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Review

Electronic tongues employing electrochemical sensors

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Abstract

This review presents recent advances concerning work with electronic tongues employing electroanalytical sensors. This new concept in the electroanalysis sensor field entails the use of chemical sensor arrays coupled with chemometric processing tools, as a mean to improve sensors performance. The revision is organized according to the electroanalytical technique used for transduction, namely, potentiometry, voltammetry/amperometry or electrochemical impedance. The significant use of biosensors, mainly enzyme-based is also presented. Salient applications in real problem solving using electrochemical electronic tongues are commented.

Keywords: Electronic tongues, Sensor arrays, potentiometry, voltammetry, amperometric sensors, impedimetric sensors, biosensors.

1. Introduction

This review is dedicated to electronic tongues in electroanalysis; therefore its scope is limited to the use of electrochemical sensors. It spans approximately the last decade, since this is the time elapsed since their birth. Anterior developments, cantered in the keyword “sensor arrays” may be consulted in two ancient reviews by Diamond [1] and Stefan et al [2]. The revision presented does not intend to be exhaustive; although complete literature searches have been performed, it has been preferred to limit it to the main research literature sources and books, pointing out significant contributions and sketching the landscape rather than depicting it in full detail. For extensive revisions, readers may consult the recent review of Ciosek and Wróblewski [3], that comprise most of the work related to the field. Recently, Zeravik and co-workers presented also an interesting review, in this case directed towards the analysis of wines [4].

To introduce the contents of this review, first it is necessary to comment what is understood for an electronic tongue. According to the recent IUPAC definition [5], an electronic tongue is “a multisensor system, which consists of a number of low-selective sensors and uses advanced mathematical procedures for signal processing based on Pattern Recognition and/or Multivariate data analysis – Artificial Neural Networks (ANNs), Principal Component Analysis (PCA), etc”. Therefore, the electronic tongue is an analytical system applied to liquid analysis formed by a sensor array in order to generate multidimensional information, plus a chemometric processing tool to extract meaning from these complex data. As agreed by the first laboratories working in this field [6], an electronic tongue has some specific connotations, namely, “it comprises an array of non-specific, poorly selective chemical sensors with partial specificity (cross sensitivity) to different compounds in a solution, and an appropriate chemometric tool for data processing”. In fact, the feature of the cross-response (or cross-sensitivity) is of main relevance here, as there is where the power of the proposed approach lies in. Conceptually it is also of main concern, as cross-sensitivity is a characteristic of the organization of animal senses. For example, in the sense of smell, animals do not possess

the thousands of receptors needed to distinguish between thousands of aromas and scents; in fact, the situation is completely the opposite: receptors group together and just differ slightly, merely giving a bit more response to certain species than its neighbour. To a certain scent, the smell sense supplies a complex response pattern, formed by a large set of individual, cross-sensitive responses. After this, it is the brain the one that processes the complex data, and accomplishes the recognition, identification or quantification. In this fashion, receptors may respond to several stimuli of different nature, being this global approach the way to identify hundreds or thousands of situations from a limited number of receptors [7]. This underlying strategy, also present in the taste or touch senses, is the one that inspires electronic noses and electronic tongues, to furnish a very rich information landscape of sensor readings that makes possible the accomplishment of special applications [8]. Electronic noses were the first biomimetic sensor systems, because they were clearly inspired in animal olfaction, and quickly permitted the development of interesting applications in different fields [9].

There is no doubt that main stream research in the sensor field is the improvement of selectivity, the foremost analytical property if one needs to integrate sensor use in a stand-alone operation, without any sample pretreatment or elimination of interferents. The novel complementary, bioinspired way to do this is the use of an array of non-specific sensors, responding to primary ions and interferents, coupled with the multivariate chemometric treatment of the obtained complex data to extract the different components present [10]. The purpose of the processing tool differs depending on the application, it can be to identify a chemical species or to determine its concentration without having to eliminate interferences or quantify them at all. The key point is that the multidimensional generated data has to comprise the needed information about the system and that the high order data has no colinearity (thanks to the cross-sensitivity assumption). Since all the sensors used in the array may respond to all the analytes, a great amount of complex data is generated that must be processed using a multivariate calibration approach. Chemometrics is in charge then, for the extraction of significant features and for the transformation into the sought information. This coupling of

chemometrics and electrochemical sensors, already identified as one of the recommended ways to improve sensor performance [11], is representing also a consolidated line of research in the electroanalysis field.

