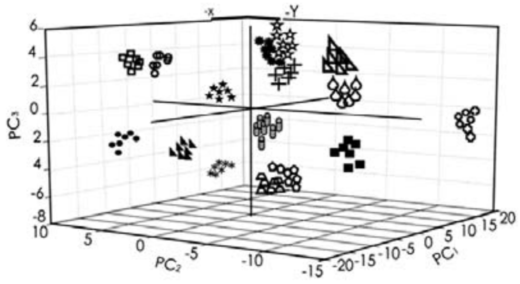


Fig. 2. PCA scores plot illustrating the discrimination between the wines using e-nose. PC1, PC2 and PC3 bring the 68%, 18% and 6% of the variance respectively. The samples are marked with the following symbols: W1 (⏢), W2 (∗), W3 (+), W4 (✹), X1 (□), X2 (○), X3 (◬), X4 (☆), Y1 (●), Y2 (◣), Y3 (▯), Y4 (⬡), Z1 (⬠), Z2 (■), Z3 (★), Z4 (△).

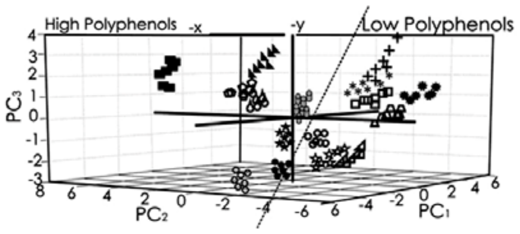


Fig. 3. PCA scores plot illustrating the discrimination between the wines using e-tongue (3 variables per sensor). PC1, PC2 and PC3 bring the 35%, 18% and 11% of the variance respectively.The samples are marked with the following symbols: W1 (⏢), W2 (∗), W3 (+), W4 (✹), X1 (□), X2 (○), X3 (◬), X4 (☆), Y1 (●), Y2 (◣), Y3 (▯), Y4 (⬡), Z1 (⬠), Z2 (■), Z3 (★), Z4 (△).

easy discrimination of wines. This demonstrated that fusion of the three arrays of sensors improved the discrimination of wines. Moreover, the PCA scores plot allows simultaneous discrimination of wines according to the closure OTR, the polyphenol content and the occurrence of micro-oxygenation. In the front part of the graph

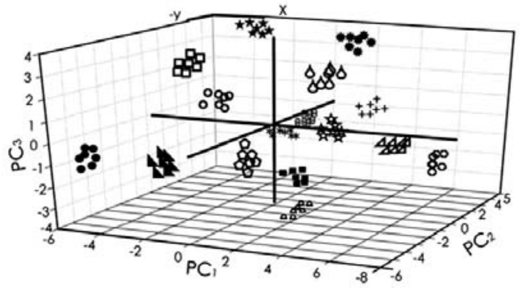
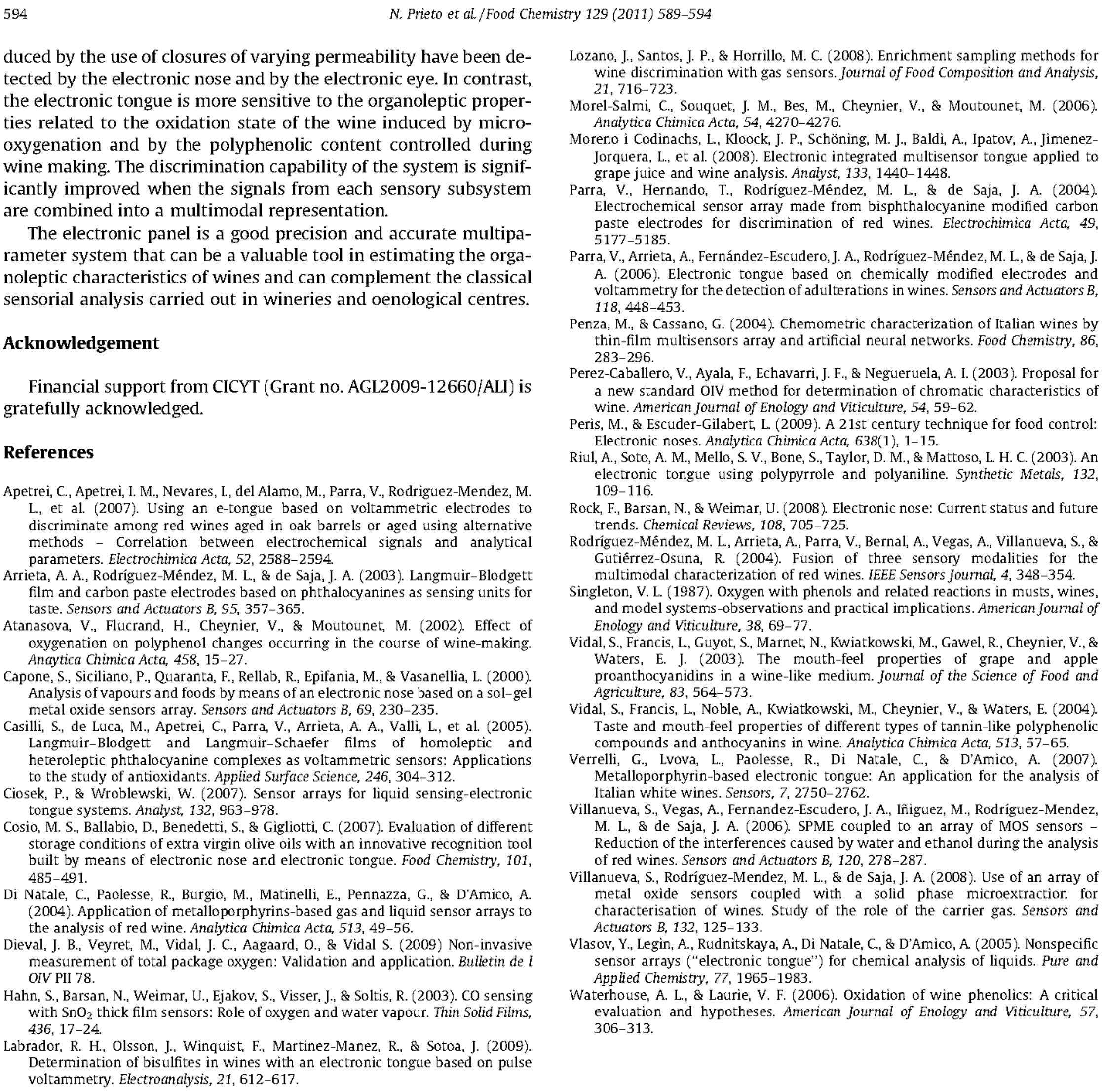


Fig. 4. PCA scores plot illustrating the discrimination between the studied wines using the e-panel. PC1, PC2 and PC3 bring the 29%, 19% and 12% of the variance respectively.The samples are marked with the following symbols: W1 (⏢), W2 (∗), W3 (+), W4 (✹), X1 (□), X2 (○), X3 (◬), X4 (☆), Y1 (●), Y2 (◣), Y3 (▯), Y4 (⬡), Z1 (○), Z2 (■), Z3 (★), Z4 (△).

(negative *X* axis) wines bottled with stoppers of high OTR to the oxygen (wines 1 and 2) could be observed, while wines bottled with low OTR stoppers (wines 4 and 5) appear in the positive *X* axis region (bottom of the image). At the same time clusters corresponding to micro-oxygenated wines (wines X and Y) tend to appear on the right part of the graph (negative *Y* axis), while wines with different polyphenols content appear in well-separated regions of the PCA scatter plot (wines Y and Z on the right side of the graph and wines X and W on the left part of the graph). This means that the combined system could discriminate simultaneously according to the type of closure (thanks to the information provided by the electronic nose and the electronic eye systems) and according to the polyphenol levels (due to the information provided by the electronic tongue). The improvement on the discrimination and prediction capabilities was confirmed by the PLS-DA models shown in Table 2.

4. Conclusions

Wines treated with different amount of oxygen have been successfully analysed using an electronic nose, an electronic tongue and an electronic eye. The changes in the sensory attributes in-



duced by the use of closures of varying permeability have been detected by the electronic nose and by the electronic eye. In contrast, the electronic tongue is more sensitive to the organoleptic properties related to the oxidation state of the wine induced by micro-oxygenation and by the polyphenolic content controlled during wine making. The discrimination capability of the system is significantly improved when the signals from each sensory subsystem are combined into a multimodal representation.

The electronic panel is a good precision and accurate multiparameter system that can be a valuable tool in estimating the organoleptic characteristics of wines and can complement the classical sensorial analysis carried out in wineries and oenological centres.

Acknowledgement

Financial support from CICYT (Grant no. AGL2009-12660/ALI) is gratefully acknowledged.

References

Apetrei, C., Apetrei, I. M., Nevares, I., del Alamo, M., Parra, V., Rodriguez-Mendez, M. L., et al. (2007). Using an e-tongue based on voltammetric electrodes to discriminate among red wines aged in oak barrels or aged using alternative methods – Correlation between electrochemical signals and analytical parameters. *Electrochimica Acta, 52*, 2588–2594.

Arrieta, A. A., Rodríguez-Mêndez, M. L., & de Saja, J. A. (2003). Langmuir–Blodgett film and carbon paste electrodes based on phthalocyanines as sensing units for taste. *Sensors and Actuators B, 95*, 357–365.

Atanasova, V., Flucrand, H., Cheynier, V., & Moutounet, M. (2002). Effect of oxygenation on polyphenol changes occurring in the course of wine-making. *Anaytica Chimica Acta, 458*, 15–27.

Capone, S., Siciliano, P., Quaranta, F., Rellab, R., Epifania, M., & Vasanellia, L. (2000). Analysis of vapours and foods by means of an electronic nose based on a sol–gel metal oxide sensors array. *Sensors and Actuators B, 69*, 230–235.

Casilli, S., de Luca, M., Apetrei, C., Parra, V., Arrieta, A. A., Valli, L., et al. (2005). Langmuir–Blodgett and Langmuir–Schaefer films of homoleptic and heteroleptic phthalocyanine complexes as voltammetric sensors: Applications to the study of antioxidants. *Applied Surface Science, 246*, 304–312.

Ciosek, P., & Wroblewski, W. (2007). Sensor arrays for liquid sensing-electronic tongue systems. *Analyst, 132*, 963–978.

Cosio, M. S., Ballabio, D., Benedetti, S., & Gigliotti, C. (2007). Evaluation of different storage conditions of extra virgin olive oils with an innovative recognition tool built by means of electronic nose and electronic tongue. *Food Chemistry, 101*, 485–491.

Di Natale, C., Paolesse, R., Burgio, M., Matinelli, E., Pennazza, G., & D'Amico, A. (2004). Application of metalloporphyrins-based gas and liquid sensor arrays to the analysis of red wine. *Analytica Chimica Acta, 513*, 49–56.

Dieval, J. B., Veyret, M., Vidal, J. C., Aagaard, O., & Vidal S. (2009) Non-invasive measurement of total package oxygen: Validation and application. *Bulletin de l OIV* PII 78.

Hahn, S., Barsan, N., Weimar, U., Ejakov, S., Visser, J., & Soltis, R. (2003). CO sensing with SnO_2 thick film sensors: Role of oxygen and water vapour. *Thin Solid Films, 436*, 17–24.

Labrador, R. H., Olsson, J., Winquist, F., Martinez-Manez, R., & Sotoa, J. (2009). Determination of bisulfites in wines with an electronic tongue based on pulse voltammetry. *Electroanalysis, 21*, 612–617.

Lozano, J., Santos, J. P., & Horrillo, M. C. (2008). Enrichment sampling methods for wine discrimination with gas sensors. *Journal of Food Composition and Analysis, 21*, 716–723.

Morel-Salmi, C., Souquet, J. M., Bes, M., Cheynier, V., & Moutounet, M. (2006). *Analytica Chimica Acta, 54*, 4270–4276.

Moreno i Codinachs, L., Kloock, J. P., Schöning, M. J., Baldi, A., Ipatov, A., Jimenez-Jorquera, L., et al. (2008). Electronic integrated multisensor tongue applied to grape juice and wine analysis. *Analyst, 133*, 1440–1448.

Parra, V., Hernando, T., Rodríguez-Mêndez, M. L., & de Saja, J. A. (2004). Electrochemical sensor array made from bisphthalocyanine modified carbon paste electrodes for discrimination of red wines. *Electrochimica Acta, 49*, 5177–5185.

Parra, V., Arrieta, A., Fernández-Escudero, J. A., Rodríguez-Mêndez, M. L., & de Saja, J. A. (2006). Electronic tongue based on chemically modified electrodes and voltammetry for the detection of adulterations in wines. *Sensors and Actuators B, 118*, 448–453.

Penza, M., & Cassano, G. (2004). Chemometric characterization of Italian wines by thin-film multisensors array and artificial neural networks. *Food Chemistry, 86*, 283–296.

Perez-Caballero, V., Ayala, F., Echavarri, J. F., & Negueruela, A. I. (2003). Proposal for a new standard OIV method for determination of chromatic characteristics of wine. *American Journal of Enology and Viticulture, 54*, 59–62.

Peris, M., & Escuder-Gilabert, L. (2009). A 21st century technique for food control: Electronic noses. *Analytica Chimica Acta, 638*(1), 1–15.

Riul, A., Soto, A. M., Mello, S. V., Bone, S., Taylor, D. M., & Mattoso, L. H. C. (2003). An electronic tongue using polypyrrole and polyaniline. *Synthetic Metals, 132*, 109–116.

Rock, F., Barsan, N., & Weimar, U. (2008). Electronic nose: Current status and future trends. *Chemical Reviews, 108*, 705–725.

Rodríguez-Mêndez, M. L., Arrieta, A., Parra, V., Bernal, A., Vegas, A., Villanueva, S., & Gutiêrrez-Osuna, R. (2004). Fusion of three sensory modalities for the multimodal characterization of red wines. *IEEE Sensors Journal, 4*, 348–354.

Singleton, V. L. (1987). Oxygen with phenols and related reactions in musts, wines, and model systems-observations and practical implications. *American Journal of Enology and Viticulture, 38*, 69–77.

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E. J. (2003). The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *Journal of the Science of Food and Agriculture, 83*, 564–573.

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V., & Waters, E. (2004). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Analytica Chimica Acta, 513*, 57–65.

Verrelli, G., Lvova, L., Paolesse, R., Di Natale, C., & D'Amico, A. (2007). Metalloporphyrin-based electronic tongue: An application for the analysis of Italian white wines. *Sensors, 7*, 2750–2762.

Villanueva, S., Vegas, A., Fernandez-Escudero, J. A., Iñiguez, M., Rodríguez-Mendez, M. L., & de Saja, J. A. (2006). SPME coupled to an array of MOS sensors – Reduction of the interferences caused by water and ethanol during the analysis of red wines. *Sensors and Actuators B, 120*, 278–287.

Villanueva, S., Rodríguez-Mendez, M. L., & de Saja, J. A. (2008). Use of an array of metal oxide sensors coupled with a solid phase microextraction for characterisation of wines. Study of the role of the carrier gas. *Sensors and Actuators B, 132*, 125–133.

Vlasov, Y., Legin, A., Rudnitskaya, A., Di Natale, C., & D'Amico, A. (2005). Nonspecific sensor arrays ("electronic tongue") for chemical analysis of liquids. *Pure and Applied Chemistry, 77*, 1965–1983.

Waterhouse, A. L., & Laurie, V. F. (2006). Oxidation of wine phenolics: A critical evaluation and hypotheses. *American Journal of Enology and Viticulture, 57*, 306–313.

2.6 ARTÍCULO 5. “Biogenic amines and fish freshness assessment using a multisensory system based on voltammetric electrodes. Comparison between CPE and screen-printed electrodes.”

M.L. Rodríguez-Méndez, M. Gay, C. Apetrei, J.A. de Saja.

Electrochimica Acta 2009. 54, 7033-7041.

2.6.1. Motivación y objetivos.

El presente trabajo surgió como colaboración con la empresa Tencas de Casaseca S.L. (Zamora), empresa dedicada a la cría y distribución de Tencas, con el objetivo de desarrollar un método para evaluar la frescura de sus pescados en función del tiempo de almacenamiento en el frigorífico después su muerte.

Para abordar dicho cometido se propuso realizar un seguimiento de los productos de descomposición emitidos por el pescado (fundamentalmente aminas biógenas) con el paso de los días. Para la monitorización de las aminas biógenas resultantes de la degradación de la materia orgánica se utilizó una red clásica compuesta por CPEs modificados con distintas ftalocianinas como materiales sensibles. Por otra parte, se decidió desarrollar paralelamente una red formada por nuevos electrodos miniaturizados, del tipo screen printed (SPE), que fueron modificados con ftalocianinas.

En este trabajo se persiguió como segundo objetivo el seguimiento de la frescura en muestras reales de pescado con las dos lenguas electrónicas, basadas en CPEs y SPEs, para finalmente comparar los resultados obtenidos con ambas.

2.6.2. **Resumen de los resultados obtenidos.**

i) Los materiales modificadores seleccionados para los dos tipos de sensores, CPE y SPE, han sido tres bisftalocianinas: $LuPc_2$, $GdPc_2$ y $LuPc_2^t$ y una monoftalocianina: CoPc. También se ha incluido en la red un sensor de carbón sin modificar. Con el fin de evaluar la respuesta de las redes de sensores en presencia de aminas biógenas, se han estudiado disoluciones de amoniaco (NH_3), dimetilamina (DMA), trimetilamina (TMA), cadaverina (CAD) e histamina (HIS).

ii) En el caso de los sensores CPEs y SPEs modificados, se han llevado estudios iniciales en KCl con el objetivo de comparar sus características electroquímicas. Aunque las curvas voltamétricas de ambos sensores son similares, existen algunas diferencias entre ellos.

