Analysis of grapes and wines using a voltammetric bioelectronic tongue

Correlation with the phenolic and sugar content

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Abstract—**Arrays of voltammetric sensors and biosensors based on phthalocyanines have been developed and used to analyze wines and grapes. Nanostructured sensors prepared by the Langmuir-Blodgett (LB) , Layer by Layer (LbL) or electro-deposition are superior because the high number of active sites. The LB and the LbL techniques can be used to co-deposit electrocatalytic materials to obtain a synergistic electrocatalytic effect. Moreover, LB and LbL can be used to immobilize enzymes in a biomimetic structure formed by amphiphilic lipids. Such sensors show an enhanced selectivity. The presence of phthalocyanines as electron mediators improves the performance of the sensors. Combining sensors and biosensors in the same array provides the advantages of classical phthalocyanine based-sensors, with the specificity of the enzyme-substrate reaction typical of biosensors. The selectivity of the electronic tongue and its capability of discrimination are clearly improved when biosensors containing glucose oxidase or tyrosinase are included in the array.**

Keywords—bioelectronic tongue; wine; must; voltammetric sensor; phthalocyanine

I. INTRODUCTION

The use of quick, reliable and cheap sensors for the detection of chemical compounds represents an important need in the wine sector. Polyphenols and glucose are among the analytes that need to be monitored and measured in order to guarantee the quality of final food products**.** A promising approach in food analysis consists in the use of electronic tongues which are multisensor systems formed by a number of low-selective sensors and use advanced mathematical procedures for processing the electrochemical signals based on pattern recognition and/or multivariate data analysis. The state of the art of electronic tongues has been the subject of many recent reviews [1]. During the last years, arrays of voltammetric sensors dedicated to the analysis of wines and grapes have been developed. They use a variety of electroactive materials such as conducting polymers or phthalocyanines among others [2]. Phthalocyanines have demonstrated to be a good choice as sensitive materials for voltammetric sensors due to their amazing electrocatalytic properties [3]. They can be deposited onto electrode surfaces using a variety of techniques that include simple and cheap procedures or advanced methods that produce nanostructured electrodes with enhanced performances [2]. Phthalocyanines can also be used as electron mediators in biosensors containing enzymes [4]. In this paper, the electronic and bioelectronics tongues based on phthalocyanines, are revised. The new strategies to improve the performance of the sensors using nanotechnology will be presented. Particular interest will be devoted to their performance and applications in the assessment of phenolic compounds and glucose and in the analysis of wines and musts. The working principle of a voltammetric electronic tongue based on phthalocyanines is illustrated in Fig. 1.

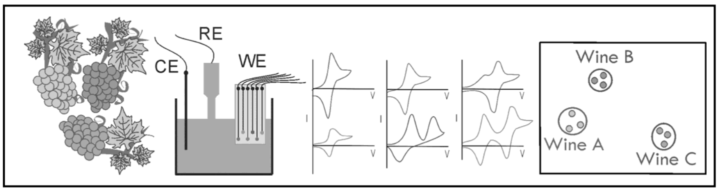

Fig. 1. Scheme of the working principle of a voltammetric electronic tongue based on electrodes chemically modified with phthalocyanines.

II. ELECTRONIC TONGUE BASED ON NON-NANOSTRUCTURED SENSORS

The first voltammetric electronic tongues based on chemically modified electrodes dated from 2003-2004 and involved sensors prepared by means of simple methods such as the carbon paste technique (CPE) where phthalocyanines are mixed with carbon powder and a mineral oil (Nujol) [5,6]. Deposition by spin-coating, drop casting onto conductive surfaces (platinum, carbon, ITO) or chemical modification of

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screen printed electrodes (SPE) was also used [7]. More recently, other simple electrodes have been prepared by immobilizing the phthalocyanines in an epoxy matrix [8].

Arrays of voltammetric electrodes chemically modified with phthalocyanines have demonstrated their ability to detect phenolic compounds [7-9]. When using such electrodes, voltammograms show redox peaks produced by the electrode material and peaks due to the redox compounds present in the sample. On the top of this, the interactions between the electrode and the solution (for instance, antioxidant properties of the solution affecting the redox properties of the electrode or electrocatalytic activity of the electrode modifying the redox behavior of the solution) produce shifts in the peak positions and changes in their intensity. Using these non-nanostructured sensors, each phenolic compound found in wines show a characteristic voltammogram [7,9]. The intensity of the peaks increases with the concentration. The limits of detection (LOD) calculated following the $3s_d/m$ criterion (where m is the slope of the calibration graph, and s_d was estimated as the standard deviation of the voltammetric signals at the concentration level corresponding to the lowest concentration of the calibration plot) attained for the detection of phenols is in the range of 10^{-5} mol.L^{-1} [7-9].

