EVALUATION OF BITTERNESS IN LIQUID SOLUTIONS BY USING AN ARRAY OF VOLTAMMETRIC SENSORS

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ABSTRACT

In this work, voltammetric sensors based on electrodes chemically modified with electroactive substances (conducting polymers and on bisphthalocyanines), capable to classify bitter liquids have been developed. Polymeric sensors have been obtained by electrodeposition of the corresponding monomers (3-methylthiophene, pyrrole and aniline) in presence of different doping agents. Phthalocyanines sensors have been prepared using carbon paste technique. The sensors have been immersed in model solutions of bitterness including $MgCl_2$, quinine, and four phenolic compounds extracted from olive oils (monoaldehydic form of oleuropein, dialdehydic form of oleuropein, monoaldehydic form of ligstroside and dialdehydic form of ligstroside). The influence of the chemical modifiers of each electrode in its electrochemical response has been studied. Finally, a sensors array has been constructed using those sensors with the best performances. The Principal Component Analysis of the registered signals has allowed the discrimination of the studied solutions.

KEYWORDS: *Electrochemical sensor, Conducting polymer, phthalocyanine, electronic tongue, bitterness*

1.Introduction

Taste and aroma development and selection of oral dosage formulations are challenging tasks for formulators in the pharmaceutical industry. To date, a main measurement method of taste intensity is the human sensory test. However, the use of sensory panelists is very difficult, not only because of the subjectivity of panel members, but also because of the potential toxicity of drugs. [1]

During the last years, multisensor systems for liquid analysis based on chemical sensor arrays and pattern recognition, which are known as Electronic tongues, have been developed [2-6].

Sensors exploiting various physico-chemical principles, such as optic, gravimetry and electrochemistry, can be employed in electronic tongues [7-11]. The sensor arrays most widely used for liquid sensing are based on electrochemical techniques like potentiometry [12–14], voltammetry [15–17], conductivity [18] and faradic impedance [19].

The literature shows that data analysis is typically performed via principal component analysis

(PCA), although some papers have even used artificial neural networks (ANN) [13–15, 19] and a few articles have employed partial least squares–discriminant analysis (PLS-DA) [16, 20, 21]. As regards the amperometric sensors employed, the literature typically reports that bare electrodes are used [9, 17, 22–25]. However, these electrodes suffer from many drawbacks when used in real matrices, especially those related to the electrode fouling. Electrode surface modification appears to be a promising route to overcoming such limitations [16]. Moreover, it seems reasonable to suppose that the presence of modified electrodes in a multisensor system could lead to a response pattern that contains useful complementary information.

These instruments employ electrochemical sensors coupled with chemometric methodologies, and they have generated great interest in laboratories as a fast, simple, and reliable method of taste analysis for beers [26], mineral water [27], coffee [28], milk [29] and environmental monitoring [30].

The objective of this work is to develop chemically modified voltammetric electrodes based on phthalocyanines and on conducting polymers, able to be used as sensing units of an electronic tongue and in particular, to discriminate bitterness. For this purpose, polymeric sensors based on polypyrrole (Ppy) have been obtained by electrodeposition of the corresponding monomer in presence of different doping agents. Sensors based on a series of bisphthalocyanines (LnPc$_2$) have been prepared using the carbon paste electrode technique (CPE). A multichannel taste sensor has been constructed after selecting those sensors with the best performances. The array of sensors has been exposed to model solutions of bitter flavour prepared from a range of compounds responsible of the bitterness of different foods and beverages. The capability of discrimination of the array has been evaluated by using Principal Component Analysis (PCA) of the obtained signals.

2. Materials and methods

Bisphthalocyaninate of lutetium (III) (LuPc$_2$) and the bis-[tetrakis (tertbutyl) phthalocyaninate] of praseodymium (III) (PrPc$_2^t$) were synthesised and purified in our laboratory following previously published procedures. The carbon paste electrodes were prepared by mixing the corresponding phthalocyanine (15% w:w) with carbon powder (Ultracarbon, Ultra F purity). Nujol was used as binder. Once prepared, 0.5 grams of the mixture were introduced in a plastic syringe (1 ml), and compressed. A copper wire was used as a contact. The CPEs were finally smoothed manually by a clean filter paper.