    Cuando se emplean los SPEs modificados se obtienen corrientes de fondo mayores que en el caso de los CPEs, la intensidad de los picos redox asociados al material modificador también es mayor y la ΔE entre los picos ánodicos y cátodicos es también superior que en el caso de los CPEs.

iii) La curva voltamétrica registrada en presencia de aminas presenta los procesos redox relacionados con el material modificador, aquellos relativos a la amina bajo estudio (si es que los presenta) y refleja las interacciones existentes entre el material sensible y la disolución de amina en el cual está inmerso. En el caso del NH_3 se ha observado que los procesos de oxidación del anillo de ftalocianina se dificultan, apareciendo a valores mayores de potencial. La CAD presenta una fuerte interacción con el electrodo, lo que hace imposible la oxidación de la ftalocianina. Por ello no se observa ningún pico redox en la respuesta. En presencia de DMA, TMA o HIS, se observa un pico de oxidación ancho, resultante de la superposición de la oxidación del anillo de ftalocianina y de la oxidación de la amina en cuestión.

iv) Para ambos tipos de sensores, SPE-$LuPc_2$ y CPE-$LuPc_2$ en TMA, se ha observado una dependencia lineal entre la intensidad de los picos redox del modificador y la raíz cuadrada de la velocidad de barrido, indicando que los procesos están controlados por difusión. En el caso del CPE-$LuPc_2$ los picos redox asociados la oxidación del anillo de ftalocianina y de la TMA están mejor resueltos que en el caso de emplear el sensor SPE-$LuPc_2$.

v) Teniendo en cuenta los coeficientes de variación de los picos asociados al material modificador, se ha determinado que la reproducibilidad de los sensores CPEs es mayor que la de los SPEs. En el caso de éstos últimos, los límites de detección alcanzados para diferentes aminas son de un orden de magnitud inferior que los obtenidos en el caso de los CPEs.

vi) Se ha realizado un PCA con las respuestas electroquímicas proporcionadas por los dos tipos de redes de sensores. En ambos casos, CPEs y SPEs existe una clara discriminación de las aminas bajo estudio. El porcentaje de información explicado por el modelo es similar para ambas lenguas electrónicas. En el gráfico de PCA se observa que el cluster asociado al NH_3 aparece separado del resto, mientras que los clusters pertenecientes a las aminas alifáticas aparecen todos en la misma región. Por otra parte, dado el peculiar comportamiento electroquímico de la amina aromática HIS es posible su discriminación con respecto al NH_3 y a las aminas alifáticas, ya que su cluster aparece separado del resto.

vii) El seguimiento de la frescura de los Tencas se ha realizado con las dos redes de sensores, CPEs y SPEs, a través de la monitorización de los productos de descomposición, los cuales incluyen aminas biógenas.

viii) En el caso de emplear un sensor de carbono sin modificar en el seguimiento de la frescura de los Tencas, se ha observado la aparición de un pico redox en la señal voltamétrica después de 2 días de almacenamiento de los peces en el frigorífico. Este pico está asociado a la generación de aminas biógenas, principalmente histamina durante el proceso de degradación. En los días sucesivos, la intensidad del pico redox aumenta, y a partir del séptimo día su intensidad decrece. Más allá del décimo día de almacenamiento no se observa ningún proceso redox. Durante los primeros días de almacenamiento del pescado los niveles de aminas biógenas crecen, y por ello aumenta la intensidad del pico asociado a la histamina. Después de los días 7-10, las aminas biógenas se descomponen y la intensidad de sus picos redox decrece drásticamente.

Cuando se utiliza un sensor modificado con bisftalocianina, se observan respuestas similares. A medida que se produce la descomposición del pescado, los potenciales asociados a la oxidación del anillo de ftalocianina aumentan, así como la intensidad de los mismos. A partir del séptimo día de evolución, se observa como la señal observada se recupera, debido a la descomposición de las aminas. El mismo comportamiento se ha observado cuando se ha empleado un sensor modificado con CoPc como electrodo de trabajo.

ix) Las señales electroquímicas obtenidas durante la evolución del pescado se han tratado mediante PCA con el propósito de evaluar la capacidad de discriminación de ambas redes de sensores en la evolución de la descomposición de los Tencas. El gráfico PCA obtenido con los dos tipos de lenguas electrónicas, CPEs y SPEs, es similar. En ellos se pueden observar clusters diferenciados asociados con cada uno de los días de estudio. El primer cluster corresponde con las muestras analizadas los días 1 y 2 y están asociados al producto fresco. Las muestras estudiadas los días 3 y 4 no han presentado olor y pueden clasificarse también como producto fresco. Los clusters correspondientes a los días 5 y 6 de evolución presentaron olores desagradables, relacionados con la degradación de los peces. Los clusters asociados a los días 7-12 se relacionan con el deterioro del producto. Dicha discriminación se basa en los diferentes patrones electroquímicos observados durante la descomposición del pescado.

x) El modelo PLS-DA muestra valores de correlación altos cuando las clases establecidas son día 0, días 1 y 2, días 3 y 4, días 5 y 6 y días 7-12 de evolución del pescado. Se confirma de esta manera la posibilidad de emplear ambas redes de sensores en el estudio de la degradación del pescado en función del tiempo.

2.6.3. Conclusiones

Se han empleado con éxito dos sistemas multisensoriales basados en CPEs y en sensores miniaturizados, SPEs, en el estudio de aminas biógenas y seguimiento de la frescura del pescado.

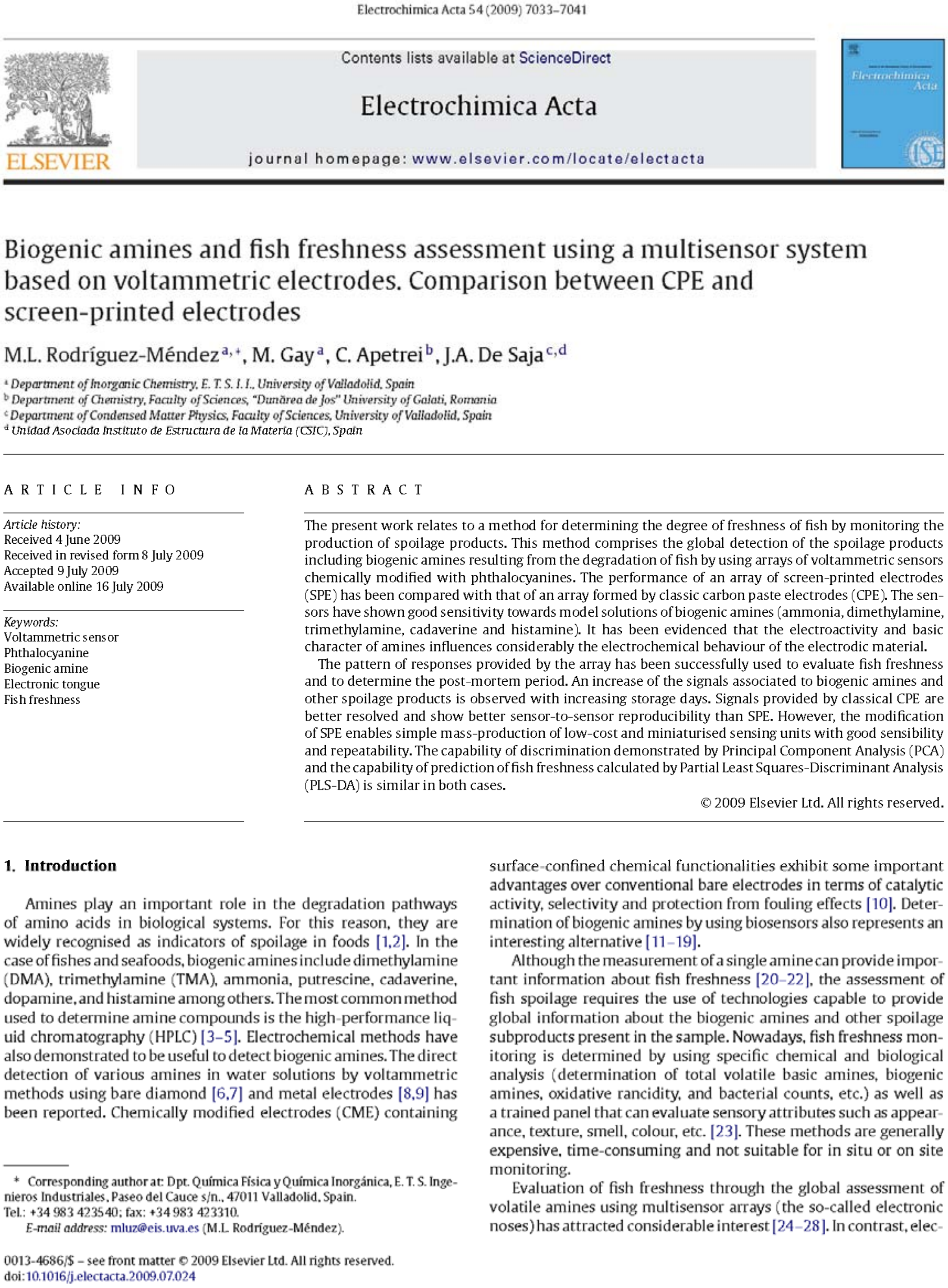
Se ha desarrollado por primera vez una lengua electrónica basada en electrodos SPE modificados con bisftalocianinas. La reproducibilidad de los mismos es menor que la de los sensores CPEs, no obstante proporcionan la ventaja de presentar en el mismo dispositivo los tres electrodos necesarios para un experimento electroquímico.

Por otra parte, debe señalarse que dichos sensores son de un único uso, dado que su superficie no se puede renovar, al contrario que en el caso de los CPE. En el caso de las unidades miniaturizadas, el electrodo de referencia debe considerarse como un pseudoelectrodo, debido a que las características de la disolución en el cual se sumerge pueden afectar a dicho electrodo y en consecuencia modificar su potencial redox.

Las señales electroquímicas obtenidas con los dos tipos de sensores son similares, si bien en el caso de los SPEs las corrientes de fondo observadas son mayores que en el caso de los CPEs. Por otra parte, los potenciales observados en los sensores miniaturizados son ligeramente inferiores que los registrados con los CPEs. Además, las intensidades de los picos redox de los SPEs son de un orden de magnitud superior que las presentadas por los CPEs, alcanzando así límites de detección inferiores en la detección de aminas biógenas.

La discriminación de aminas biógenas por parte de ambas redes de sensores es similar, siendo ambas capaces de distinguir entre NH_3, aminas alifáticas y aminas aromáticas y de monitorizar la evolución de degradación de los peces, comprobándose que la descomposición de los Tencas comienza a partir de los días 5-6 de su almacenamiento en el frigorífico. Dichos resultados se han confirmado mediante el modelo PLS-DA, el cual muestra altos coeficientes de correlación para cada uno de los periodos de descomposición, con valores de pendiente cercanos a 1 y ordenada en el origen próxima a cero, acompañados de errores residuales bajos.

Estos resultados demuestran la posibilidad de emplear redes de sensores voltamétricos basados en CPEs y SPEs modificados con bisftalocianinas en la evaluación de la frescura del pescado a través del estudio de las aminas biógenas emitidas como consecuencia del proceso de descomposición.



Electrochimica Acta 54 (2009) 7033–7041

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

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

M.L. Rodríguez-Méndez[a,*], M. Gay[a], C. Apetrei[b], J.A. De Saja[c,d]

[a] *Department of Inorganic Chemistry, E. T. S. I. I., University of Valladolid, Spain*
[b] *Department of Chemistry, Faculty of Sciences, "Dunărea de Jos" University of Galati, Romania*
[c] *Department of Condensed Matter Physics, Faculty of Sciences, University of Valladolid, Spain*
[d] *Unidad Asociada Instituto de Estructura de la Materia (CSIC), Spain*

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.

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-

---

[*] Corresponding author at: Dpt. Química Física y Química Inorgánica, E. T. S. Ingenieros Industriales, Paseo del Cauce s/n., 47011 Valladolid, Spain. Tel.: +34 983 423540; fax: +34 983 423310.
E-mail address: mluz@eis.uva.es (M.L. Rodríguez-Méndez).

0013-4686/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.electacta.2009.07.024

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 multivariant 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} mol L^{-1}). To improve the conductivity, KCl was added to the solutions (10^{-1} mol L^{-1}). All solutions were prepared with deionized water (18.3 MΩ 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 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 V, by using the setup: f = 15 Hz; E_{sw} = 100 mV; ΔEs = 7 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).

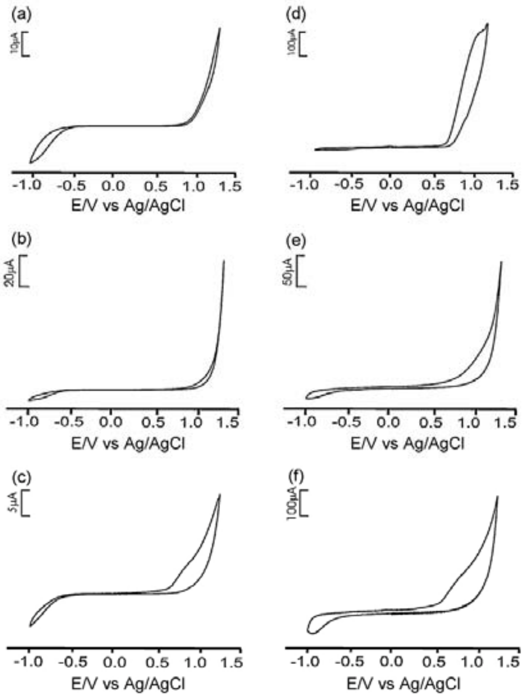


Fig. 1. Cyclic voltammogram of unmodified CPE (left side) and SPE (right side) immersed in 10^{-1} mol L^{-1} (a and d) NH_3; (b and e) CAD; (c and f) HIS. All solutions were prepared in 10^{-1} mol L^{-1} KCl.

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. ($E = 1.24$ V for DMA and $E = 1.03$ 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.

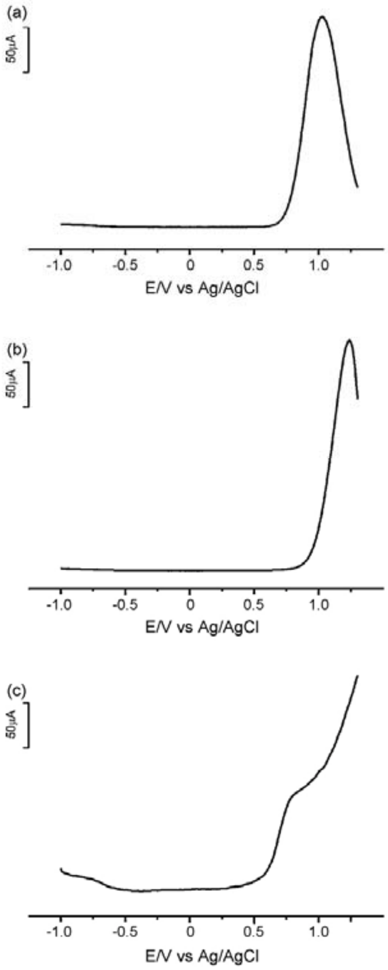


Fig. 2. SWV curves of an unmodified CPE towards 0.1 mol L^{-1} solution of (a) TMA; (b) DMA; (c) HIS. All solutions were prepared in 10^{-1} mol L^{-1} KCl.

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.

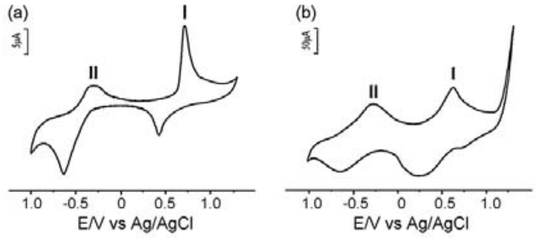


Fig. 3. The responses of a $LuPc_2$-CPE (a) and a $LuPc_2$-SPE (b) immersed in KCl 0.1 mol L^{-1}.

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 $LuPc_2$-CPE and a $LuPc_2$-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} NH_3 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 $LuPc_2$ 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 NH_3 solution.

The responses produced by other biogenic amines are illustrated in Fig. 5 for an electrode based on $GdPc_2$ immersed in the five amines under study. The response towards each amine is different due to the particular interactions occurring between the electrodic 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 an 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 an 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

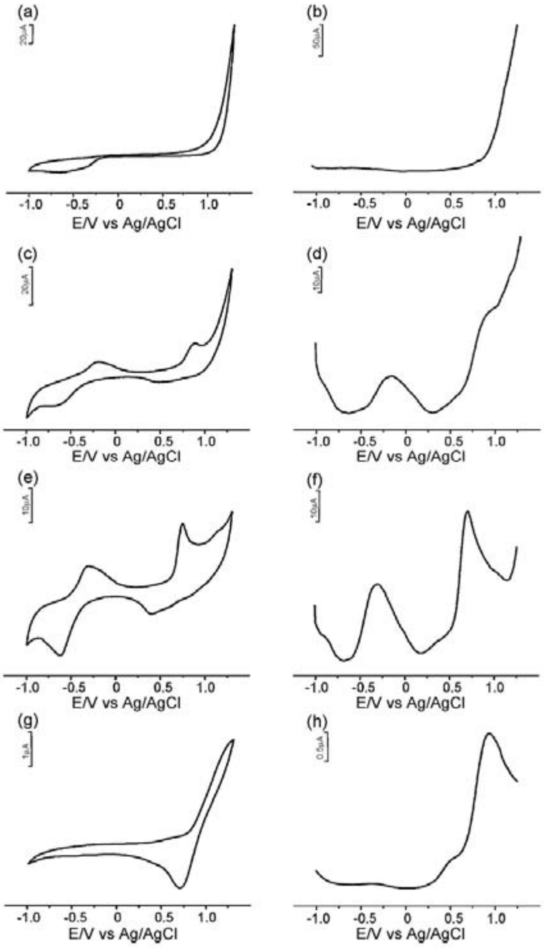


Fig. 4. CV and SWV curves in NH_3 solution 0.1 mol L^{-1} of (a and b) CoPc-CPE; (c and d) $GdPc_2$-CPE; (e and f) $LuPc_2$-CPE; (g and h) $LuPc_2{}^t$-CPE. All solutions were prepared in 10^{-1} mol L^{-1} KCl.