The variety of peaks and the cross-selectivity of the signals generated by an array of voltammetric electrodes are an advantage because curves provide improved information about the sample. For this reason, arrays of voltammetric sensors based on phthalocyanines, have been extensively used to analyze foods and beverages. These systems have been particularly successful in the field of wines. The reason is that voltammetric sensors are sensitive to acids and electroactive materials (i.e. polyphenols) and these compounds play an important role in the organoleptic characteristics of wines. E-tongues based on voltammetry have been able to discriminate grapes [10], red wines with different organoleptic characteristics [6], wines elaborated with different varieties of grapes [11], to detect adulterations **[**11**]** to discriminate among red wines with different antioxidant capability [8,13-14], to follow the ageing of red wines using oak chips [15] or to discriminate cava wines [16]. Good correlations have been found with chemical parameters related to the polyphenolic content and the pH [9, 13, 14].

The performance of the voltammetric electronic tongue can be improved following different strategies. They include (1) use of nanostructured sensors to improve the number of active sites (2) combination of electrocatalytic materials to obtain a synergistic effect and (3) introduction in the array of biosensors specific for the detection of phenols and sugars.

III. Electronic Tongues based on Nanostructured Sensors

A. Electronic Tongue based on Nanostructured Films

Nanostructured thin films have shown great potential in improving the sensitivity of chemical sensors. Ultra-thin films with high surface uniformity and enhanced surface to volume ratio show an increased number of active sites while allowing the analyte molecules to adsorb or desorb from the molecular sites more readily.

Arrays of sensors based on the nanostructured layers have been prepared by Langmuir-Blodgett (LB), Layer by Layer (LbL), Self-assembling (SAM) or by electrodeposition (EDP) [17-19]. The voltammograms are similar to those obtained in non-nanostructured sensors, but the peaks are better defined and the separation between the anodic and cathodic waves is reduced as a result of the improvement of the reversibility (Fig.2). Such nanostructured sensors show higher sensitivity, faster kinetics and better reproducibility than the corresponding non-nanostructured sensors [20]. The LOD attained are in the range of 10^{-6} mol.L^{-1}. Arrays of sensors have demonstrated to be able to detect and to discriminate among mono- di-and tri-phenols present in wines.

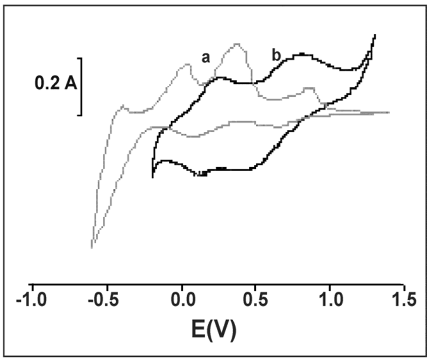

Fig. 2. Phthalocyanine electrodes immersed in citric acid 0.1 M: (a) LB film, (b) CPE.

B. Nanostructured Sensors combining Electrocatalytic Materials: Synergistic Effects

Metallic nanoparticles, carbon nanotubes or graphene have demonstrated to be efficient electrocatalysts for sensing applications [21, 22].The design of hybrid systems combining different electrocatalytic materials is very attractive for the development of electrochemical sensors [23,24]. Recently, it has been demonstrated that SWCNT and phthalocyanines have a synergistic effect in terms of improving electrocatalysis for the detection of ascorbic acid [25]. Similarly, AuNPs and phthalocyanines have been successfully combined to obtain electrochemical sensors with improved electrocatalytic properties [26]. The applications of these composite systems require the development of methods producing structures with controlled distribution of the two components. The LbL or the LB techniques can be an alternative to produce nanostructured multilayers where two or more compounds can be co-deposited in mixed films [27, 28]. The synergistic effect is promoted in nanostructured sensors as it has been demonstrated in LB films containing nanoparticles and phthalocyanines exposed to polyphenols. The electrochemical response was characterized by an increase in the intensity of the redox peaks and a reduction of the overpotential. LODs in the range of 10^{-7} mol.L^{-1} were attained. In spite of the interest of these systems, electronic tongues where sensors combine electrocatalytic materials have not been developed yet.

C. Biomimetic Electrodes: Bioelectronic Tongue

Electrochemical biosensors are an interesting alternative for the analysis of phenols and sugars due their high sensitivity and selectivity. They contain enzymes such as tyrosinase or glucose oxidase combined with appropriate electron mediators [29, 30]. Some attempts have been carried out to develop arrays of biosensors containing phenol oxidases for the detection of phenols (the so-called bioelectronics tongues) [31]. It has been demonstrated that arrays of biosensors combine the advantages of classical arrays of electrochemical sensors that provide global information about the sample, with the specificity of the enzyme-substrate reaction typical of biosensors. An electronic tongue formed by voltammetric sensors and biosensors containing phthalocyanines has been developed and used to analyze grapes of different varieties. The sensors are prepared using the carbon paste technique and have been chemically modified with different metallophthalocyanines. In turn, biosensors consist of carbon paste electrodes modified with phthalocyanines combined with tyrosinase or glucose oxidase. The capability of the system to discriminate grapes according to their sugar and their polyphenolic content has been evidenced using Principal Component Analysis (PCA) [32].