Sensors based on polypyrrole, were obtained by electrochemical polymerization onto a platinum disk (1mm diameter) from an 0.1 M aqueous solution of the corresponding monomer using potassium ferrocyanide (II) trihydrate (K$_4$Fe(CN)$_6$·3H$_2$O), p-toluenesulfonic acid monohydrate (pTOL), sodium 1-decanesulfonate, (DSA) and sodium tetrasulphonate-phthalocyaninate of nickel (II) (NiPcTs) as doping agents. Electrochemistry was performed using an EG&G PARC Model 263 potentiostat/galvanostat. The chemically modified sensors were used as the working electrode in a conventional three electrode cell. Pt plate was used as the counter electrode and Ag/AgCl electrode as the reference electrode.

The artificial bitter solutions were obtained by solving well known substances responsible of bitterness in foods and beverages in ultra pure water (MilliQ). As bitter substances, MgCl$_2$, quinine-hydrochloride, and four important phenolic compounds (monoaldehydic form of oleuropein (MAOL), dialdehydic form of oleuropein (DAOL), monoaldehydic form of ligstroside (MALI) and dialdehydic form of ligstroside (DALI) solved in 0.2M KCl were chosen. Principal Component Analysis (PCA) was carried out using Matlab V. 5.3.

3. Results and discussions

The initial experiments were focused to develop preparation procedures of the electrodes directed to optimise the method in terms of stability and reproducibility of the signals. Then, work was carried out to characterize the electrodes and to explore whether electrodes produced characteristic responses to the compounds under study. In Figure 1 were presented the CVs of voltammetric electrodes immersed in aqueous 0.2 M KCl.
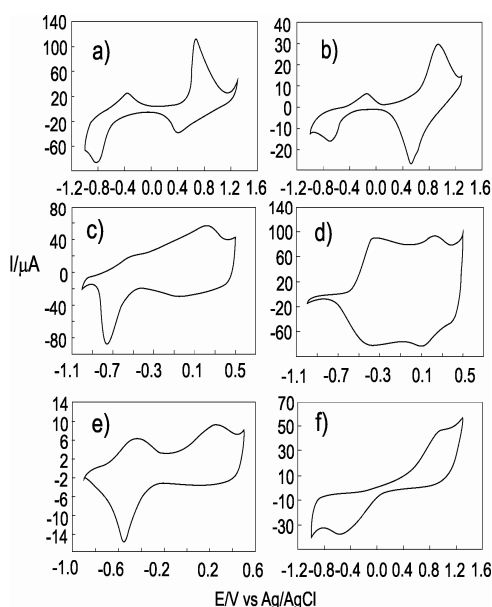

Figure 1. *Cyclic voltammetry of (a) LuPc$_2$ (b) PrPc$_2^t$ (c) Ppy-Tol (d) Ppy-FCN (e) Ppy-DSA (f)Ppy-NiPcTs electrodes immersed in KCl solution. Scan rate 50 mV s^{-1} .*

As expected, the CVs of the bisphthalocyanine-based electrodes showed definite anodic and cathodic waves due to the oxidation and reduction of the phthalocyanine rings. In good correlation to the results found in gas sensors, the electrochemical behavior is directly correlated to the nature of the metal complexed in the phthalocyanine core and to the presence of substituents in the phthalocyanine ring.

The redox transformations observed in phthalocyanine CPE electrodes 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 test solution and the electrochemical responses can be used to distinguish the model bitter solutions.

For instance, the response of a sensor based on LuPc$_2$ immersed in a 0.1 M solution of MgCl$_2$ is different to that observed for KCl. In this case, the peaks corresponding to the reduction of the phthalocyanine ring are much less intense due to the difficulty of bivalent ions Mg^{2+} to diffuse inside the electrode to compensate the negative charge. Important changes are also observed when immersing the CPE electrodes in bitter solutions obtained from organic compounds such as quinine, MAOL, DAOL, MALI and DALI. In this case, the shifts are more pronounced and also new peaks are observed, due to redox processes concerning the organic compounds (Figure 2.a and 2.b).
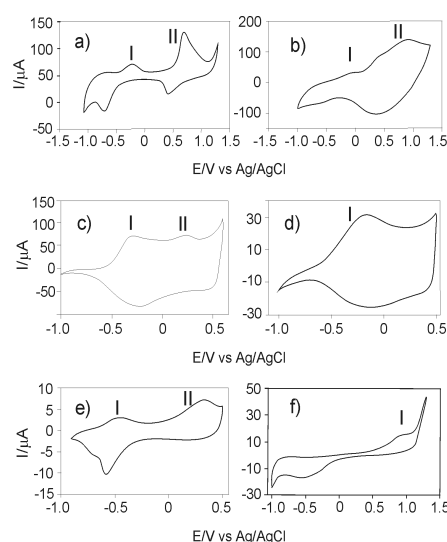