Table 1
Peak potentials of $LuPc_2$ 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

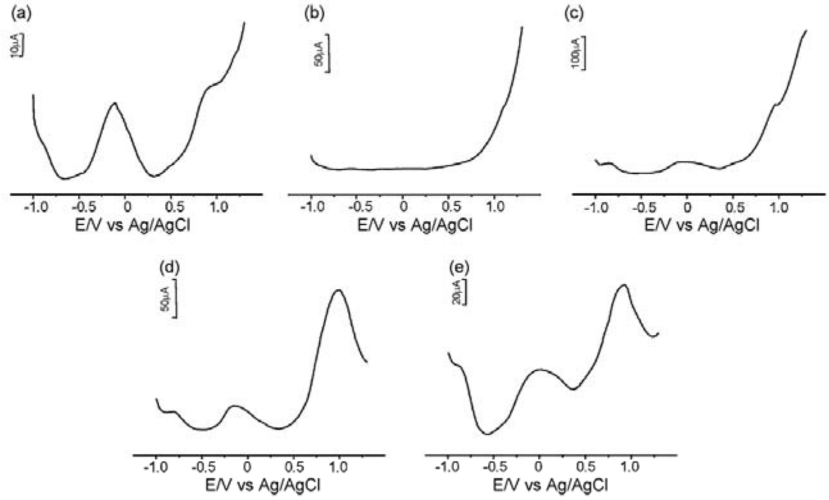


Fig. 5. SWV curves of $GdPc_2$-CPE towards 0.1 mol L^{-1} solution of (a) NH_3; (b) CAD; (c) DMA; (d) TMA; (e) HIS. All solutions were prepared in 10^{-1} mol L^{-1} KCl.

the peak potentials of $LuPc_2$-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 $LuPc_2$-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

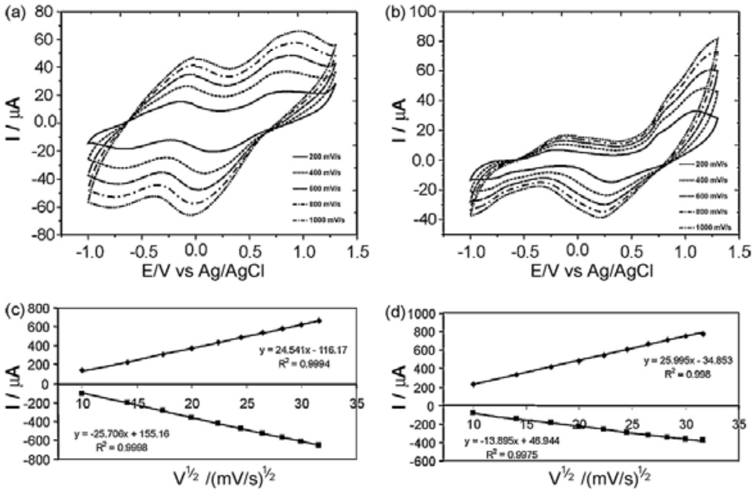


Fig. 6. Cyclic voltammograms of (a) $LuPc_2$-SPE and (b) $LuPc_2$-CPE, respectively, at various scan rates: (200–1000 mV s^{-1}) in 0.1 mol L^{-1} TMA solution. Plot of I_{pa} versus $v^{1/2}$, respectively I_{pc} versus $v^{1/2}$ of (c) $LuPc_2$-SPE and (d) $LuPc_2$-CPE. All solutions were prepared in 10^{-1} mol L^{-1} KCl.

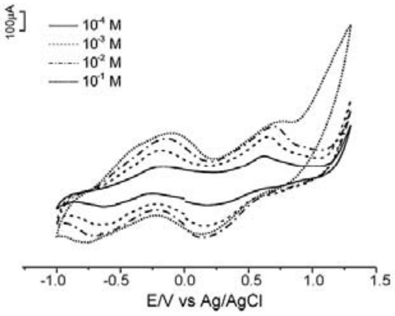


Fig. 7. CVs of a $LuPc_2$-SPE immersed in NH_3 solution with different concentration. All solutions were prepared in 10^{-1} mol L^{-1} KCl.

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 $LuPc_2$ 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 $LuPc_2$ were used to evaluate the detection limit by registering voltammetric measurements in solutions containing increasing amounts of TMA and NH_3 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 $LuPc_2$-SPE immersed in NH_3 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 NH_3 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.

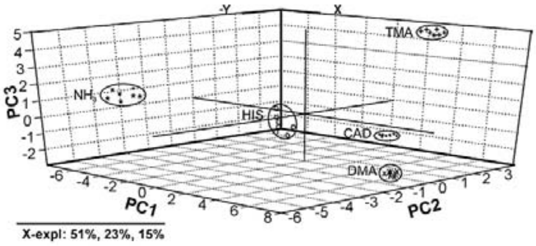


Fig. 8. Scores plot of a PCA on amine using the voltammetric sensors array.

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).

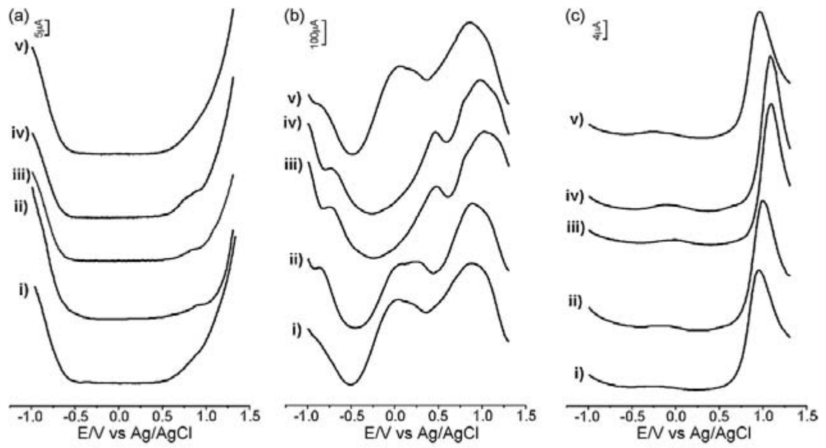


Fig. 9. SWV curves of (a) unmodified-CPE, (b) $GdPc_2$-CPE and (c) $LuPc_2^t$-CPE during storage time. (i) Day 0; (ii) day 4; (iii) day 5; (iv) day 7; (v) day 10.

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 $LuPc_2^t$ (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).

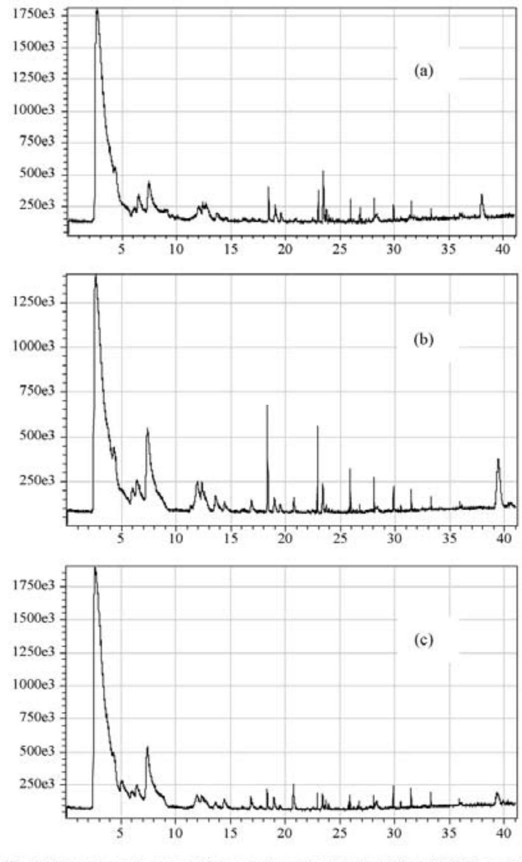


Fig. 10. Gas chromatograms of fish samples collected the (a) first (b) fifth and (c) tenth day after death.

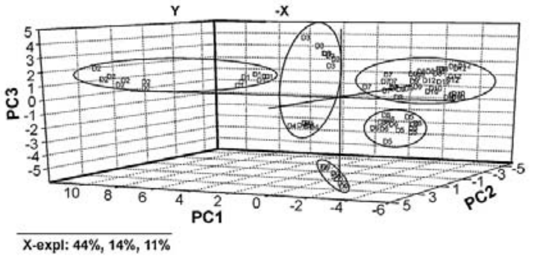


Fig. 11. PCA score plot as a function of time. The days 0, 1–2, 3–4, 5–6, and 7–12 form grouped clusters.

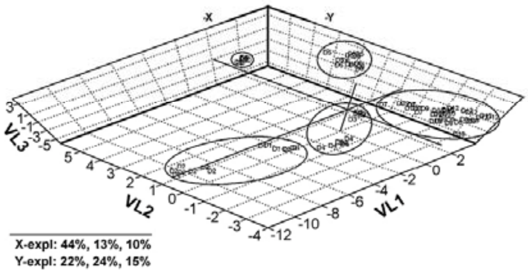


Fig. 12. PLS-DA score plot corresponding to fish freshness monitoring.

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

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 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".

References

[1] H. Yamanaka, Food Rev. Int. 6 (1990) 591.
[2] T.A. Gill, J. Conway, J. Evrovski, Changes in fish muscle proteins at high and low temperature, in: G.J. Flick, R.E. Martin (Eds.), Advances in Seafood Biochemistry-Composition and Quality, Technomic Publishing, Lancaster, Pennsylvania, 1992.
[3] S. Suzuki, K. Kobayashi, J. Noda, T. Suzuki, K. Takama, J. Chromatogr. 508 (1990).
[4] J.C. Hoekstra, D.C. Jonson, Anal. Chim. Acta 390 (1999) 45.
[5] I.G. Casella, M. Gatta, E. Desimoni, Food Chem. 73 (2001) 367.
[6] M.D. Koppang, M. Witek, J. Blau, G.M. Swain, Anal. Chem. 71 (1999) 1188.
[7] B.V. Sarada, T.N. Rao, D.A. Tryk, A. Fujishima, Anal. Chem. 72 (2000) 1632.
[8] J.C. Hoekstra, D.C. Johnson, Anal. Chem. 70 (1998) 83.
[9] D.A. Dobberpuhl, J.C. Hoekstra, D.C. Jonson, Anal. Chim. Acta 322 (1996) 55.
[10] V. Carralero, A. Gonzalez-Cortes, P. Yanez-Sedeno, J.M. Pingarron, Electroanalysis 17 (2005) 289.
[11] G.C. Yen, C.L. Hsieh, J. Food Sci. 56 (1991) 158.
[12] C.X. Xu, S.A.M. Marzouk, V.V. Cosofret, R.P. Back, M.R. Neuman, R.H. Sprinkle, Talanta 44 (1997) 1625.
[13] R. Stevanato, B. Mondovi, S. Sabatini, A. Rigo, Anal. Chim. Acta 273 (1990) 391.
[14] M. Gutiérrez, S. Alegret, M. del Valle, Biosens. Bioelectron. 22 (2007) 2171.
[15] J.M. Hungerford, A.A. Arefyev, Anal. Chim. Acta 261 (1992) 351.
[16] M.K. Alam, M. Sasaki, T. Watanabe, K. Maeyama, Anal. Biochem. 229 (1995) 26.
[17] J. Lange, C. Wittmann, Anal. Bioanal. Chem. 372 (2002) 276.
[18] C. Loechel, A. Basran, J. Basran, N.S. Scrutton, E.A.H. Hall, Analyst 128 (2003) 166.
[19] A. Pacquit, K.T. Lau, H. McLaughlin, J. Frisby, B. Quilty, D. Diamond, Talanta (2006) 515.
[20] M. Niculescu, C. Nistor, I. Frebort, P. Pec, B. Mattiasson, E. Csolregi, Anal. Chem. 72 (2000) 1591.
[21] G.C. Chemnitius, U. Bilitewski, Sens. Actuators B 32 (1996) 107.
[22] K. Mitsubayashi, Y. Kubotera, K. Yano, Y. Hashimoto, T. Kon, S. Nakakura, Y. Nishi, H. Endo, Sens. Actuators B 103 (2004) 463.
[23] G. Olafsdóttir, E. Martinsdóttir, J. Oehlenschläger, P. Dalgaard, B. Jensen, I. Undeland, I.M. Mackie, G. Henehan, J. Nielsen, H. Pilsen, Trends Food Sci. Technol. 8 (1997) 258.
[24] J. Hammond, B. Marquis, R. Michaels, B. Oickle, B. Segee, J. Vetelino, A. Bushway, M.E. Camire, K. Davis-Dentici, Sens. Actuators B 84 (2002) 113.
[25] J.E. Haugen, E. Chanie, F. Westad, R. Jonsdottir, S. Bazzo, S. Labreche, P. Marcq, F. Lundby, G. Olafsdottir, Sens. Actuators B 116 (2006) 72.
[26] C. Di Natale, G. Olafsdottir, S. Einarsson, E. Martinelli, R. Paolesse, A. D'Amico, Sens. Actuators B 77 (2001) 572.
[27] G. Olafsdottir, P. Nesvadba, C. Di Natale, M. Careche, J. Oehlenschlager, S.V. Tryggvadottir, R. Schubring, M. Kroeger, K. Heia, M. Esaiassen, A. Macagnano, B.M. Jorgensen, Trends Food Sci. Technol. 15 (2004) 86.
[28] N. El Barbri, E. Llobet, N. El Bari, X. Correig, B. Bouchikhi, Mater. Sci. Eng. C 28 (2008) 666.
[29] A. Legin, A. Rudnitskaya, B. Seleznev, Yu. Vlasov, Int. J. Food Sci. Technol. 37 (2002) 375.
[30] L. Gil, J.M. Barat, E. Garcia-Breijo, J. Ibanez, R. Martinez-Manez, J. Soto, E. Llobet, J. Brezmes, M.C. Aristoy, F. Tolera, Sens. Actuators B 131 (2008) 362.
[31] L. Gill, J.M. Barat, I. Escriche, E. Garcia-Breijo, R. Martinez-Manez, J. Soto, Sens. Actuators B 131 (2008) 362.
[32] C. Krantz-Rülcker, M. Stenberg, F. Winquist, I. Lundström, Anal. Chim. Acta 426 (2001) 217.
[33] G. Verrelli, L. Francioso, R. Paolesse, P. Siciliano, C. Di Natale, A. D'Amico, A. Logrieco, Sens. Actuators B 123 (2007) 191.
[34] A. Arrieta, M.L. Rodríguez-Méndez, J.A. de Saja, Sens. Actuators B 95 (2003) 357.
[35] A. Gutés, F. Céspedes, S. Alegret, M. del Valle, Talanta 66 (2005) 1187.
[36] C. Apetrei, M.L. Rodríguez-Méndez, V. Parra, F. Gutiérrez, J.A. de Saja, Sens. Actuators B 103 (2004) 145.
[37] V. Parra, A.A. Arrieta, J.A. Fernández-Escudero, H. García, C. Apetrei, M.L. Rodríguez-Méndez, J.A. de Saja, Sens. Actuators B 115 (2006) 54.
[38] C. Apetrei, I.M. Apetrei, I. Nevares, M. del Alamo, V. Parra, M.L. Rodriguez-Mendez, J.A. De Saja, Electrochim. Acta 52 (2007) 2588.
[39] M. Linaje, M.C. Quintanilla, A. Gonzalez, J.L. del Valle, G. Alcaide, M.L. Rodriguez-Mendez, Analyst 125 (2000) 341.
[40] K. Pihel, S. Hsieh, J.W. Jorgenson, R.M. Wightman, Anal. Chem. 67 (1995) 4514.
[41] A. Adenier, M.M. Chehimi, I. Gallardo, J. Pinson, N. Vila, Langmuir 20 (2004) 8243.
[42] Analytical Methods Committee, Analyst 112 (1987) 199.
[43] A.V. Legin, A.M. Rudnitskaya, Y.G. Vlasov, C. Di Natale, A. D'Amico, Sens. Actuators B 58 (1999) 464.

2.7 ARTÍCULO 6. “Films of lutetium bisphthalocyanine nanowires as electrochemical sensors.”

M. Gay Martín, M.L. Rodríguez-Méndez, J.A. de Saja.

Langmuir 2010, 26 (24), 19217-19224.

2.7.1. Motivación y objetivos.

Según la experiencia previa de nuestro Grupo, el empleo de sensores basados en películas delgadas preparadas por la técnica de Langmuir-Blodgett basados en bisftalocianinas ofrece la posibilidad de obtener sensores voltamétricos nanoestructurados con un alto control a nivel molecular. Este tipo de sensores proporciona señales con bajas corrientes de fondo y picos redox con alta simetría, característica asociada a la alta ordenación estructural. Sin embargo, debe señalarse que los sensores LB son de un único uso y los tiempos necesarios para su preparación son excesivamente largos (horas), lo que hace de la técnica LB una herramienta poco competitiva a la hora de producir sensores a escala industrial. Además, la instrumentación requerida para obtener los films LB supone un alto coste económico.

Por otra parte la posibilidad de obtener películas nanoestructuradas de materiales electroactivos con morfología y espesor controlable mediante deposición electroforética (EPD), nos pareció muy atractiva como una posible técnica alternativa de la clásica LB. Con esta filosofía, se prepararon películas delgadas EPD de $LuPc_2$, debido a que hasta la fecha no existía ningún trabajo en el que se hubiera electrodepositado dicha molécula.

El avance alcanzado en esta parte de la investigación se centra en la obtención por primera vez de películas delgadas de bisftalocianina de lutecio mediante la técnica EPD y su aplicación como sensor voltamétrico para la detección de antioxidantes, compuestos clave en la composición de vinos.

2.7.2. Resumen de los resultados obtenidos.

i) La obtención de películas delgadas basadas en $LuPc_2$ mediante la técnica EPD se ha realizado a partir de una disolución clorofórmica de $LuPc_2$ a la cual se ha añadido ácido trifluoroacético (TFA) para protonar la bisftalocianina. En dicha disolución, se han introducido dos electrodos, una placa de platino y un vidrio recubierto de ITO, que actúan como ánodo y cátodo, respectivamente. La aplicación de un determinado voltaje (20V) entre ambos electrodos hace que la bisftalocianina protonada en disolución migre hacia el electrodo correspondiente (ITO) y se reduzca sobre él, dando lugar a una película homogénea de color verde constituida por nanohilos de bisftalocianina.

ii) Tras la preparación, se ha realizado una caracterización microscópica y espectroscópica, con el fin de determinar la morfología y características estructurales de las películas.

El espectro UV-Vis de la película EPD es similar al de la disolución, excluyendo de esta manera cualquier tipo de proceso de polimerización durante la electrodeposición. El espectro UV-Vis de la película muestra bandas más anchas, característica del estado sólido. La banda Q aparece a mayores longitudes de onda en estado sólido que en disolución, indicando la existencia de agregados tipo J. Además, se ha observado que existe una relación lineal entre la absorbancia de la película y el tiempo de deposición, lo que demuestra que la cantidad de compuesto depositado sobre el ITO es mayor a medida que aumenta el tiempo. Por otra parte, en el NIR se observa una banda ancha a 1424nm, asociada a una transferencia de carga intramolecular, donde uno de los anillos de ftalocianina (dianión) actúa como dador de electrones y el otro (anión radical) como aceptor. La presencia de esta banda confirma de nuevo que la $LuPc_2$ se encuentra en estado neutro.

iii) Las imágenes SEM y AFM muestran que las películas están formadas por nanohilos, cuyo diámetro y longitud dependen de las condiciones experimentales. El aumento del tiempo de deposición o el tratamiento térmico (se comentará posteriormente), produce una coalescencia entre hilos que da lugar a nanohilos con diámetros mayores y estructuras ramificadas tipo V.

iv) La difracción de rayos X (DRX) muestra dos picos, a $2\theta = 29.60$ y $2\theta = 6.97$, asociados con una disposición en la que las moléculas se disponen con interacción cofacial dentro de los nanohilos (d = 3.01Å) y con una disposición tipo herringbone (d = 12.67Å), en estructuras tipo V. Después del tratamiento térmico, el pico a $2\theta = 6.97$ aparece con mayor intensidad, por lo que se puede concluir que debido al calentamiento del film, se forman mayor número de estructuras tipo V.