About the different processing tools used with electronic tongue systems, although being one of their chief parts, their detailed description is out the scope of this paper and they will be just mentioned here; a proper exposition can be consulted in the review by Richards et al. [12]. Chemometric tools used in electronic tongues are essentially the same as those used in electronic nose systems. Basically, the non introduced reader needs to know that certain processing algorithms are more indicated to identification/classification purposes, such as Principal Component Analysis (PCA), and are therefore used for qualitative applications. Other procedures, for example regression using Partial Least Squares (PLS), are specially devised for quantification purposes, and mainly used for multidetermination applications [13]. The mentioned chemometric variants are conventional pattern recognition techniques, mainly linear in nature, which can be somehow limited if the sensors considered show non-linear response trends. To improve results, researchers have proposed the use of Artificial Neural Networks (ANNs), which is a massively parallel computing technique, specially suited to non-linear sensor responses and very much related to human pattern recognition. Concerning the experience in our laboratory, we are most in favour of the use of ANNs, as these are very powerful modelling tools, amenable to both qualitative and quantitative applications [14]. An interesting chemometric tool of recent use in electronic nose/electronic tongue applications is Support Vector Machines, a radically different type of “learning machine” with high identification/classification capability [15].

1.1 Advantages of using sensor arrays

Sensor arrays are a clear example of integration in electroanalysis. Apart from joining together different analytical elements in one piece, the use of sensor arrays leads to

certain advantages [16]. In first place, sensor arrays provide multicomponent data at no extra effort. For the same sample processing, different analytes may be determined in parallel if selectivity is a premise. If not, the use of multicomponent data makes possible the interpretation of complex compositions, the resolution of mixtures, the deconvolution of the contributions from primary species and their interferents, or even, to distinguish between spurious responses and the true ones. An additional reason for the popularization of sensor arrays may be due to the recent availability of multichannel electrochemical instrumentation, probably installed in a laboratory PC, facilitating in this way storage, visualization and processing of complex data.

In the use of sensor arrays, one may distinguish three main variants, depending on their main purpose, and this is determined if sensors responses obtained are equivalent, completely independent or cross-selective.

1.1.1 Arrays of redundant sensors

The use of redundancy in the sensor field is a sample of intelligent use of sensors in order to diagnose malfunctions and/or detect the degradation of the sensor responses. It normally employs a set of equivalent sensors, that is, responding to the same species, that can be identical or with different transduction schemes. Its use can provide extra confidence in the final measurements, validation of results or confirmation that no technique-dependent matrix effect is present. A second possibility, somehow connected to the previous one, is to perform fault detection of an analytical system. In principle, a redundant sensor system provides results with improved precision and stability than the individual sensor counterpart. In fact, the use of a redundant sensor array can provide means for self-diagnosing the obtained results. If a single sensor is malfunctioning (for instance, through a faulty electrical connection, its membrane degradation, presence of air bubbles, etc.), it can be identified as an individual fault, the related response can be automatically discarded from the pattern, and its replacement/maintenance can be called for. The opposite case may be when the whole set is yielding abnormal responses, that

may be attributable to the fault of the operating system (liquid leakage, faulty sample introduction, abnormal electrical noise, etc.). The intelligent use of this information in real-time, employing automatic control routines may improve substantially the performance of analytical systems [17]. There is still a third possibility, which implies the use of a set of equal sensors in parallel, which can be used for concentration mapping. If the sensors are distributed in space according to a predefined pattern, after the measurement, a concentration profile or 2D imaging of a certain analyte can be obtained.

1.1.2 Arrays of independent sensors

The typical multidetermination variant uses a set of sensors, meaning each to be specific in order to perform a number of analysis in parallel, thus with a gain in sample processing. A single operation with a sample will yield a battery of results, an interesting variant when the objective is its characterization. Additional advantages include reduced use of reagents, power and space, and lower cost. A typical example may be the use of an array of Ion Selective Electrodes (ISEs) placed in series to perform a multiple determination, i.e. the determination of alkaline and alkaline-earth cations. As a certain degree of interference (or cross-response) occurred among the different sensors used, separation (for example chromatographic) can be employed to minimize any cross-response effect [18]. A simpler example was the three-analyte determination of Na^+, K^+ and Cl^- in sweat, in search for the diagnostic of cystic fibrosis from newborn babies, which provided a much more confident diagnostic than the normally performed tests based only in conductivity [19]. As commented, this approach will only yield correct results if the condition of specificity or absence of interferences is fulfilled, i.e. each sensor responds to a single component in a multi-component mixture. Nevertheless, there are the novel successful variants of this approach, the parallel affinity arrays, both of nucleic acids (DNA or RNA) or of antibodies; of these, the most successful devices are the automated microarray systems, normally using optical transduction, i.e. fluorescence, although electrochemical sensor based variants have been also described [20].