Figure 2. *Cyclic voltammetry of (a) LuPc$_2$ (b) PrPc$_2^t$ (c) Ppy-Tol (d) Ppy-FCN (e) Ppy-DSA (f)Ppy-NiPcTs electrodes immersed in the DAOL solution. Scan rate 50 mV s^{-1}*

In order to obtain polymeric sensors with improved electrochemical response, a series of Ppy electrodes doped with a range of doping agents were prepared. When Ppy films were doped with conventional counterions such as ClO_4^-, $CF_3SO_3^-$, or BF_4^-, the cyclic voltammograms obtained showed small and irreproducible peaks in the studied range (-1.0 V to 0.5V). The process of reduction and oxidation of the PPy is also understood in terms of the transport of ions charge carriers in and out of the polymeric film necessary for preserving the macroscopic electroneutrality of the layer. These ionic movements can lead to the dedoping of the polymeric layer causing the irreproducibility of the observed peaks.

In order to obtain reproducible polymeric films one possibility is to prepare sensors by using large doping anions which can be permanently captured in the polymer layer. In order to explore this possibility, large surfactant doping anions such as sodium 1-decanesulfonate (DSA) or other large doping agents including acid p-toluenesulfonic (pTOL and sodium tetrasulphonate-phthalocyaninate of nickel (II) (NiPcTs) have been used.

Ppy electrodes doped with large doping anions show two redox processes. In the first process, a partial oxidation of the PPy film takes place with cations (K^+) being expelled. The film behaves as a cation exchanger. In the second process, occurring at higher voltages, the PPy film is gets highly oxidized and anions (Cl^-) from the electrolyte are inserted into the film to maintain the charge balance. Thus, during this process, the film behaves as an anion exchanger.

An interesting new type of Ppy is where redox anions such as hexacyano-ferrate are used as dopant. It can be expected that these films not only behave as a good redox electrodes but also display the electroactivity of the dopant anion and show sensitivity to the size of cations present in the solution phase. In this case, when the film was immersed in KCl, it was observed that a well defined, stable oxidation-reduction pair appeared at $E_{1/2}= 0.16$ V on top of a large current connected to the partial redox transformation of the polymeric film itself.

When Ppy films are cycled in aqueous electrolytes, movements of both anions and cations take place during redox reactions For this reason, it can be expected that the nature of the tested solution influences greatly the response of the polymeric electrodes when immersed in liquids.

This is illustrated in Figure 2, where the responses of polymeric electrodes towards DAOL are shown. It can be observed that each electrode produces a particular response towards the model solution providing a certain degree of selectivity.

In order to evaluate the repeatability of the sensors consecutive cycles were recorded in 0.2 M KCl. The first 1-5 scans were slightly different from the subsequent ones. It is important to point out that regardeless the doping agent introduced in the Ppy films, the stability of the electrodes is strongly related to the scan range studied: when the vertex potential does not exceeds + 0.50 V, subsequent voltammograms does not differ from each other. However, at potentials higher than +0.60 V, overoxidation of polypyrrol film takes place. The polypyrrole is oxidised to a higher oxidation state in which it is susceptible to nucleophilic attack.

In Figure 3 were presented six subsequent CVs of Ppy/ BF_4^- and Ppy/DSA, respectively, immersed in KCl solution. As can be observed, the response of the sensor is stable when the counter anion is a large anion, which cannot be expelled from the polymer matrix during redox processes. An unstable response is observed in the case of Ppy doped with BF_4^- anion.