Además, se ha comprobado que el pico a $2\theta = 6.97$ (d = 12.67Å) aumenta de intensidad cuando el tiempo de deposición aumenta, debido a que a mayores tiempos existen más estructuras tipo V.

v) Los estudios electroquímicos demuestran que para aumentar la adhesión del material al sustrato y obtener respuestas electroquímicas reproducibles es necesario realizar un tratamiento térmico previo. Todos los estudios electroquímicos se han realizado con las películas tratadas térmicamente. Empleando la película EPD-$LuPc_2$ como electrodo de trabajo en KCl se ha observado que el perfil voltamétrico es similar al obtenido empleando sensores LB, si bien existen algunas diferencias relacionadas con la organización molecular de los mismos. En el caso de los sensores LB, los picos redox que se observan son simétricos, mientras que en el caso de los sensores EPD, el pico anódico presenta un pequeño hombro, relacionado con la presencia de distintos entornos en la película delgada (asociación cofacial y estructuras tipo V).

vi) Se ha estudiado la respuesta frente a disoluciones de electrolitos simples tales como KCl, $MgCl_2$, $KClO_4$, HCl y NaOH, con el fin de explorar la respuesta electroquímica del sensor EPD. Los perfiles voltamétricos observados revelan que para cada electrolito se obtiene una respuesta distinta y específica.

vii) Por último, se ha estudiado el ácido cafeico como ejemplo de antioxidante presente en la industria alimentaria. El sensor EPD-$LuPc_2$ inmerso en una disolución de dicho antioxidante presenta dos tipos de señales; por una parte los picos redox asociados con la electroactividad de los nanohilos de $LuPc_2$ y por otra parte los asociados a los procesos redox del antioxidante. Además, se ha observado un efecto electrocatalítico de los nanohilos, el cual favorece la oxidación del ácido cafeico en comparación con el sustrato de ITO virgen. Por su parte, el antioxidante, dificulta la oxidación de la $LuPc_2$, haciendo que ésta se produzca a mayores potenciales. El límite de detección alcanzado por el sensor EPD-$LuPc_2$ es de 10^{-5}M, el cual está en el rango en cual dicho compuesto está presente en la mayoría de los alimentos.

2.7.3. Conclusiones

En el presente trabajo se han preparado por primera vez películas delgadas basadas en $LuPc_2$ mediante la técnica de deposición electroforética y se ha estudiado su viabilidad como sensores voltamétricos.

Las técnicas espectroscópicas han permitido determinar la estructura de este tipo de películas. Se ha demostrado que las moléculas se disponen con los anillos aromáticos en disposición cofacial, existiendo además estructuras tipo herringbone.

Las técnicas microscópicas han demostrado que la morfología de las películas está basada en nanohilos, cuyo diámetro y longitud depende de los parámetros experimentales.

Los experimentos de voltametría cíclica han demostrado que las películas EPD-$LuPc_2$ pueden emplearse como sensores voltamétricos, debido su reproducibilidad y estabilidad. La respuesta voltamétrica es similar a la que presentan los sensores LB con algunas diferencias en cuanto a la posición, intensidad y forma de los picos redox, asociada a la diferente estructura de cada una de las películas delgadas. La respuesta voltamétrica de las películas EPD-$LuPc_2$ está íntimamente relacionada con el electrolito en el cual se sumerge la película. En el caso del ácido cafeico, se ha observado un efecto electrocatalítico en comparación con un electrodo de ITO sin recubrir.

La deposición electroforética es una técnica que permite obtener de forma reproducible sensores nanoestructurados con un coste bajo y empleando tiempos de preparación mucho menores que en el caso de los sensores LB. Dichas características hacen de la EPD una técnica muy competitiva a escala industrial.

Langmuir
Article
pubs.acs.org/Langmuir

Films of Lutetium Bisphthalocyanine Nanowires As Electrochemical Sensors

Mónica Gay Martín,[†] Maria Luz Rodríguez-Méndez,*[,†] and Jose Antonio de Saja[‡]

[†]*Inorganic Chemistry Department, ETS Ingenieros Industriales and* [‡]*Condensed Matter Physics Department, Faculty of Science, University of Valladolid, 47011 Valladolid, Spain*

Received June 26, 2010. Revised Manuscript Received November 3, 2010

Lutetium bisphthalocyanine ($LuPc_2$) nanowires have been successfully obtained by electrophoretic deposition (EPD). The influence of the deposition conditions and annealing in the structure of the films has been studied by AFM, SEM, X-ray diffraction (XRD), UV−vis absorption, and near-infrared (NIR). The electrochemical properties of the EDP films immersed in different electrolytic solutions (KCl, $MgCl_2$, $KClO_4$, HCl, and NaOH) indicate that anions diffuse inside the film to maintain the electroneutrality and the kinetics follows the Randles-Sevcik equation. The stability of the response increases strongly upon annealing due to the improvement of the adhesion of the sensitive material to the substrate. The EPD films have been successfully used to detect caffeic acid (an antioxidant of interest in the food industry). The anodic peak associated with the oxidation of caffeic acid appears at 0.54 V and is linearly dependent on the caffeic acid concentration in the 6×10^{-5} M to 5×10^{-4} M range with a detection limit of 3.12×10^{-5} M. The electrochemical behavior of the annealed $LuPc_2$ EPD films is similar to that observed using Langmuir−Blodgett (LB) nanostructured films. However, the different molecular organization of the molecules inside the film causes differences in the shape and position of the peaks. Although $LuPc_2$ sensors prepared with both EPD and LB techniques provide stable and reproducible responses, the use of EPD is preferred for real sensing applications because of its lower cost, shorter preparation time, and longer lifetime.

1. Introduction

Phthalocyanine compounds are among the most interesting materials for sensing applications because their optical, electronic, and electrochemical properties can be modified under different conditions.[1−3] The electrochemical behavior of electrodes chemically modified with phthalocyanines has been exploited to construct potentiometric or amperometric sensors for the analysis of a variety of liquids.[4−6] The control of the structure at the nanometric level (size, orientation, alignment, thickness, etc.) is an important tool used to modulate the sensor response. Nanostructured thin films have shown great potential in improving the sensitivity and reliability of chemical sensors.[7,8]

Typical methods used to fabricate nanostructured phthalo cyanine films include drop-cast,[9] self-assembly monolayer,[10] self-assembly multilayer (or layer-by-layer),[11−13] the Langmuir−Blodgett technique,[14−17] and the thermal vacuum evaporation technique.[18] Compared with these methods, which are accomplished at high cost and long experimental time, electrophoretic deposition (EPD) can be an alternative way to prepare thin films with low cost, simple apparatus, little restriction of the shape of substrate and with a short formation time. EPD has recently been used to deposit unique nanostructured films consisting of nanowires of metallophthalocyanines such as copper phthalocyanine (CuPc)[19−22] or aluminum phthalocyanine (AlPc).[23] Studies of their morphology and their optical properties have been done. Attempts have also been made to prepare nanowires of gadolinium and europium double-decker phthalocyanines.[24−26]

Lanthanide bisphthalocyanines ($LnPc_2$) are of special interest as sensitive materials due to their rich electrochemistry, which is

---

*Corresponding author. Phone: +34 983423540. E-mail: mluz@eis.uva.es.

(1) Kadish, K. M.; Smith, K. M.; Guilard, R. *The Porphyrin Handbook*; Academic Press: San Diego, 2000; Vols. *1−10*.

(2) Giancane, G.; Guascito, M. R.; Malitesta, C.; Mazzotta, E.; Picca, R. A.; Valli, L. *J. Porphyrins Phthalocyanines* **2009**, *13*, 1129–1139.

(3) Jiang, J.; Kasuga, K.; Arnold, D. P. In *Supramolecular Photoactive and electroactive materials*, Nalwa, H. S., Eds.; Academic Press: New York, 2001; p 113.

(4) Ou, Z. P.; Jiang, Z.; Chen, N. S.; Huang, J.; Shen, J.; Kadish, K. M. *J. Porphyrins Phthalocyanines* **2008**, *12*, 1123–1133.

(5) De Saja, J. A.; Rodríguez-Méndez, M. L. *Adv. Colloid Interface Sci.* **2005**, *116*, 1–11.

(6) Damos, F. S.; Luz, R. C. S.; Tanaka, A. A.; Kubota, L. T. *Anal. Chim. Acta* **2010**, *664*, 144–150.

(7) Rodríguez-Méndez, M. L. In *Encyclopedia of Sensors*, Grimes, C. S.; Dickey, E. C.; Pishko, M. V., Eds.; American Scientific Publishers: New York, 2006; Vol *9*, p 111.

(8) Penza, M.; Rossi, R.; Alvisi, M.; Signore, M. A.; Serra, E.; Paolesse, R.; D'Amico, A.; Di Natale, C. *Sens. Actuators, B* **2010**, *144*, 387–394.

(9) Yang, Z.-Y.; Gan, L.-H.; Lei, S.-B.; Wan, L.-J.; Wang, C.; Jiang, J.-Z. *J. Phys. Chem. B.* **2005**, *109*, 19859–19865.

(10) Matemadombo, F.; Durmus, M.; Togo, C.; Limson, J.; Nyokong, T. *Electrochim. Acta* **2009**, *54*, 5557–5565.

(11) Santos, A. C.; Zucolotto, V.; Constantino, C. J. L.; Cunha, H. N.; Dos Santos, J. R., Jr; Eiras, C. *J. Solid State Electrochem.* **2007**, *11*, 1505–1510.

(12) Alessio, P.; Rodríguez-Méndez, M. L.; De Saja Sáez, J. A.; Constantino, C. J. L. *Phys. Chem. Chem. Phys.* **2010**, *12*, 3972–3983.

(13) Zhao, W.; Tong, B.; Pan, Y.; Shen, J.; Zhi, J.; Shi, J.; Dong, Y. *Langmuir* **2009**, *25*, 11796–11801.

(14) Arrieta, A.; Rodríguez-Méndez, M. L.; De Saja, J. A. *Sens. Actuators, B* **2003**, *95*, 357–365.

(15) Rodríguez-Méndez, M. L.; De Saja, J. A. *J. Porphyrins Phthalocyanines* **2009**, *13*, 606–615.

(16) Gorbunova, Y.; Rodríguez-Méndez, M. L.; Kalashnikova, I. P.; Tomilova, L.; De Saja, J. A. *Langmuir* **2001**, *17*, 5004–5010.

(17) Liu, Y. L.; Wang, W. J.; Gao, X. X.; Li, S. H.; Li, Y. *Spectrosc. Spect. Anal.* **2008**, *28*, 2375–2379.

(18) Rodríguez-Méndez, M. L.; Aroca, R.; De Saja, J. A. *Spectrochim. Acta* **1993**, *49A*, 965–973.

(19) Yamanouchi, H.; Irie, K.; Saji, T. *Chem. Lett.* **2000**, *29*, 10–11.

(20) Takada, M.; Yoshioka, H.; Tada, H.; Matsushige, K. *Jpn. J. Appl. Phys.* **2002**, *41*, L73–L75.

(21) Xu, H.-B.; Chen, H.-Z.; Xu, W.-J.; Wang, M. *Chem. Phys. Lett.* **2005**, *412*, 294–298.

(22) Tong, W. Y.; Djurisic, A. B.; Ng, A. M. C.; Chan, W. K. *Thin Solid Films* **2007**, *515*, 5270–5274.

(23) Yang, Z.; Pu, H. *Mater. Chem. Phys.* **2005**, *94*, 202–206.

(24) Chen, H.-Z.; Cao, L.; Zhou, H.-B.; Rong, Y.; Wang, M. *J. Cryst. Growth* **2005**, *281*, 530–537.

(25) Bai, R.; Shi, M. M.; Ouyang, M.; Cheng, Y.; Zhou, H.; Yang, L.; Wang, M.; Chen, H. *Thin Solid Films* **2009**, *517*, 2099–2105.

(26) Bai, R.; Chen, H.-Z.; Zhou, H.-B.; Shi, M.-M.; Wang, M. *J. Cryst. Growth* **2005**, *285*, 183–190.

Langmuir **2010,** *26(24)*, 19217–19224 **DOI:** 10.1021/la1025894

related to the accessibility of a range of oxidation states centered on the ligand.[15,27−30] Voltammetric sensors based on Langmuir−Blodgett (LB) films of a number of bisphthalocyanine derivatives have been prepared. The electrodes show distinct responses when immersed in different solutions, and this is the basis of their use as electrochemical sensors.[14,31,32] It has been demonstrated that nanostructured $LnPc_2$ LB films show faster kinetics and better reproducibility than non-nanostructured electrodes,[14] and these properties make them more suitable for sensing applications. However, the high cost and long time needed to prepare LB electrodes are major constraints for practical applications.

The objective of this work is to obtain and characterize nanowires of a lanthanide bisphthalocyanine containing lutetium as the central metal atom ($LuPc_2$) using electrophoretic deposition (EPD) and to analyze the sensing properties of the obtained film. Therefore, films have been generated under different electrochemical conditions. After the structural characterization, the films have been employed as the working electrode in cyclic voltammetry experiments. The electrochemical responses toward different ionic solutions and the kinetics of the responses have been analyzed and compared with that observed in nanostructured LB films. Finally, the electrodes have been used to detect caffeic acid (an antioxidant of great interest in the food industry), and their sensing properties have been evaluated.

2. Materials and Methods

The $LuPc_2$ (with a sandwich structure) was synthesized as previously reported.[27,33] The lutetium bisphthalocyanine was submitted for elemental analysis and the results obtained for $LuC_{64}H_{32}N_{16}$ were C, 64.06%; H, 2.68%; N, 18.68%; and C, 64.07%; H, 2.70%; N, 18.70% for calculated and found, respectively. According to previous works,[33] the IR (KBr) spectra obtained for the $LuPc_2$ shows the main peaks at 724 cm^{-1}, 1115 cm^{-1}, 1320 cm^{-1}, 1450 cm^{-1}, and 1516 cm^{-1}. The ^1H NMR of the lutetium bisphthalocyanine (dissolved in $CDCl_3$) is included in the Supporting Information. The chemical shifts obtained were δ = 9.20 ppm (atom a, 1H), δ = 7.95 ppm (atom d, 1H), δ = 7.89 ppm (atom c, 1H), and δ = 7.74 ppm (atom b, 1H). All these experiments confirm the purity of the $LuPc_2$. 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 consisted of a lutetium bisphthalocyanine (10^{-4} M) and trifluoroacetic acid (0.06M) mixture diluted in chloroform. The electrodes were kept at a constant distance (1 cm) and a direct current density of 20 $V.cm^{-1}$ was applied between the two electrodes. $LuPc_2$ films were prepared at three different deposition times: 40 s, 60 s, and 180 s. After deposition, films were annealed at 150 °C for 1 h (at atmospheric pressure) in order to improve their electrochemical stability.

The Langmuir−Blodgett (LB) films were prepared in a KSV 2000 LB trough equipped with a Wilhelmy plate to measure the surface pressure. Arachidic acid was used for reducing the rigidity of the Langmuir films. A mixture of bisphthalocyanine and arachidic acid (1:1) was dissolved in chloroform (10^{-4} M) and spread onto ultrapure water, which was kept at a constant temperature (20 °C). The floating molecules were compressed at a speed of 5 $mm.min^{-1}$, with a surface pressure of 40 $mN \cdot m^{-1}$; LB films were deposited onto ITO glass substrates, with a substrate speed of 3 $mm.min^{-1}$. A Z deposition type was observed. Under these conditions, 20 ML films were obtained.

UV−vis absorption spectra were recorded using a Shimadzu UV-1603 spectrophotometer. NIR transmittance spectra were carried out in a Nicolet Magna-IR 760 spectrophotometer. AFM images were registered in tapping mode using a microscope from Digital Instruments (multimode MMAFM-2). SEM images were recorded in a Jeol JSM-S820. XRD data were obtained using a Philips PW1830 diffractometer. ^1H NMR spectrum was recorded with a Bruker Avance 400 Ultrashield instrument and was referred to TMS. Elemental analysis was performed with a Perkin-Elmer 2400B microanalyzer. Electrochemical measurements were carried out using a Parstat 2273 potentiostat (EG&G) using a conventional three electrode cell. The $LuPc_2$ nanowires deposited on the ITO were used as the working electrode, a platinum sheet was used as the counter electrode and a Ag/AgCl electrode was used as the reference electrode. Cyclic voltammograms were registered in 0.1 M aqueous solutions of KCl, $MgCl_2$, $KClO_4$, HCl, and NaOH from −1.0 to 1.3 V (except in the case of HCl, which was from −0.5 to 1.3 V), at a scan rate of 0.05 $V.s^{-1}$. Caffeic acid (5×10^{-4} M) was dissolved in a solution of 12% (v/v) ethanol and 0.033 M L-tartaric acid, pH 3.6. The voltammograms were recorded from −0.5 to 1.3 V (vs Ag/AgCl). For the detection limit studies, the caffeic acid concentrations used were 6×10^{-5} M, 1×10^{-4} M, 3×10^{-4} M, and 5×10^{-4} M.

3. Results and Discussion

3.1. Film Morphology of $LuPc_2$ Electrodeposited Films.

EPD films were prepared at deposition times of 40, 60, and 180 s. Initially, the UV−vis spectra of the prepared films showed a purple color (maximum at 520 nm) that corresponded to the protonated species.[7,25−27] After preparation, the films were washed with deionized water. Then, spectra were recorded every 2.5 min (Figure 1a). It could be observed that the neutral green species (with a maximum at 667 nm) was progressively formed. After 30 min, the formation of the green form was completed.

When the green neutral form was obtained, the spectra were similar to those recorded in chloroform solutions with an intense Q-band at 663, 666, and 667 nm for films prepared at 40, 60, and 180 s, respectively (Figure 1b). The similarity to the spectra registered in solution (Q-band at 659 nm) excluded the occurrence of polymerization processes. According to the exciton theory,[35] the observed bathochromic shift of the Q-band can be associated with the formation of J-aggregates. This result is in good agreement with the UV−vis spectra previously published of $LuPc_2$ LB films where the formation of J-aggregates has been demonstrated.[36] In such films, the molecules are arranged with their main axis perpendicular to the substrate.[37,38]

The films were then annealed (1 h, 150 °C, atmospheric pressure) to improve the electrochemical stability (see Electrochemical Results section). The thermal treatment caused a drastic

---

(27) Tomilova, L. G.; Chernykh, E. V.; Gavrilov, V. I.; Shelepin, I. V.; Derkacheva, V. M.; Luk'yanets, E. A. *Zh. Obshch. Khim.* **1982**, *52*, 2606.