1.1.3 Arrays of cross-sensitive sensors

This third variant, the bioinspired one, is the base of electronic tongues. In an ideal situation, a sensor array containing a set of sensors with slightly different sensitivities (those non autocorrelated) may bring an overdetermined system which should make possible the simultaneous determination of a number of analytes in a complex mixture. For this purpose, the limited selectivity of each individual sensor may be compensated through the data processing, thus allowing the determination of a species in presence of its interferents. Apart, we must take into account that some of the recent needs for analytical information do not refer to a high precision quantitative determination of a certain compound, but to an overall effect or perception, in order to take a quick decision; examples of these are freshness of food, toxicity, smell, taste, pollution, etc. These situations are to be ruled by identification or classification algorithms, i.e. data processing treatments pertaining to chemometrics. In this way, initial electronic tongue systems, employed sensor arrays of non-specific solution sensors together with pattern recognition tools, such as PCA, and were applied to qualitative analysis of different beverages, in an application mimicking human taste.

The first electronic tongue contributions originated from the collaboration of two sensor groups in Europe, Prof. Vlasov laboratories in Saint Petersburg University and D'Amico laboratories in Tor Vergata University in Rome [21], who employed arrays of ISEs. Shortly after, the Swedish group from Prof. Winquist in Linköping proposed an equivalent system that permitted classification of solutions with different redox properties, this time from voltammetric measurements originated in a number of different metallic electrodes [22]. These initial systems showed an interesting possibility, which can be termed the *software sensor;* when there is a need of information from a species whose sensor is unavailable, and in absence of any other possibility, one can employ information from a number of sensors, and try to correlate available information with concentration sought. This indirect methodology can be suited for complex analytical determinations (for example, organic matter), or for those of general nature, such as

toxicity, degree of maturity, etc., and obviously is forced by a need of quick, automated generation of chemical information. From this moment on, a main field of application of electronic tongues (and also electronic noses) was the food and beverage analysis. Most applications described included identification and classification of beverages and food variants or perceptions of food quality, and have been specially applied in the food industry as a mean to provide an automated equivalent to the sensory panel of human experts [23].

The uses of electronic tongues extended quickly to neighbour fields, for example to water monitoring applications. The functionality also gathered new variants, including the recognition, identification, classification and quantitative determination of multiple component concentrations [24]. Thanks to these range of possibilities, electronic tongues are starting to prove useful as quality control devices in the food industry/environment along with a few other applications such as process monitoring and clinical analysis. This review will focus from now on in this type of biomimetic sensor systems; and what has picked up special attention is when the use of an array of different sensors brings any added value, i.e. some new information, different to the individual concentrations yielded by each single sensor. This can be the imaging of a concentration profile from a multichannel portrait, or some error prevention ability gained along the process. The potential of these new tools lies in the new types of analytical information obtained, different from the simple sum of individual signals; examples of these are the correction of an interference effect, the identification of a sample variety or the correlation with a perception from a panel of sensory experts.

2. Types of electronic tongues

In an electronic tongue, an array of sensors generates multidimensional information after presenting the sample; the generated information, complex in nature, needs to be processed according to the sought chemical information. As several possibilities exist on

the nature of sensors forming the array, the general response shown by the different sensors used to the studied case is of paramount importance; that is, the electronic tongue application needs the cross-selectivity features in order to take profit of the multidimensional aspects of the information [5]. The type of application can be qualitative, like identification of species, classification of sample varieties, or recognition of an adulteration episode; on the other side, the application can be also quantitative, normally the multidetermination of a set of chemical species, an interesting objective for process control. A different, more bioinspired trend is the *artificial taste* concept [25] devised with the goal of performing automated taste perception, especially in the industrial field. This idea, initiated by the works of Prof. Toko in Japan [26], consists in developing a sensor array inspired in the tongue's papillae and/or responding to the basic taste types (sweet, sour, salty and bitter, plus the far-east concept *umami*, which meaning is delicious); its goal is to mimic analogues of human taste assessment in cases where a human expert is not a possible choice, like in automatic control, poisonous/extreme condition samples, etc. Again, in order to keep this review focused on the most conventional electronic tongue systems, this variant will be just treated as a conventional electronic tongue with sensors (normally ISEs) trying to reproduce human taste perception [27].

Therefore, the present review will be organised according the different families of chemosensors and biosensors that may form the sensor array, which comprises potentiometric, voltammetric and impedimetric transduction. This classification is done according to the nature of the primary signal, whether after an interaction with the analyte, a potential, electrochemical current or resistivity/capacitance change is generated. The potentiometric type can contemplate sensor arrays using ISEs and/or ISFETs. The voltammetric variant implies the use of voltammetric sensors, that can generate the multidimensionality in different ways, for example, with a number of electrodes polarized at different fixed potentials, or with a full voltammetric scan per sensor used. This variability may be also found with impedimetric sensors, as a number of the latter can be used at a fixed AC frequency, or a full frequency spectrum can be recorded per sensor used.