Subsequent scans were highly reproducible and after 100 scans the only noticeable change was a small and gradual decrease of the intensity of the peaks.
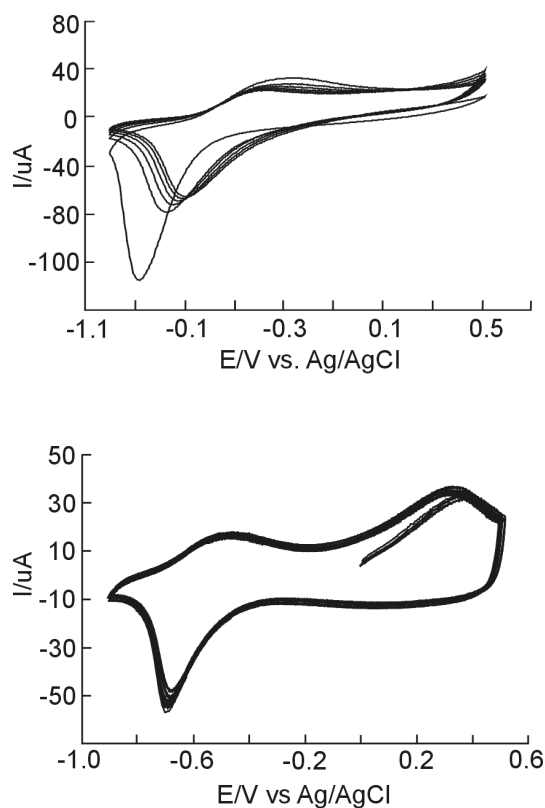
Figure 3. *Six subsequent CVs of Ppy/ BF₄⁻ (left) and Ppy/DSA (right) immersed in KCl solution.*

As it has been previously demonstrated, each electrode provides a particular signal when immersed in each model solution, that could be used as a fingerprint to discriminate the six types of bitterness (Figure 2). In order to evaluate the discrimination capabilities of the voltammetric sensors, PCA was conducted by combining the information obtained from electrodes based on phthalocyanines and on conducting polymers.

The high amount of information displayed by the voltammograms, makes desirable to use the whole curve as the input variable for PCA. The use of the whole curve has the disadvantage of the high number of I,E pair values to be processed, making difficult the data analysis and increasing the processing time. A method has been developed in our laboratory that allows reducing the number of variables to a few representative values by using mathematical functions

(kernels) that capture the information along the dynamic characteristics of the global response.

As shown in Figure 4, using this system, the PCA analysis allow to obtain well defined ellipses. The separated clusters indicate that the six bitter solutions can be clearly discriminated from each other. Is has to be noticed that the cluster corresponding to the ionic MgCl₂ appears far apart from the clusters corresponding to the organic solutions. The cluster corresponding to quinine is also well separated from the rest. Finally, clusters corresponding to the four MALI, DALI, MAOL, DAOL appear close to each other, but well separated.
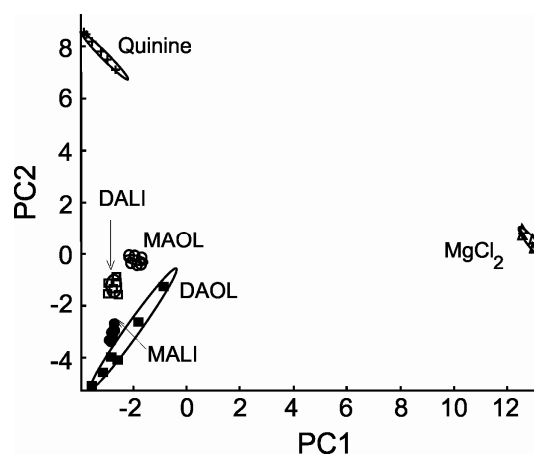

Figure 4. *PCA score plot of the response of the array of sensors towards bitter solutions*

4. Conclusions

A new type of voltammetric sensors based on electrodes chemically modified with electroactive compounds such as phthalocyanines or doped polypyrrole has been reported. Such sensors give rise to complex curves that show peaks related not only to the oxidation-reduction of the chemical species present in the tested solution but also to the oxidation-reduction of the sensitive material deposited onto the electrode. In addition, the chemical properties and

hence the sensing properties of these electroactive materials can be modified at the chemical level to form sensors with cross-selectivity and good stability. For instance, Ppy based sensors with improved electrochemical response can be obtained, by doping the electrode with large anions such DSA or with redox ions such as $[Fe(CN)_6]^{2-}$.

An array of sensors has been constructed by selecting those sensors with the best performance in terms of stability and cross-selectivity. The array has been exposed to model solutions of bitter flavor prepared from a range of organic and ionic compounds. The PCA analysis has confirmed the good performance of these electrodes towards bitter solutions.

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