(28) Bouvet, M.; Parra, V.; Locatelli, C.; Xiong, H. *J. Porphyrins Phthalocyanines* **2009**, *13*, 84 91.

(29) Acikbas, Y.; Evyapan, M.; Ceyhan, T.; Çapan, R.; Bekaroglu, O. *Sens. Actuators, B* **2009**, *135*, 426 429.

(30) Selector, S. L.; Arslanov, V. V.; Gorbunova, Y. G.; Raitman, O. A.; Sheinina, L. S.; Birin, K. P. *J. Porphyrins Phthalocyanines* **2008**, *12*, 1154 1162.

(31) Apetrei, C.; Rodríguez-Méndez, M. L.; Parra, V.; Gutierrez, F.; De Saja, J. A. *Sens. Actuators, B* **2004**, *103*, 145 152.

(32) Casilli, S.; De Luca, M. A.; Apetrei, C.; Parra, V.; Arrieta, A.; Valli, L.; Jiang, J.; Rodríguez-Méndez, M. L.; De Saja, J. A. *Appl. Surf. Sci.* **2005**, *246*, 304 313.

(33) Linaje, M.; Quintanilla, M. C.; González, A.; Del Valle, J. L.; Alcaide, G.; Rodriguez-Méndez, M. L. *Analyst* **2000**, *125*, 341 346.

(34) Rodríguez-Méndez, M. L.; Aroca, R.; De Saja, J. A. *Spectrochim. Acta* **1993**, *49A*, 965 973.

(35) Mc Rae, E. G.; Kasha, M. In *Physical Processes in Radiation Biology*, Augenstein, L.; Manson, R.; Rosenberg, B., Eds.; Academic Press: New York, 1964.

(36) Rodríguez-Méndez, M. L.; Gorbunova, Y.; De Saja, J. A. *Langmuir* **2002**, *18*, 9560 9565.

(37) Moreira, W. C.; Aroca, R. *Spectrochim. Acta* **1995**, *51*, 2325 2334.

(38) Souto, J.; de Saja, J. A.; Aroca, R.; Rodríguez-Méndez, M. L. *Synth. Met.* **1993**, *54*, 229 235.

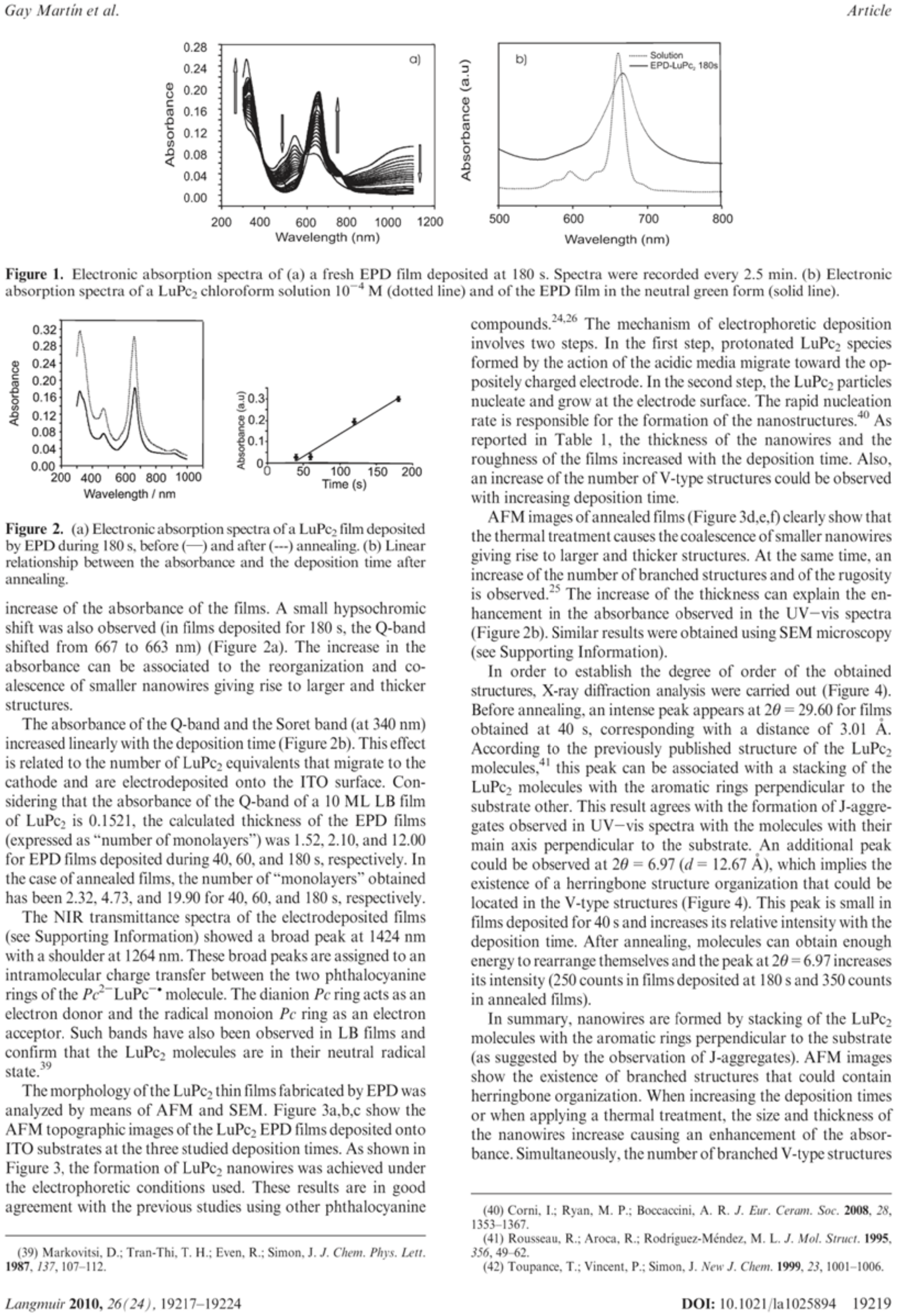


Figure 1. Electronic absorption spectra of (a) a fresh EPD film deposited at 180 s. Spectra were recorded every 2.5 min. (b) Electronic absorption spectra of a $LuPc_2$ chloroform solution 10^{-4} M (dotted line) and of the EPD film in the neutral green form (solid line).

Figure 2. (a) Electronic absorption spectra of a $LuPc_2$ film deposited by EPD during 180 s, before (—) and after (---) annealing. (b) Linear relationship between the absorbance and the deposition time after annealing.

increase of the absorbance of the films. A small hypsochromic shift was also observed (in films deposited for 180 s, the Q-band shifted from 667 to 663 nm) (Figure 2a). The increase in the absorbance can be associated to the reorganization and coalescence of smaller nanowires giving rise to larger and thicker structures.

The absorbance of the Q-band and the Soret band (at 340 nm) increased linearly with the deposition time (Figure 2b). This effect is related to the number of $LuPc_2$ equivalents that migrate to the cathode and are electrodeposited onto the ITO surface. Considering that the absorbance of the Q-band of a 10 ML LB film of $LuPc_2$ is 0.1521, the calculated thickness of the EPD films (expressed as "number of monolayers") was 1.52, 2.10, and 12.00 for EPD films deposited during 40, 60, and 180 s, respectively. In the case of annealed films, the number of "monolayers" obtained has been 2.32, 4.73, and 19.90 for 40, 60, and 180 s, respectively.

The NIR transmittance spectra of the electrodeposited films (see Supporting Information) showed a broad peak at 1424 nm with a shoulder at 1264 nm. These broad peaks are assigned to an intramolecular charge transfer between the two phthalocyanine rings of the $Pc^{2-}LuPc^{-\bullet}$ molecule. The dianion *Pc* ring acts as an electron donor and the radical monoion *Pc* ring as an electron acceptor. Such bands have also been observed in LB films and confirm that the $LuPc_2$ molecules are in their neutral radical state.[39]

The morphology of the $LuPc_2$ thin films fabricated by EPD was analyzed by means of AFM and SEM. Figure 3a,b,c show the AFM topographic images of the $LuPc_2$ EPD films deposited onto ITO substrates at the three studied deposition times. As shown in Figure 3, the formation of $LuPc_2$ nanowires was achieved under the electrophoretic conditions used. These results are in good agreement with the previous studies using other phthalocyanine compounds.[24,26] The mechanism of electrophoretic deposition involves two steps. In the first step, protonated $LuPc_2$ species formed by the action of the acidic media migrate toward the oppositely charged electrode. In the second step, the $LuPc_2$ particles nucleate and grow at the electrode surface. The rapid nucleation rate is responsible for the formation of the nanostructures.[40] As reported in Table 1, the thickness of the nanowires and the roughness of the films increased with the deposition time. Also, an increase of the number of V-type structures could be observed with increasing deposition time.

AFM images of annealed films (Figure 3d,e,f) clearly show that the thermal treatment causes the coalescence of smaller nanowires giving rise to larger and thicker structures. At the same time, an increase of the number of branched structures and of the rugosity is observed.[25] The increase of the thickness can explain the enhancement in the absorbance observed in the UV−vis spectra (Figure 2b). Similar results were obtained using SEM microscopy (see Supporting Information).

In order to establish the degree of order of the obtained structures, X-ray diffraction analysis were carried out (Figure 4). Before annealing, an intense peak appears at $2\theta = 29.60$ for films obtained at 40 s, corresponding with a distance of 3.01 Å. According to the previously published structure of the $LuPc_2$ molecules,[41] this peak can be associated with a stacking of the $LuPc_2$ molecules with the aromatic rings perpendicular to the substrate other. This result agrees with the formation of J-aggregates observed in UV−vis spectra with the molecules with their main axis perpendicular to the substrate. An additional peak could be observed at $2\theta = 6.97$ ($d = 12.67$ Å), which implies the existence of a herringbone structure organization that could be located in the V-type structures (Figure 4). This peak is small in films deposited for 40 s and increases its relative intensity with the deposition time. After annealing, molecules can obtain enough energy to rearrange themselves and the peak at $2\theta = 6.97$ increases its intensity (250 counts in films deposited at 180 s and 350 counts in annealed films).

In summary, nanowires are formed by stacking of the $LuPc_2$ molecules with the aromatic rings perpendicular to the substrate (as suggested by the observation of J-aggregates). AFM images show the existence of branched structures that could contain herringbone organization. When increasing the deposition times or when applying a thermal treatment, the size and thickness of the nanowires increase causing an enhancement of the absorbance. Simultaneously, the number of branched V-type structures

---

(39) Markovitsi, D.; Tran-Thi, T. H.; Even, R.; Simon, J. *J. Chem. Phys. Lett.* **1987**, *137*, 107–112.

(40) Corni, I.; Ryan, M. P.; Boccaccini, A. R. *J. Eur. Ceram. Soc.* **2008**, *28*, 1353–1367.

(41) Rousseau, R.; Aroca, R.; Rodríguez-Méndez, M. L. *J. Mol. Struct.* **1995**, *356*, 49–62.

(42) Toupance, T.; Vincent, P.; Simon, J. *New J. Chem.* **1999**, *23*, 1001–1006.

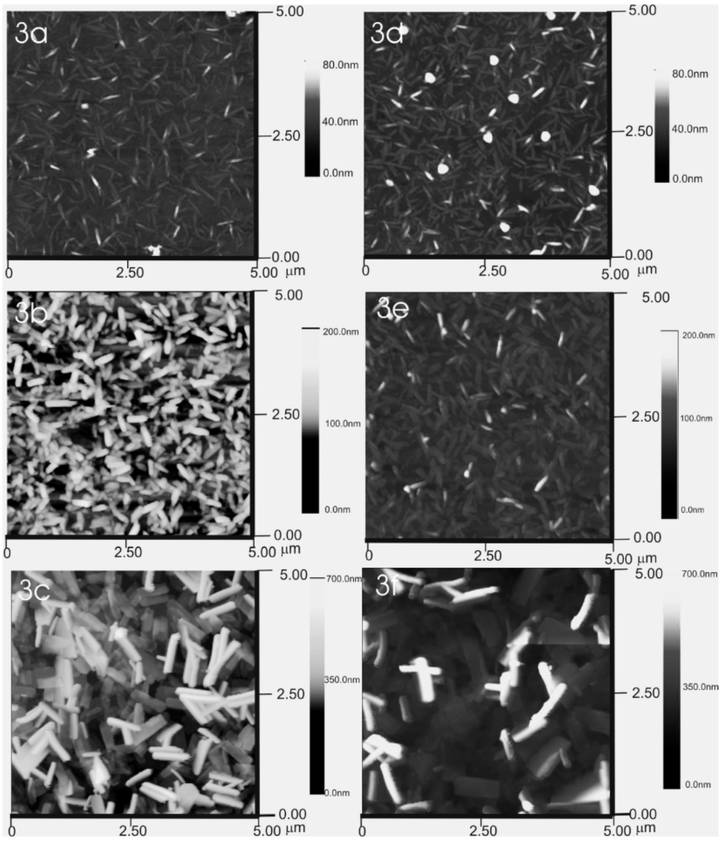


Figure 3. AFM images of $LuPc_2$ nanowires formed at 40, 60, and 180 s (a,b,c) before and (d,e,f) after annealing at 150 °C for 1 h.

Table 1. Size of the Nanowires and Structural Data of Films Obtained at Different Deposition Times[a]

	40		60		180	
deposition time (s)	a	b	a	b	a	b
diameter (nm)	30−60	80−100	95−100	90−130	100−120	200−400
length (nm)	200−400	250−300	300−450	350−500	600−800	900−1100
roughness (nm)	4.518	7.364	11.525	10.810	49.015	87.787
R_{max} (nm)	46.627	31.339	67.688	49.721	44.002	43.941
R_z (nm)	20.937	20.289	51.568	33.473	27.336	31.679

[a] a, before annealing; b, after annealing.

increases (observed in the AFM images), enhancing the intensity of the X-ray peak at $2\theta = 6.97$.

3.2. Electrochemical Response of the $LuPc_2$ Nanowires: Kinetics, Reproducibilty, and Stability. Electrochemical experiments were carried out by immersing the ITO electrodes in a variety of ionic solutions and registering the corresponding cyclic voltammograms. A clear voltammogram was obtained for the first cycle, but with successive cycles, the films became detached from the electrode and reproducible results could not be obtained. In contrast, highly reproducible voltammograms were obtained when using annealed films due to the improvement of the adhesion of the sensitive material to the substrate. The results described in the following paragraphs correspond to annealed films.

The electrochemical behavior of the $LuPc_2$ films electrodeposited for 40, 60, and 180 s is illustrated in Figure 5 using KCl as an electrolyte. The voltammograms displayed two quasireversible processes corresponding to the one electron ring oxidation $Lu(III)Pc_2/Lu(III)Pc_2^+$ (peak I): ($E_{1/2} = 0.56$ V) and the one electron ring reduction $Lu(III)Pc_2/Lu(III)Pc_2^-$ (peak II) ($E_{1/2} = -0.42$ V) of the bisphthalocyanine molecule (In Figure 5, I_a means anodic wave and I_c means cathodic wave). As expected, the intensities of the peaks increase with the deposition time due to the increasing amount of phthalocyanine that migrates to the cathode.

This behavior is quite similar to the well-known electrochemical activity previously described for LB films[14] (Figure 6), with a few notable exceptions. Peak I appears at slightly higher potentials ($E_{1/2} = 0.64$ V) in LB films due to the presence of the arachidic acid used to facilitate the deposition of the floating monolayers. In EPD films, a shoulder in the anodic wave of peak I (at ca. 0.5 V) was observed in thick films as a reflection of the coexistence of two

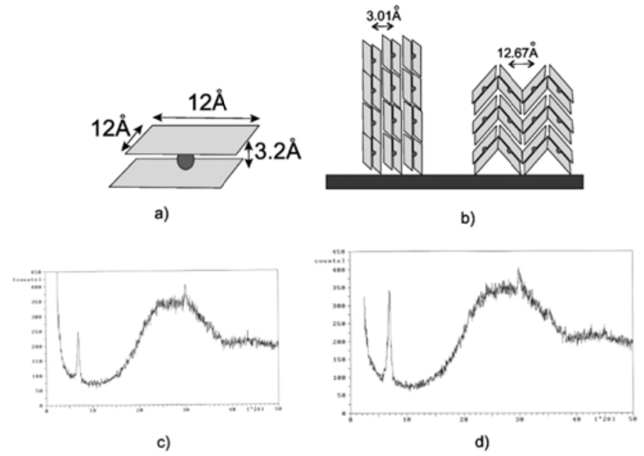


Figure 4. (a) Molecular structure of $LuPc_2$ and its dimensions. (b) Possible structure of the nanowires electrodeposited on the ITO substrate. (c) X-ray diffractogram of the film deposited at 180 s. (d) X-ray diffractogram of the annealed film deposited at 180 s.

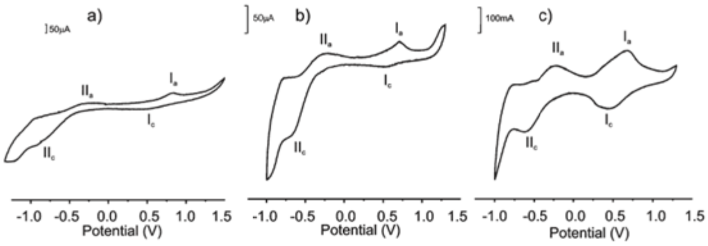


Figure 5. Cyclic voltammetry of $LuPc_2$ electrodeposited films at (a) 40 s, (b) 60 s, and (c) 180 s immersed in 0.1 M KCl.

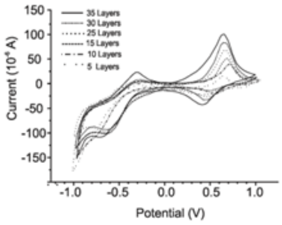


Figure 6. Electrochemical response of LB films of increasing thicknesses (5−35 monolayers) recorded at 0.050 V.s^{-1} in a 0.1 M KCl solution.

structures within the film that has been observed in the XRD experiments. The existence of two classes of environments can give rise to the oxidation of *Pc* molecules at two different (although very close) electrochemical potentials. This shoulder increases in intensity when the deposition time is increased. For this reason, in films deposited at 40 s, only a certain asymmetry of the peak is noticed, whereas in films deposited for 180 s, the shoulder is clearly observed. In contrast, for the LB films, the intensity of peak I increased with the number of monolayers, but the shape of the curve did not change due to the homogeneity of films obtained by the deposition of successive monolayers.