However, in these previous works only non-nanostructured sensors (prepared by classical techniques) have been used. LbL, SAM or the LB techniques are of special interest in the field of biosensors because using these methods enzymes can be immobilized in a nanostructured lipidic layer with structures similar to those of the biological membranes. This biomimetic environment can help to preserve the functionality of the enzyme [4,33]. A final advantage is that using these methods the enzyme and the electron mediator can be co-immobilized in a single sensitive layer, facilitating the electron transfer between the enzyme and the electrode.

An electronic tongue formed by nanostructured voltammetric biosensors (based on biomimetic LB films containing phthalocyanines combined with tyrosinase, laccase or glucose oxidase in an amphiphilic matrix) has been developed. The improvement in the performance has been evaluated by testing model solutions of catechol, glucose and musts prepared from grapes of different varieties: Tempranillo (T), Prieto Picudo (PP), Mencía (M), Cabernet (C) and Garnacha (G).

The biomimetic structure of these films enhances the performance of the biosensors. The electron mediator behavior of the phthalocyanines has been evidenced by an increase in the sensitivity of the sensors towards model solutions of catechol and glucose. For instance, using nanostructured biosensors based on tyrosinase, detection limits of 10^{-8} mol.L^{-1} for the detection of catechol have been attained. The PCA scores plot has demonstrated that the multisensor system is able to discriminate phenols according to the number of phenolic groups attached to the structure. In addition, each sensor provides a distinguishable electrochemical response when exposed to musts (Fig. 3). Using PLS1, good correlations have been found with the phenolic and glucose content measured by means of chemical analysis with correlations superior to 0.97.

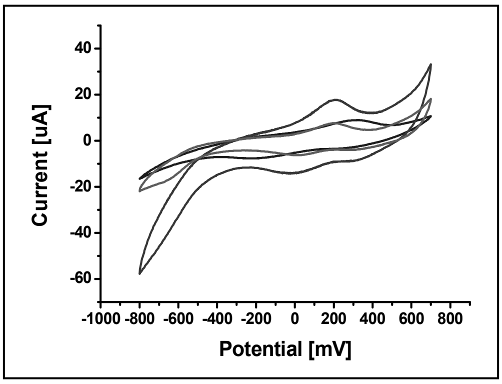

Fig. 3. Voltammetric response towards musts prepared from grapes of different varieties using a biosensor containing tyrosinase imbibed in a LB film.

The ability of the system to discriminate grapes according to their sugar and polyphenolic content has been evidenced using Principal Component Analysis where the positions of the clusters corresponding to each variety of grape is correlated to the polyphenolic content (Fig. 4). The selectivity of the multisensor system and its capability of discrimination have been clearly improved when biomimetic biosensors are included in the array. This good performance is due to the combination of four factors: the high functionality of the enzyme obtained using a biomimetic immobilization, the signal enhancement caused by the electron mediator, the improvement in the selectivity induced by the enzymes and the complementary activity of the enzymatic sensors demonstrated in the loading plots.

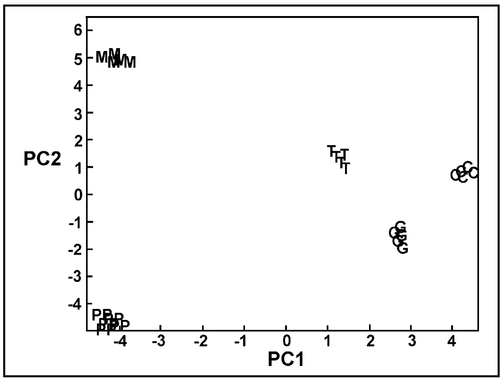

Fig. 4. PCA scores plot corresponding to the classification of the musts: T-Tempranillo, PP-Prieto Picudo, M-Mencía, C-Cabernet, G-Garnacha.

IV. CONCLUSIONS

Arrays of voltammetric sensors and biosensors have demonstrated to be a good alternative for the analysis of wines and musts. Sensors can be prepared following different strategies, but nanostructured sensors have demonstrated to be superior due to the enhanced sensitivity. The selectivity of the multisensor systems and their capability of discrimination is clearly improved when biosensors are included in the array. In particular, glucose oxidase or tyrosinase have been successfully immobilized in lipidic membranes using the LB or LbL techniques. This biomimetic environment preserved the enzymatic activity while improving the sensitivity. In summary, multisensor systems containing sensors and biosensors combine the advantages of classical arrays of sensors (which provide global information about the samples) plus the advantages of the selectivity provided by biosensors (which provide specific information about sugars and polyphenolic content).

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