Kinetic studies were carried out by recording the cyclic voltammograms of the EPD electrodes at different scan rates (from 0.025 to 1.2 V.s^{-1}). In all cases, the intensity of the redox peaks incresed linearly with the square root of the scan rate (Figure 7) indicating the dominance of the diffusion controlled processes according to the Randles−Sevcik equation

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

where I is the peak current, A is the electrode surface area, D is the diffusion coefficient, and C is the bulk concentration. The diffusion coefficient (D) can be calculated from the I versus $v^{1/2}$ plot (Table 2). The Diffusion coefficients calculated are consistent with the results found in Langmuir−Blodgett films.[14]

In order to evaluate the stability of the EPD films, consecutive cycles were recorded in 0.1 M KCl solution.The nonannealed films were quite unstable and reproducible voltammograms could not be obtained. Nevertheless, the stability was significantly improved by using annealed films. The first scan of the thermally treated films was, expectedly, slightly different from the second cycle; subsequent scans were highly reproducible and the only noticeable change was a gradual decrease of the intensity of the peaks. The decrease of the intensity was more significant in the EPD films than in the LB films. In the EPD films, the intensity decreased by 50% after 75 scans, whereas in the LB films, the intensity decreased by 50% after 500 scans. When the cyclic voltammetry treatment was stopped, the EPD films were withdrawn from the solution and washed with milli-Q water to remove any adsorbed particle on the surface of the electrode. The films

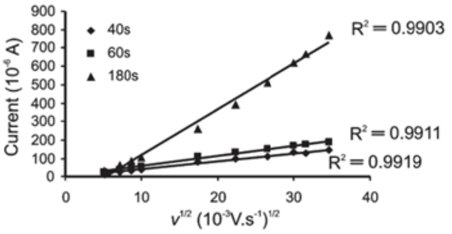


Figure 7. Plot of the intensity of the anodic wave of peak I vs the square root of the scan rate at the various annealing times (40, 60, and 180 s).

Table 2. Diffusion Coefficient of the Films Deposited at Differents Deposition Times

deposition time (s)	diffusion coefficient (cm^2/s)
40	2.11×10^{-8}
60	3.46×10^{-8}
180	6.84×10^{-7}

were dipped again in a KCl solution and produced voltammograms similar to those previously obtained, except at lower intensities. In contrast, when the LB films were removed and again immersed in the test solution, considerable changes occurred and the films could not be reused.

In order to study the reproducibility of the EPD technique, nine identical electrodes were prepared and tested in a 0.1 M KCl solution. The values of the peak potentials were highly reproducible with standard deviations of 2.94%, 8.70%, 4.54%, and 1.64% for peaks I anodic (I_a), I cathodic (I_c), II anodic (II_a), and II cathodic (II_c), respectively (Figure 8a). These values were similar to those obtained in the LB films where the standard deviations were 1.14%, 0.89%, 1.58%, and 0.84% for peaks I_a, I_c, II_a, and II_c respectively.

The influence of the nature of the electrolytic solution on the electrochemical response of the EPD $LuPc_2$ films was tested by immersing the electrodes in solutions containing KCl, $MgCl_2$, $KClO_4$, HCl, and NaOH (all the concentrations were 0.1 M). The studies were carried out using the EPD electrodes deposited at 180 s. The results are depicted in Figure 8.

Using $MgCl_2$ as an electrolyte (Figure 8b), the voltammograms obtained were similar to those recorded in the KCl solution (Figure 8a). In this case (when $MgCl_2$ is used as an electrolyte), the peaks appear at $E_{1/2} = 0.57$ V for peak I and $E_{1/2} = -0.34$ V for peak II. The similarities in the responses in KCl and in $MgCl_2$ indicate that, during oxidation and reduction, anions (and not cations) are incorporated into the film to compensate the generated positive charges. Similarly, during reduction, anions are expelled from the film to maintain the electroneutrality of the electrode.

In the presence of $KClO_4$ (Figure 8c), the oxidizing character of the ClO_4^- anion incorporated into the film facilitates the oxidation of the phthalocyanine molecules. The values obtained for peak I and peak II were $E_{1/2} = 0.44$ V and $E_{1/2} = -0.45$ V, respectively. In acid media (HCl) (Figure 8d), the response of the electrode differed significantly from those observed in neutral solutions. It is well-known that acidic solutions may protonate the reduced form of $LuPc_2$ and then induce a disproportionation reaction causing the production of two oxidation processes: the expected peak at 0.75 V and of a new peak at 0.28 V associated with the oxidation of the protonated form.[41] In basic media (NaOH) (Figure 8e), the redox activity of the bisphthalocyanine was not observed. This result is in accordance with previously published results, where carbonous electrodes chemically modified with lanthanide bisphthalocyanines have been exposed to biogenic amines.[43] In strongly basic pHs, the oxidation and reduction of the phthalocyanine ring occur at potentials out of the working range.

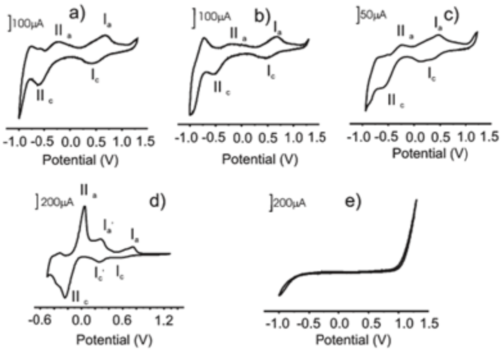


Figure 8. CV curves of $LuPc_2$ film electrode (180 s) inmmersed in 0.1 M aqueous solutions: (a) KCl; (b) $MgCl_2$; (c) HCl; (d) $KClO_4$; and (e) NaOH.

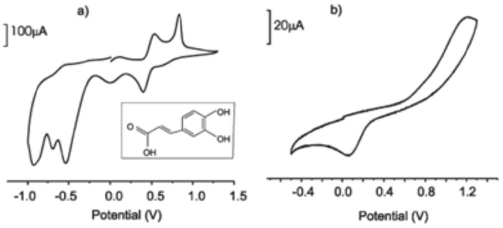


Figure 9. Cyclic voltammogram of (a) EPD electrode modified with $LuPc_2$ and (b) a bare ITO electrode, immersed in a caffeic acid solution (5×10^{-4} M). The inset shows the formula of the caffeic acid.

In summary, depending on the nature of the electrolytic solution, the electrode covered with $LuPc_2$ nanowires showed characteristic electrochemical responses. This variety of responses makes these electrodes appropriate to use as a sensing unit of an electronic tongue (an array of sensing units with cross-selectivity, coupled with pattern recognition techniques).[44] In these systems, the electrochemical signals produced by the array immersed in different solutions (training set) are used to train a pattern recognition software. Using predictive chemometric techniques, it is possible to recognize unknown solutions by comparing the responses of the analyte with the signals of the training set.[45]

3.3. Detection of Caffeic Acid. One of the main challenges in the field of multisensor systems (e-tongues) is the development of new sensors able to detect non-ionic analytes. In order to evaluate the possible application of the $LuPc_2$ films as sensor of non-ionic solutions, the electrochemical response toward caffeic acid was tested.

Figure 9 shows the electrochemical response of the EPD-film and a bare ITO film immersed in a solution of 5×10^{-4} M caffeic

---

[43] Rodriguez-Méndez, M. L.; Apetrei, C.; Gay, M.; De Saja, J. A. *Electrochim. Acta* **2009**, *54*, 7033–7041.

[44] Gutierrez, J. M.; Moreno-Baron, L.; Pividori, M. I.; Alegret, S.; Del Valle, M. *Microchim. Acta* **2010**, *169*, 261–268.

[45] Escuder-Gilabert, L.; Peris, M. *Anal. Chim. Acta* **2010**, *665*, 15–25.

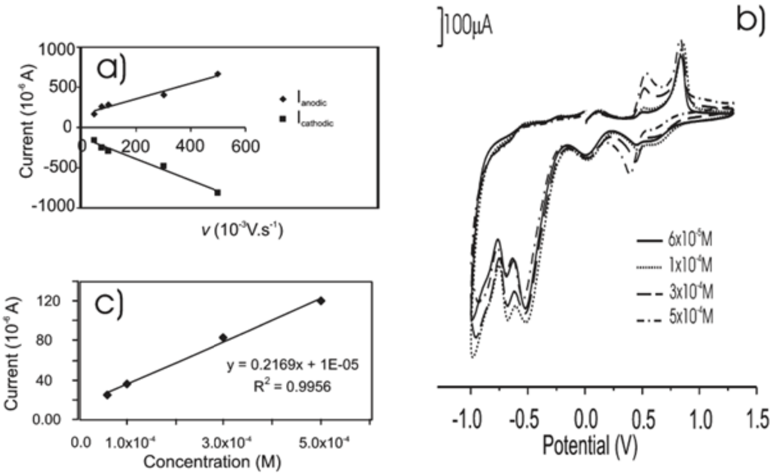


Figure 10. (a) Plot of the intensity of the peaks associated with caffeic acid vs the scan rate. (b) Linear relationship of the intensity of the peaks associated to caffeic acid vs the concentration. (c) Cyclic voltammograms of the EPD film immersed in caffeic acid from 6×10^{-5} M to 5×10^{-4} M.

acid (pH = 3.6). The voltammogram obtained (Figure 9a) differs significantly from the voltammograms recorded in the ionic solutions shown in Figure 8. The curve is characterized by (i) a peak at $E_{1/2} = 0.47$ V associated with the two-electron reversible oxidation of caffeic acid;[46] (ii) the characteristic peaks associated with the oxidation and reduction of the lutetium bisphthalocyanine in acidic media at $E_{1/2} = -0.11$ V, $E_{1/2} = 0.27$ V, and $E_{1/2} = 0.70$ V.

It is important to remark that the interactions between the caffeic acid and the bisphthalocyanine cause changes in the intensities and positions of the peaks expected for both components analyzed separately. For example, the antioxidant character of the caffeic acid shifts the oxidation of the phthalocyanine to higher potentials (from 0.71 V to 0.84 V). In turn, the electrocatalytic effect of the phthalocyanine favors the oxidation of the caffeic acid that shows the anodic peak at 0.54 V instead of at 1.2 V observed when using a bare ITO electrode.

All these effects and interactions responsible for the distinct response of this electrode to caffeic acid. In fact, the response is different from the responses previously published using carbon paste or LB lutetium bisphthalocyanine electrodes immersed in model solutions of basic tastes[14] and bitterness,[47] biogenic amines,[43] other antioxidants,[32] and complex solutions containing a mixture of ions and antioxidants such as wines.[48,49]

Kinetic studies have been carried out (Figure 10a) by recording the cyclic voltammograms of the EPD-LuPc$_2$ electrode immersed in 5×10^{-4} M caffeic acid at different scan rates (from 0.05 to 0.5 V.s^{-1}). The intensity of the redox peaks associated with the caffeic acid (at $E_{1/2} = 0.47$ V) increased linearly with the scan rate, indicating the dominance of the surface confined processes. The surface coverage (Γ) can be calculated using the Laviron equation

$$I = nF^2 vA\Gamma/4RT \qquad (2)$$

where n is the number of electrons, F the Faraday constant, v the rate, A the area of the sensor, Γ the surface coverage, R the gas constant, and T the temperature. The surface coverage of the EPD-LuPc$_2$ film calculated using eq 2 for the anodic wave (at 0.54 V) was 2.45×10^{-10} mol.cm^{-2}. This value is higher than that obtained using a bare ITO electrode (2.37×10^{-11} mol.cm^{-2}) indicating that, when the nanostructured sensor was employed as the electrode, the oxidation of the caffeic acid was favored.

As shown in Figure 10b,c, the intensity of the peak at 0.54 V was linearly dependent with the concentration of caffeic acid in the range from 6×10^{-5} M to 5×10^{-4} M with a detection limit of 3.12×10^{-5} M.

These results demonstrate that the LuPc$_2$ film provides a distinct and reproducible response to caffeic acid, and that the sensor can be used to quantify the presence of the antioxidant in the range usually present in foods.

4. Conclusions

LuPc$_2$ nanowires have been successfully deposited onto ITO substrates by the EPD technique. The structure of the nanowires has been analyzed by means of spectroscopic and microscopic techniques confirming the stacking of the molecules perpendicular to the substrate and the coexistence of a herringbone structure. The size of the nanowires and the roughness of the films can be controlled by the deposition time.

Highly stable and reproducible cyclic voltammograms can be obtained by annealing the films, because the thermal process increases the adhesion of the films to the substrate. The electrochemical behavior is similar to that previously observed in LB films with some differences in the position, intensity, and shape of the peaks. Such differences are associated with the different organization of the molecules in EPD films. The electrochemical responses depend on the nature the electrolytic solution. As expected for diffusion-controlled processes, the intensities of the peaks are linearly dependent with the square root of the scan rate.

The electrodes have been successfully used as sensors for the detection of caffeic acid. The interactions between the antioxidant and the electrodic material are responsible of the specific response for the electrodes to caffeic acid and the voltammograms can be considered as a fingerprint of this compound. A detection limit of

---

(46) Giacomelli, C.; Ckless, K.; Galato, D.; Miranda, F. S.; Spinelli, A. *J. Braz. Chem. Soc.* **2002**, *13*, 332–338.

(47) Apetrei, C.; Rodriguez-Méndez, M. L.; Parra, V.; Gutierrez, F.; de Saja, J. A. *Sens. Actuators, B* **2004**, *103/1−2*, 145–152.

(48) Apetrei, C.; Apetrei, I; Nevares, I.; del Alamo, M.; Parra, V.; Rodriguez-Méndez, M. L.; De Saja, J. A. *Electrochim. Acta* **2007**, *52*, 2588–2594.

(49) Parra, V.; Arrieta, A.; Fernández-Escudero, J. A.; Rodríguez-Méndez, M. L.; de Saja, J. A. *Sens. Actuators, B* **2006**, *118*, 448–453.

3.12×10^{-5} M has been attained, which is in the range of the concentration of caffeic acid found in several foods.

In comparison with $LuPc_2$ LB films, the EPD $LuPc_2$ films allow the development of nanostructured sensors at lower costs and in a shorter time. Also, the EPD technique is reliably reproducible.

Acknowledgment. Financial support by the Spanish Ministry of Science (grant AGL2009-12660/ALI) is gratefully acknowledged. MGM also thanks the Spanish Ministry of Education for a fellowship (F.P.I, AGL2006-05501). Central de Tecnología del Instituto de Sistemas Optoelectrónicos y Microtecnología (CT-ISOM) is also gratefully acknowledged.

Supporting Information Available: Additional figures as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

2.8 ARTÍCULO 7. “Multisensor system based on bisphthalocyanine nanowires for the detection of antioxidants.”

M. Gay Martín, J.A. de Saja, R. Muñoz, M.L. Rodríguez-Méndez.

Electrochimica Acta 2012, 68, 88-94.

2.8.1. Motivación y objetivos.

El éxito alcanzado por la deposición electroforética como técnica en la preparación del sensor nanoestructurado EPD-$LuPc_2$ nos hizo plantearnos el extender el estudio a otras bisftalocianinas de lantánidos y construir una red de sensores EPD basada en tres bisftalocianinas con diferentes metales centrales: $GdPc_2$, $DyPc_2$ y $LuPc_2$. En este sentido, se ha evaluado la influencia del metal central, en términos del radio iónico, en la respuesta electroquímica de la red de sensores EPD.

Debido a la importancia de los antioxidantes en el sector alimentario, la red de sensores EPD se ha aplicado al estudio de cuatro fenoles; ácido cafeico, ácido gálico, ácido ferúlico y ácido vanílico. La elección de los mismos se ha hecho en base a la diferente estructura química, la cual desempeña un papel fundamental en las propiedades electroquímicas de dichas moléculas. El estudio de los antioxidantes se ha llevado a cabo en medio hidroalcohólico, debido a la limitada solubilidad que poseen en agua y con el fin de simular el medio líquido de un vino, ya que éste representa una futura aplicación de dicha red.

El avance alcanzado en la presente Memoria consiste en la obtención por primera vez de una red de sensores nanoestructurados basados en diferentes bisftalocianinas preparados mediante la técnica EPD y su aplicación en el estudio y discriminación de antioxidantes en medio hidroalcohólico.

2.8.2. Resumen de los resultados obtenidos.

i) Como materiales sensibles de la red de sensores se han seleccionado bisftalocianina de gadolinio ($GdPc_2$), bisftalocianina de disprosio ($DyPc_2$) y bisftalocianina de Lutecio ($LuPc_2$). La preparación de estos sensores se ha realizado mediante EPD, modificando las condiciones para adaptarse a las peculiaridades de cada molécula y obtener películas de morfología similar.

ii) La respuesta voltamétrica de los sensores EPD-$LnPc_2$ se ha estudiado inicialmente en un electrolito inerte desde el punto de vista redox (KCl), con el fin de estudiar los procesos redox asociados exclusivamente con el material electroactivo. Los voltamogramas obtenidos reflejan los dos pares de picos característicos de los procesos de oxidación y reducción del anillo de ftalocianina.

Los valores de potencial de cada uno de los procesos están íntimamente relacionados con el radio del ion del metal central (Gd^{3+}, Dy^{3+} y Lu^{3+}) debido a que éste determina la distancia entre los anillos de ftalocianina. Cuanto menor es el radio iónico del metal central, la distancia entre anillos es menor, y por tanto, la diferencia de energía entre el HOMO-LUMO del sistema también es menor, lo cual hace que la energía requerida para arrancar un electrón del HOMO sea menor que en el caso de que la energía HOMO-LUMO sea mayor. El valor de $E_{1/2}$ asociado al pico I para las tres bisftalocianinas estudiadas siguen la siguiente secuencia: $GdPc_2$ ($E_{1/2}$ =0.72V)>$DyPc_2$ ($E_{1/2}$ =0.69V)>$LuPc_2$ ($E_{1/2}$ =0.59V).

iii) Debido a que el estudio de los antioxidantes se realiza en medio hidroalcohólico, se ha estudiado la respuesta voltamétrica de la red EPD-$LnPc_2$ en dicho medio, en ausencia de antioxidantes. El perfil electroquímico observado corresponde con el esperado para una bisftalocianina en medio ácido (pH = 3.6). Además de observarse los picos que aparecían en medio neutro (KCl) se ha observado la aparición de un nuevo pico relacionado con el medio ácido en el cual está inmersa la bisftalocianina.

iv) Los antioxidantes seleccionados han sido dos orto difenoles; ácido cafeico y ácido gálico, y dos monofenoles; ácido ferúlico y ácido vanílico. El número de grupos hidroxilo y su posición relativa desempeña un papel de vital importancia en las características electroquímicas de dichos fenoles.

v) La respuesta voltamétrica de los antioxidantes está directamente relacionada con el número de grupos hidroxilos y su posición relativa en el anillo aromático. Así, los antioxidantes que presentan grupos hidroxilo en posición orto en su esqueleto aromático, tales como el ácido cafeico y gálico, se caracterizan por presentar una oxidación en una única etapa de dos electrones, dando lugar a la correspondiente quinona. Por el contrario, los monofenoles, tales como el ácido ferúlico y vanílico, sufren una oxidación que tiene lugar en dos etapas, dando lugar también al correspondiente producto de oxidación.

En el caso de los ácidos cafeico y gálico, la presencia del grupo orto hidroxilo favorece la oxidación de los mismos, lo cual se traduce en valores potencial de oxidación menores que en el caso de los monofenoles.

vi) Por otra parte, la influencia de los antioxidantes en el comportamiento electroquímico de la bisftalocianina se ha reflejado principalmente en los valores de potencial del pico III, el cual está relacionado con el medio ácido de la disolución. La suma de ambos procesos redox hace que se obtenga una curva voltamétrica con gran información acerca del sistema estudiado.

vii) Se determinado el límite de detección de la red de sensores para cada uno de los antioxidantes, obteniéndose valores en el rango de 6×10^{-5}M-2×10^{-4}M, los cuales se encuentran dentro del rango en el que se presentan estos antioxidantes en vinos.

viii) Estudios cinéticos han demostrado que la intensidad de los picos asociados a los fenoles aumenta linealmente con la velocidad de barrido. La ecuación de Lavirón ha permitido calcular el *surface coverage* (Γ) en cada uno de los casos, obteniéndose valores del orden de 10^{-10} mol cm^{-2}.

ix) Con el fin de explorar la capacidad de discriminación de la red de sensores EPD en el estudio de antioxidantes, se ha realizado un Análisis de Componentes Principales (PCA). El PCA explica el 84% del modelo con sólo dos componentes principales. En el gráfico PCA se observa que existe una discriminación de los antioxidantes en base a su estructura. Los clusters asociados a los antioxidantes que poseen un grupo orto difenol en su estructura aparecen separados de los clusters asociados a lo monofenoles. Además se observa una proximidad entre los clusteres del ácido cafeico y del ácido ferúlico debido a que ambos fenoles, presentan una respuesta electroquímica similar dado que el ácido ferúlico al oxidarse genera el mismo compuesto de oxidación que el ácido cafeico. El modelo PLS-DA ha permitido cuantificar la discriminación alcanzada con el modelo PCA, obteniéndose coeficientes de correlación altos, pendientes cercanas a la unidad y ordenadas en el origen próximas a cero, lo que se traduce en una buena capacidad de discriminación de los antioxidantes por parte de la red EPD-LnPc$_2$.

2.8.3. Conclusiones

Por primera vez se ha preparado una red de sensores EPD voltamétricos basados en bisftalocianinas de lantánidos. Dicha red se ha aplicado con éxito al estudio de cuatro antioxidantes en disolución; ácido cafeico, ácido gálico (ambos con grupos hidroxilos en posición orto en su estructura aromática), ácido ferúlico y ácido vanílico (ambos con un único grupo hidroxilo en su estructura aromática).

El PCA permite discriminar los cuatro antioxidantes estudiados de acuerdo al número de grupos –OH presentes en su estructura. El porcentaje de varianza explicado empleando únicamente dos componentes principales es de 84%, casi la información total del modelo. El análisis PLS-DA evalúa cuantitativamente la predicción del tipo de fenol, obteniéndose altos coeficientes de correlación con una pendiente cercana a la unidad y ordenada en el origen próxima a cero.

Las características de los nuevos sensores EPD obtenidos, en términos de repetitividad, reproducibilidad y límite de detección son similares a las presentadas por otros sensores basados en ftalocianinas. Sin embargo, estos sensores EPD presentan la importante ventaja de su fácil y económica preparación.

Los resultados confirman la posibilidad de emplear esta novedosa red de sensores EPD en el estudio de antioxidantes en disolución hidroalcohólica y su posible aplicación en el estudio de vinos.

Electrochimica Acta 68 (2012) 88–94

Contents lists available at SciVerse ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

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*

ARTICLE INFO

Article history:
Received 17 November 2011
Received in revised form 7 February 2012
Accepted 9 February 2012
Available online 19 February 2012

Keywords:
Nanowires
Sensor
Phthalocyanine
Antioxidant
Electronic tongue

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).

0013-4686/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.electacta.2012.02.042

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 $5 \times 10^{-5}\,mol\,L^{-1}$ for $GdPc_2$ and $DyPc_2$ and $1 \times 10^{4}\,mol\,L^{-1}$ 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 lanthanide 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 $5 \times 10^{-5}\,mol\,L^{-1}$ for $GdPc_2$ and $DyPc_2$ and $1 \times 10^{-4}\,mol\,L^{-1}$ 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).

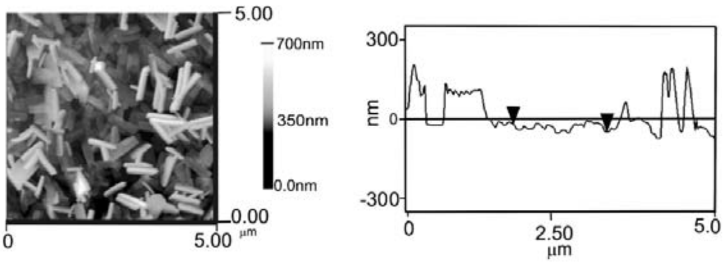


Fig. 1. (Left) AFM image and (right) profile of the EPD-$GdPc_2$ film.

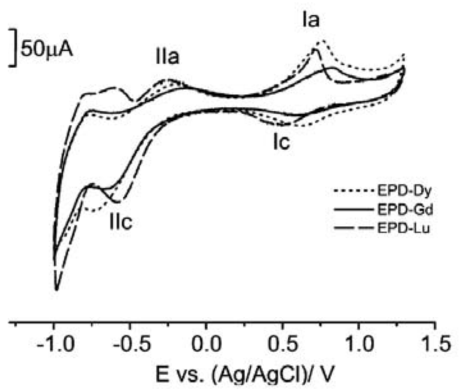


Fig. 2. Cyclic voltammograms in KCl 0.1 mol L^{-1} of the array of EPD sensors. Sweep rate 0.05 V s^{-1}.

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

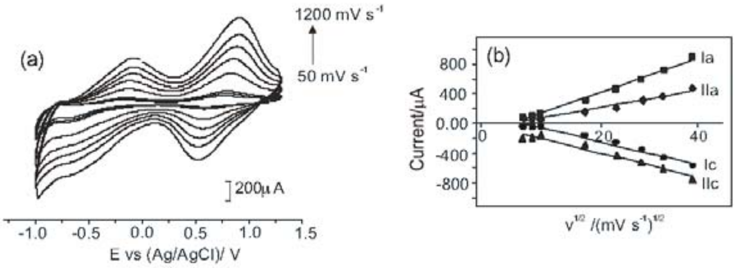


Fig. 3. (a) Cyclic voltammogram of EPD-DyPc$_2$ sensors in KCl 0.1 mol L^{-1} at increasing scan rates (50, 75, 100, 300, 500, 700, 900 and 1200 mV s^{-1}) and (b) linear dependence between the intensity and the square root of the scan rate.

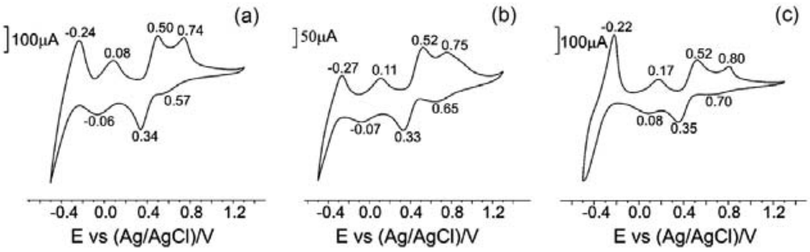


Fig. 4. Cyclic voltammograms of the array of sensors in the hydroalcoholic solution (12% (v/v) ethanol and pH = 3.6).

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

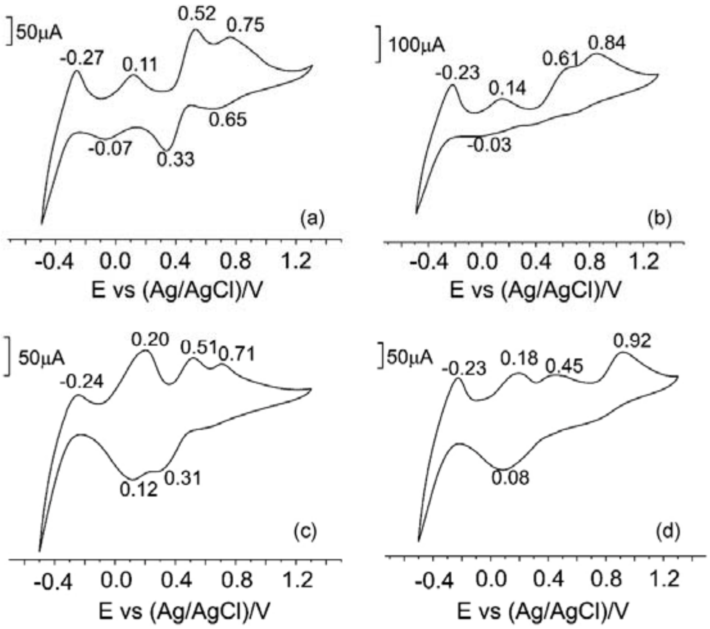


Fig. 5. Cyclic voltammograms of EPD-DyPc$_2$ sensor immersed in 0.5 mmol L^{-1} (a) caffeic, (b) gallic, (c) vanillic and (d) ferulic acid.

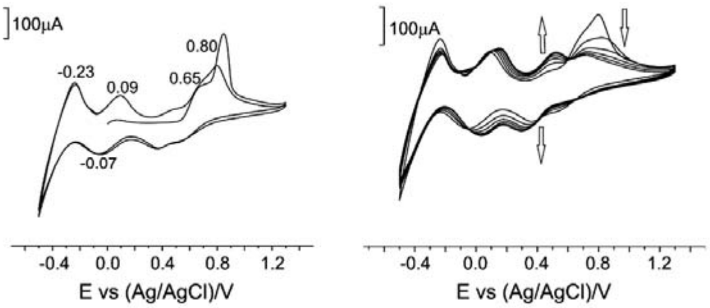


Fig. 6. CV of a $DyPc_2$ electrode immersed in a hydroalcoholic solution containing ferulic acid. Left curve shows the first and second voltammograms. Right curve shows scans from 2 to 7. Scan rate 50 mV s^{-1}.

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.

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

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).

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}

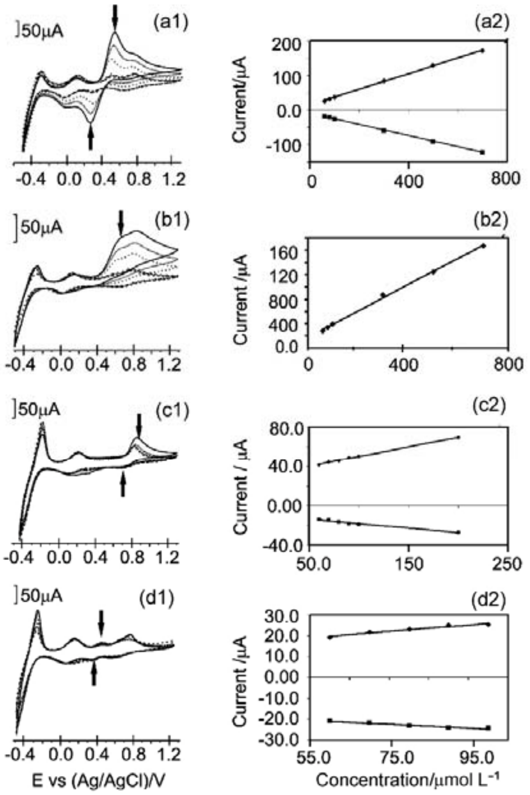


Fig. 7. Cyclic voltammograms (left) and calibration curves (right) of EPD-GdPc$_2$ immersed in (a) caffeic, (b) gallic, (c) vanillic and (d) ferulic acids.

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).

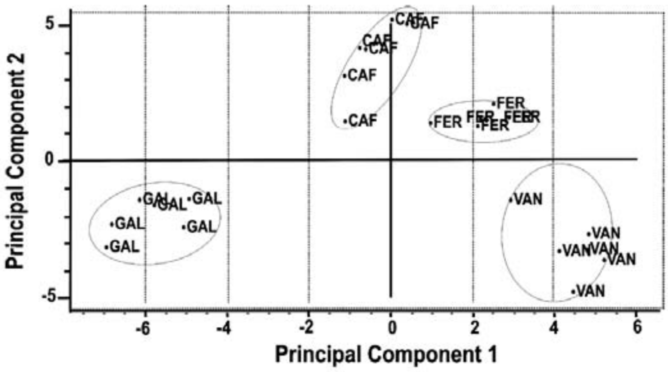


Fig. 8. PCA score plot corresponding to the classification of the antioxidants.

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.

Acknowledgments

Financial support by the Spanish Ministry of Science (Grant AGL2009-12660/ALI) is gratefully acknowledged. MGM also thanks the Spanish Ministry of Education for the fellowship (F.P.I. AGL 2006-05501).

References

[1] M. del Valle, Electroanalysis 22 (2010) 1539.
[2] A. Riul Jr., C.A.R. Dantas, C.M. Miyazaki, O.N. Oliveira Jr., Analyst 135 (2010) 2481.
[3] M. Jańczyk, A. Kutyla, K. Sollohub, H. Wosicka, K. Cal, P. Ciosek, Bioelectrochemistry 80 (2010) 94.
[4] Y.G. Vlasov, Y.E. Ermolenko, A.V. Legin, A.M. Rudnitskaya, V. Kolodnikov, J. Anal. Chem. 65 (2010) 880.
[5] G. Verrelli, L. Lvova, R. Paolesse, C. Di Natale, A. D'Amico, Sensors 7 (2007) 2750.
[6] M. Scampicchio, S. Benedetti, B. Brunetti, S. Mannino, Electroanalysis 18 (2006) 1643.
[7] M.L. Rodríguez-Méndez, V. Parra, C. Apetrei, S. Villanueva, M. Gay, N. Prieto, J. Martinez, J.A. de Saja, Microchim. Acta 163 (2008) 23.
[8] R.H. Labrador, J. Olsson, F. Winquist, R. Martínez-Máñez, J. Soto, Electroanalysis 21 (2009) 612.
[9] P.H.B. Aoki, D. Volpati, A. Riul Jr., W. Caetano, C.J.L. Constantino, Langmuir 25 (2009) 2331.
[10] R.H Labrador, R. Masot, M. Alcaniz, D. Baigts, J. Soto, R. Martinez-Manez, E. Garcia-Breijo, L. Gil, J.M. Barat, Food Chem. 122 (2010) 864.
[11] X. Cetó, F. Céspedes, M.I. Pividori, J.M. Gutiérrez, M. del Valle, Analyst 137 (2012) 349.
[12] A. Gutés, A.B. Ibánez, F. Céspedes, S. Alegret, M. del Valle, Anal. Bioanal. Chem. 382 (2005) 471.
[13] M. Ghasemi-Vamankhasti, M.L. Rodríguez-Méndez, S.S. Mohtasebi, C. Apetrei, J. Lozano, S.H. Razavi, H. Ahmadi, J.A. de Saja, Food Control 25 (2012) 216.
[14] J. Zeravik, A. Hlavacek, K. Lacina, P. Sklàdal, Electroanalysis 21 (2009) 2509.
[15] X. Cetó, J.M. Gutiérrez, L. Moreno-Barón, S. Alegret, M. del Valle, Electroanalysis 23 (2011) 72.
[16] M. Gutierrez, A. Llobera, J. Vila-Planas, F. Capdevila, S. Demming, S. Buttgenbach, S. Minguez, C. Jimenez-Jorquera, Analyst 135 (2010) 1718.
[17] V. Parra, A. Arrieta, J.A. Fernández-Escudero, H. García, C. Apetrei, M.L. Rodríguez-Méndez, J.A. de Saja, Sens. Actuators B 115 (2006) 54.
[18] A.A. Arrieta, C. Apetrei, M.L. Rodríguez-Méndez, J.A. de Saja, Electrochim. Acta 49 (2004) 4543.
[19] V. Parra, A. Arrieta, J.A. Fernández-Escudero, M. Iñiguez, M.L. Rodríguez-Méndez, J.A. de Saja, Anal. Chim. Acta B 563 (2006) 229.
[20] M.L. Rodríguez-Méndez, C. Apetrei, M. Gay, J.A. De Saja, Electrochim. Acta 54 (2009) 7033.
[21] C. Apetrei, M.L. Rodríguez-Méndez, J.A. de Saja, Electrochim. Acta 53 (2008) 5867.
[22] T. Toupance, V. Plichon, J. Simon, New J. Chem. 23 (1999) 1001.
[23] P. Mashazi, E. Antunes, T. Nyokong, J. Porphyr. Phthalocyan. 14 (2010) 932.
[24] T.V. Magdesieva, I.V. Zhukov, D.N. Kravchuk, O.A. Semenikhin, L.G. Tomilova, K.P. Butina, Russ. Chem. Bull. Int. Ed. 51 (2002) 805.
[25] E. Njanja, A. Nassi, E. Ngameni, C. Elleouet, F. Quentel, M. L'Her, Electrochem. Commun. 9 (2007) 1695.
[26] M.L. Rodríguez-Méndez, M. Gay, J.A. de Saja, J. Porphyr. Phthalocyan. 13 (2009) 1159.
[27] L. Gaffo, D. Gonçalves, A. Dhanabalan, W.C. Moreira, O.N. Oliveira Jr., Synth. Met. 124 (2001) 351.
[28] V. Zucolotto, M. Ferreira, M.R. Cordeiro, C.J.L. Constantino, W.C. Moreira, O.N. Oliveira Jr., Synth. Met. 137 (2003) 945.
[29] E.G. Ramos Fernandes, L.C. Brazaca, M.L. Rodríguez-Méndez, J.A. de Saja, V. Zucolotto, Biosens. Bioelectron. 26 (2011) 4715.
[30] L. Besra, M. Liu, Prog. Mater. Sci. 52 (2007) 1.
[31] R. Bai, H.-Z. Chen, H.-B. Zhou, M.-M. Shi, M. Wang, J. Cryst. Growth 285 (2005) 183.
[32] H.-Z. Chen, L. Cao, H.-B. Zhou, Y. Rong, M. Wang, J. Cryst. Growth 281 (2005) 530.
[33] M. Gay Martín, M.L Rodríguez-Méndez, J.A. De Saja, Langmuir 26 (2010) 19217.
[34] M. Linaje, M.C. Quintanilla, A. González, J.L. del Valle, G. Alcaide, M.L. Rodríguez-Méndez, Analyst 125 (2000) 341.
[35] L.G. Tomilova, E.V. Chernykh, N.T. Ioffe, E.A. Luk'yanets, Zh. Obshch. Khim. 53 (1983) 2594 (J. Gen. Chem. USSR 53 (1983) (Engl. Transl.)).
[36] H. Konami, M. Hatano, N. Kobayashi, T. Osa, Chem. Phys. Lett. 165 (1990) 397.
[37] R. Rousseau, R. Aroca, M.L. Rodríguez-Méndez, J. Mol. Struct. 356 (1995) 49.
[38] V. Mirčeski, J. Phys. Chem. B 108 (2004) 13719.
[39] V. Mirčeski, F. Quentel, M. L'Her, F. Sholtz, J. Electroanal. Chem. 585 (2006) 86.
[40] Y. Liu, K. Shigehara, A. Yamada, Thin Solid Films 179 (1989) 303.
[41] O. Makhotkina, P.A. Kilmartin, Anal. Chim. Acta 668 (2010) 155.
[42] P.A. Kilmartin, H. Zou, A.L. Waterhouse, J. Agric. Food Chem. 49 (2001) 1957.
[43] S.K. Trabelsi, N.B. Tahar, B. Trabelsi, R. Abdelhedi, J. Appl. Electrochem. 35 (2005) 967.
[44] C. Apetrei, P. Alessio, J.C. Constantino, J.A. de Saja, M.L. Rodríguez-Méndez, F. Pavinatto, E. Giuliani, V. Zucolotto, O.N. Oliveira, Biosens. Bioelectron. 26 (2011) 2513.

Capítulo III

Conclusiones y Perspectivas Futuras

3.1. CONCLUSIONS

According to the objectives planned and the order of presentation of the results, the following conclusions have been reached:

1. A voltammetric electronic tongue based on carbon paste electrodes chemically modified with phthalocyanines has been successfully applied to the analysis of red wines aged by two different methods: the traditional ageing method using oak barrels and the alternative method where wines are aged in stainless steal tanks where pieces of wood are added (chips and staves).

    a. The system has been able not only to detect the use of different methods of ageing, but it has been able to discriminate wines aged in barrels from the same wines aged in stainless steal tanks (with pieces of wood), in which a micro-oxygenation has been carried our in order to simulate the microoxigenation that happens through the pores of the wood barrels.

    b. The prediction model has shown that the electronic tongue is capable to detect the use of alternative methods in the bottled stage.

    c. Besides, it is possible to establish correlations between the electrochemical signals generated by the array of sensors and the chemical parameters of wines. The results showed that the best correlation was found with polyphenols. Also, it has been found excellent correlations between the results given by the array and those obtained by a human panel, especially with astringency. These excellent correlations indicate that the array of sensors presented can be employed as an analytical tool to predict the polyphenolic content in red wines.

    d. In summary, the electronic tongue based on carbon paste electrodes modified with phthalocyanines can be considered as an adequate and complementary method in the detection of using alternatives methods in the ageing of red wines.

2. In collaboration with the Company Nomacorc, dedicated to the fabrication of synthetic closures, the electronic tongue has been applied to an industrial problem: the evaluation of the effect of the oxygen levels in the organoleptic characteristics of wines.

    a. Wines prepared by different methods, micro oxygenated and bottled using closures with different porosity have been clearly discriminated by the e-tongue composed by carbon paste electrodes chemically modified with phthalocyanines and perylenes.

    b. The electronic tongue is able to discriminate the wines according to their polyphenolic content. The electrochemical profiles observed are also affected by the micro-oxygenation made in wines. Some tendencies according to the phenolic content and micro-oxygenation process in the PCA are observed. This fact is associated to the influence that oxygen has in the polyphenolic content and hence in the taste properties.

    c. The fusion of the data obtained with the electronic tongue with those given by others electronic systems, such as electronic eye and nose, permits a simultaneous discrimination according to the type of closure employed, micro-oxygenation and polyphenolic content.

3. An electronic tongue formed by miniaturized Screen Printed Electrodes modified with phthalocyanines has been developed. In collaboration with the company Tencas of Casaseca, it has been successfully used to discriminate biogenic amines and to monitor the spoilage of fishes.

    a. The performance of this array of miniaturized sensors has been compared with that of an array composed by carbon paste electrodes modified with the same molecules.

    b. The electrochemical signals provided by the two types of sensors (SPE and CPE) are similar. In case of SPE, the background current observed are higher than in case of CPE. On the other hand, the potentials observed in the miniaturized sensors are slightly lower than those observed in CPE. Besides, the intensities of the redox peaks of SPE sensors are one order of magnitude higher than those of their analogues CPEs, reaching lower detection limits in the detection of biogenic amines.

    c. The reproducibility of the SPE sensors is lower than that of CPE sensors, but the miniaturized electrodes are disposable and cheaper. In addition, they have the reference, the working and the counter electrode inserted in the same device, providing a portable system.

   d. Both types of arrays have similar capabilities of discrimination towards of biogenic amines, being both able to distinguish between NH_3, aliphatic amines and aromatic amines. Also, both of them are able to monitor the evolution of the spoilage of fishes, observing that the decomposition of the Tencas begins in the 5-6 days of their storing in the refrigerator. These results have been confirmed by means of PLS-DA model, which shows excellent correlations.

   e. All these results show the possibility of using arrays of voltammetric sensors based on CPE and SPE modified with bisphthalocyanines to monitor the spoilage of fishes through the study of the biogenic amines emitted as consequence of the decomposition process.

4. New nanostructured sensors based on phthalocyanines have been developed. The performance of the nanostructured sensors takes advantage of the enhanced surface to volume ratio of the electrodes and of the high control on the structure.

   a. For the first time, thin films based on nanowires of $LuPc_2$ as voltammetric sensor have been prepared by means of electrophoretic deposition technique.

   b. The structure has been fully characterized by means of spectroscopic and microscopic techniques. It has been demonstrated that the molecules are arranged with the aromatic rings cofacially stacked, with some branches where a herringbone structure is observed.

   c. The diameter and length of the nanowires depend on the experimental parameters, mainly the deposition times.

   d. The cyclic voltammetry experiments have shown that EPD-$LuPc_2$ films can be used as voltammetric sensors due to their reproducibility and stability. The voltammetric response is similar to that obtained with the LB sensors with some differences associated with the position, intensity and shape of the redox peaks. These differences are related to the structure of each type of sensor.

   e. The voltammetric responses of the EDP-$LuPc_2$ sensor towards ionic solutions and phenolic antioxidants have been analyzed.

   f. The electrophoretic deposition is a technique that allows obtaining reproducible nanostructured sensors with a low cost and shorter preparation time in comparison with LB sensors. These characteristics of the EPD a very competitive technique in the industry.

5. An array of EDP nanostructured sensors based on three different phthalocyanines has been constructed and successfully applied to the study of antioxidants.

    a. An array formed by three EDP films of $LnPc_2$ nanowires ($LuPc_2$, $DyPc_2$ and $GdPc_2$) has been developed for the first time.

    b. The array has been successfully used to detect hydroalcoholic solutions of four phenolic antioxidants usually found in wines: caffeic, gallic (with an ortho diphenol group in their structure), vanillic and ferulic acids (with hydroxyl group in their structure).

    c. PCA has shown a good discrimination capability according to the number of –OH group present in the aromatic ring 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.

    d. 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.

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

3.2. FUTURE TRENDS

The results and conclusions obtained during the present work suggest new research lines in the field of voltammetric sensors.

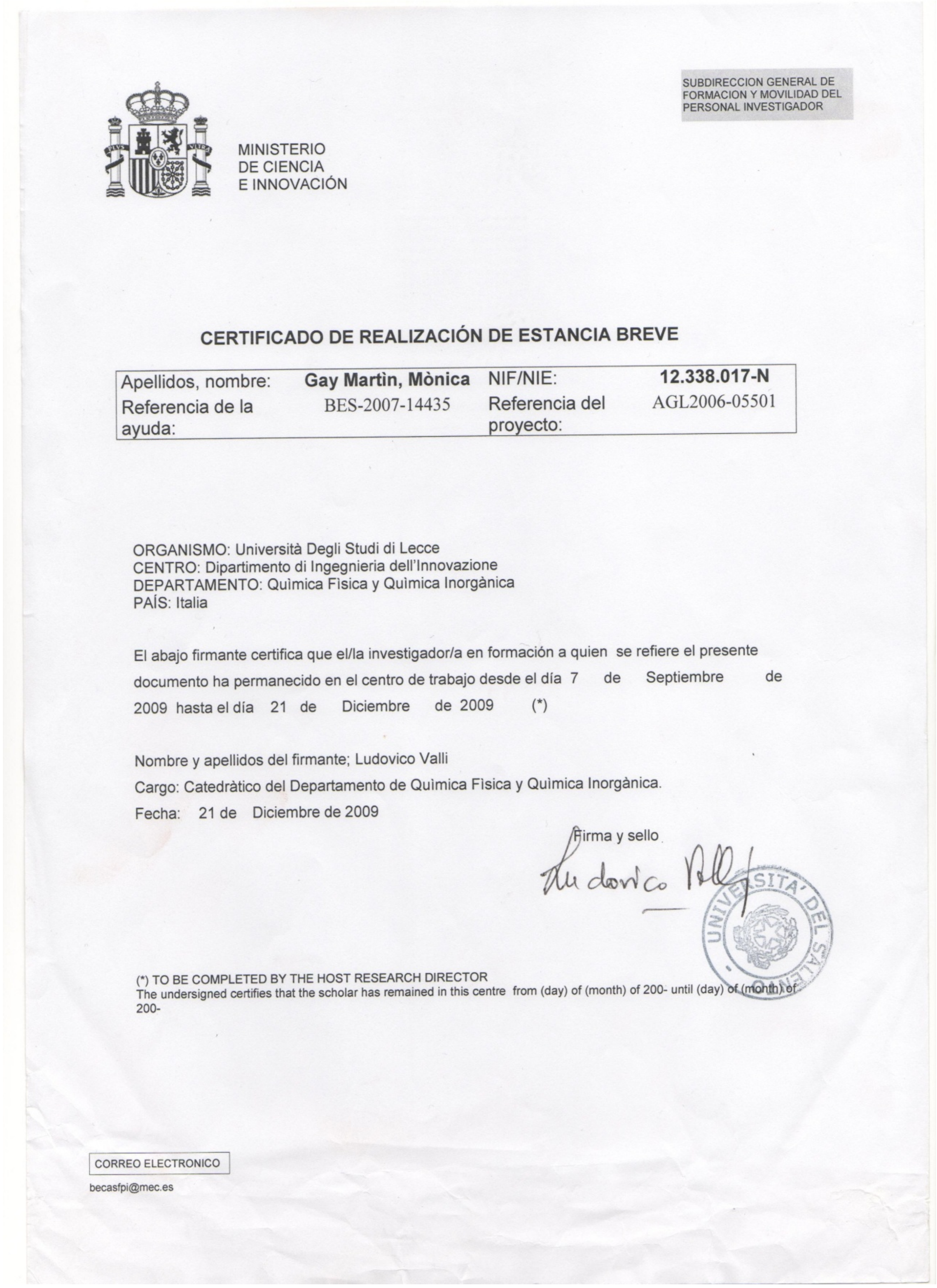
One of the interesting possibilities could be to extend the array of EPD sensors to other molecules with complementary electroactivity in order to reach a better discrimination of the studied solutions. In this sense, it would be interesting to investigate the electrodeposition of perylenes as electroactive compounds. Also, new antioxidants and other kind of complex liquids could be analyzed with these arrays of sensors.

Besides, the incorporation of enzymes as the electronic mediators between the bisphtalocyanine and the substrate can be also proposed. In this way, it would be interesting to construct a bioelectronic tongue formed by sensing units prepared by the LB technique in which different kind of enzymes combined with different kind of electroactive molecules. The specificity shows by the enzymes could be improving the selectivity of the sensors towards a certain kind of molecules.

On the other hand, to understand the mass changes that happen in the electrochemical process related to the modifier and also to the molecule analyzed, it would be interesting to use an electrochemical quartz microbalance. In this case, it would be possible to know the mass changes related to the redox process of the bisphthalocyanine deposited onto the quartz crystal as well as the antioxidant present in the solution in which is immersed the quartz crystal. In this way, the mechanism in terms of mass of both, electroactive modifier and antioxidant under study, could be analyzed.

Apéndice

Certificado de estancia en el extranjero.



SUBDIRECCION GENERAL DE
FORMACION Y MOVILIDAD DEL
PERSONAL INVESTIGADOR

MINISTERIO
DE CIENCIA
E INNOVACIÓN

CERTIFICADO DE REALIZACIÓN DE ESTANCIA BREVE

Apellidos, nombre:	**Gay Martìn, Mònica**	NIF/NIE:	**12.338.017-N**
Referencia de la ayuda:	BES-2007-14435	Referencia del proyecto:	AGL2006-05501

ORGANISMO: Università Degli Studi di Lecce
CENTRO: Dipartimento di Ingegnieria dell'Innovazione
DEPARTAMENTO: Quìmica Fìsica y Quìmica Inorgànica
PAÍS: Italia

El abajo firmante certifica que el/la investigador/a en formación a quien se refiere el presente documento ha permanecido en el centro de trabajo desde el día 7 de Septiembre de 2009 hasta el día 21 de Diciembre de 2009 (*)

Nombre y apellidos del firmante; Ludovico Valli

Cargo: Catedràtico del Departamento de Quìmica Fìsica y Quìmica Inorgànica.

Fecha: 21 de Diciembre de 2009

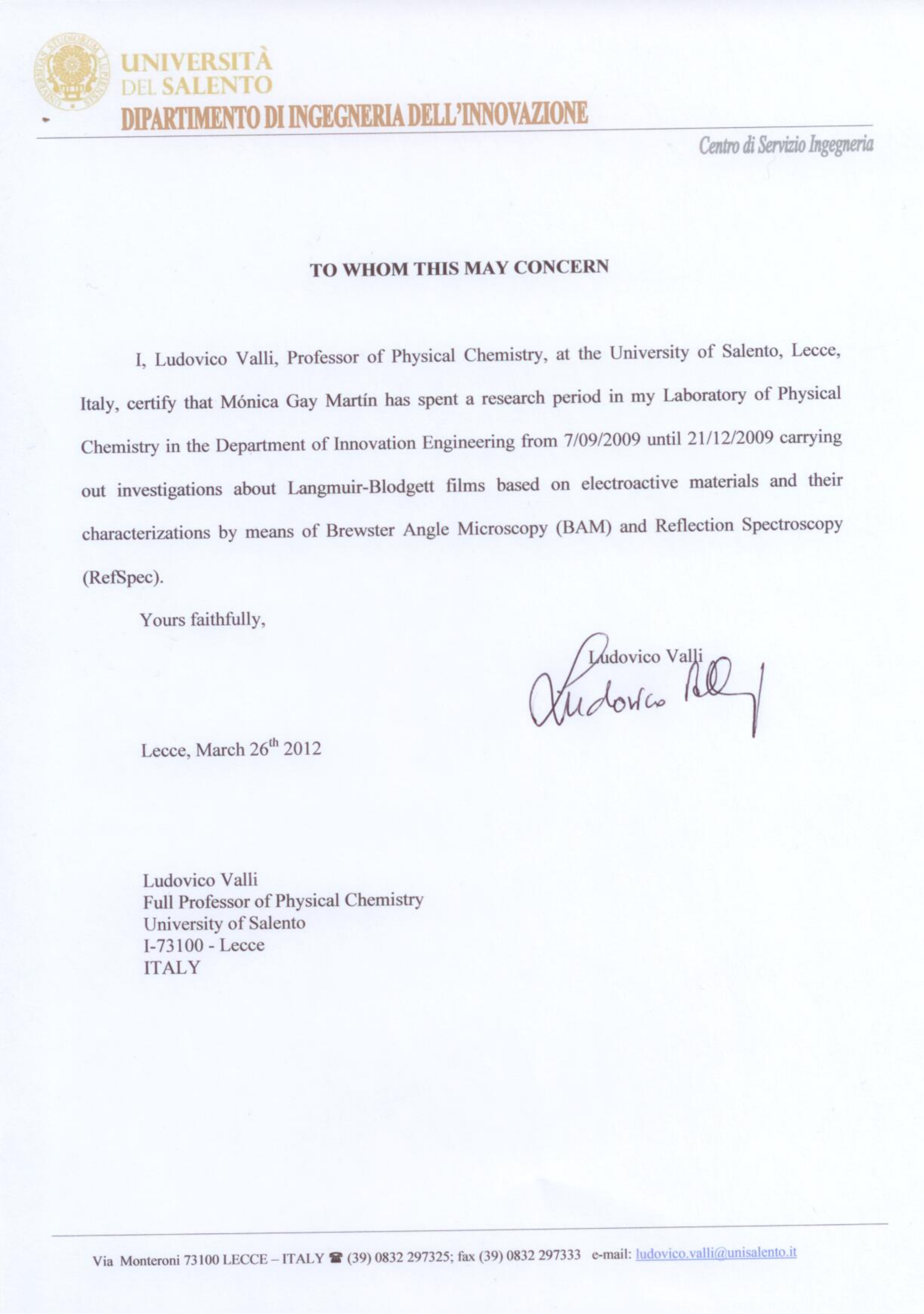
Firma y sello

Ludovico Valli

UNIVERSITA' DEL SALENTO

(*) TO BE COMPLETED BY THE HOST RESEARCH DIRECTOR
The undersigned certifies that the scholar has remained in this centre from (day) of (month) of 200- until (day) of (month) of 200-

CORREO ELECTRONICO
becasfpi@mec.es



UNIVERSITÀ
DEL SALENTO
DIPARTIMENTO DI INGEGNERIA DELL'INNOVAZIONE

Centro di Servizio Ingegneria

TO WHOM THIS MAY CONCERN

I, Ludovico Valli, Professor of Physical Chemistry, at the University of Salento, Lecce, Italy, certify that Mónica Gay Martín has spent a research period in my Laboratory of Physical Chemistry in the Department of Innovation Engineering from 7/09/2009 until 21/12/2009 carrying out investigations about Langmuir-Blodgett films based on electroactive materials and their characterizations by means of Brewster Angle Microscopy (BAM) and Reflection Spectroscopy (RefSpec).

Yours faithfully,

Ludovico Valli

Ludovico

Lecce, March 26th 2012

Ludovico Valli
Full Professor of Physical Chemistry
University of Salento
I-73100 - Lecce
ITALY

Via Monteroni 73100 LECCE – ITALY ☎ (39) 0832 297325; fax (39) 0832 297333 e-mail: ludovico.valli@unisalento.it