If a literature search is performed with the terms ‘electronic tongue’, it can be observed that the number of articles has been growing steadily since the initial works dated in 1997, although it seems to stabilize in the last years. These trends have been summarized in Figure 1. The number of articles in the most recent years, is ca. 40 per year – an additional observation is that nearly all the records documented per year correspond to electrochemical families; apart from those mentioned above, a few electronic tongues have been proposed employing optical sensors and piezoelectric mass sensors. Lastly, it can be commented that, at the very beginning, most of the works were related to arrays employing potentiometric sensors, while more recently, this variant accounts only for around the half of contributions. That is, as more research groups have entered the field, the different variants are more equally divided than at the beginning and the more diverse applications are covered. In brief, one may conclude that the research field of electronic tongues is now mature.

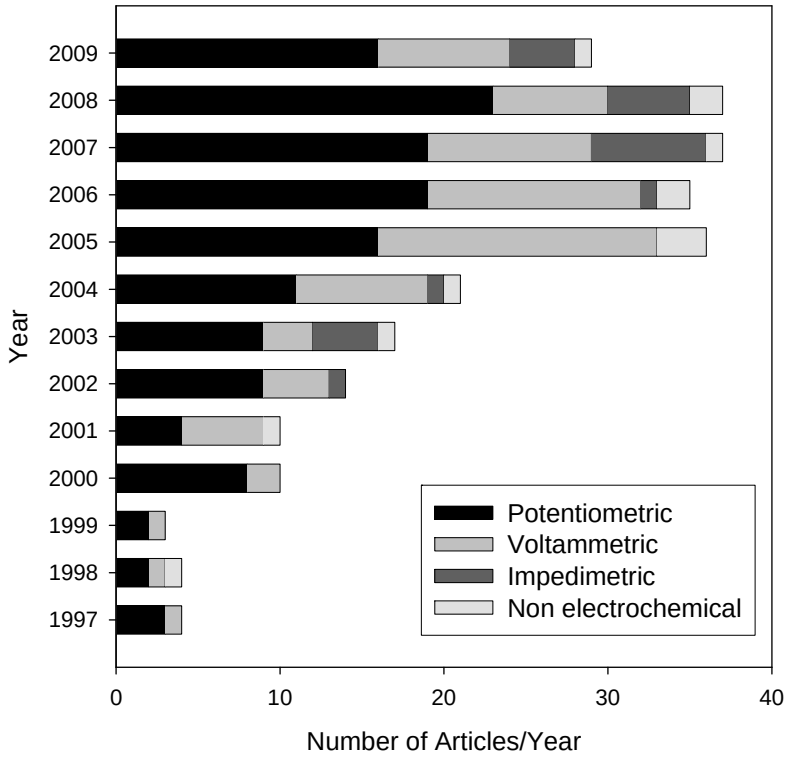

Fig. 1. Evolution of the number of publications referred to the term “electronic tongue”, with its breakdown to the different sensor families. Information extracted from Scopus (Elsevier B.V).

2.1 Electronic tongues employing potentiometric sensors

Traditional evolution in the field of sensors and biosensors has followed the path of continuous search of better selectivity, i.e., specific sensors. This goal has rarely been attained, although affinity biosensors, those employing the recognition abilities of antibodies or DNA, practically hit the mark. What is widely available to the analytical community is a vast number of reasonable combinations of sensors well performing in a specific situation, although their ideality is not always fulfilled. As paradigmatic example, there is the glass electrode, the universally used ion selective electrode for hydrogen ion determination. This paradigm in sensors commercial success has the sometimes forgotten sodium effect, caused by the interference of sodium ion at extremely alkaline pH and its use is discouraged in this situation. This reminder is then to point out that many widely accepted ISEs have known interferences, which nobody should forget when devising a new application.

For a given case study, one can devise a sensor array incorporating ISEs with appropriate membranes, taking in consideration the need for the cross-response condition. Using arrays formed by chalcogenide glass ISEs and PVC membrane ISEs, different electronic tongues were conceived, for qualitative identification of beverages or recognition of their aging and other episodes [21], normally through the use of PCA. Similarly, the simultaneous multidetermination of metals was successfully demonstrated in mine leachates, as well as physiological ions in simulated human plasma [8]. Conventional potentiometric PVC membranes can be used to form the sensor arrays if their formulation is properly considered. An example studied in our laboratories is the environmental determination of ammonium with nonactin-based ISEs, a case where the interference from other alkaline ions, mainly sodium and potassium can be expected – the approach was then to include the potential interferents in the array, and perform the multidetermination of the species of interest plus its interferents, compensating in each case the presence of its complementaries [28]. The number of sensors used was 8, and the processing tool selected was a backpropagation ANN, having as its outputs the

different concentrations of the species sought [24]. The scheme of the concept employed is sketched on Figure 2, which was later fully applied in an environmental monitoring application with unattended operation [29]. In fact, it was already known that the calibration of several ions in the presence of interferences could be carried out using an experimental design and multivariate calibration methods. But as commented, when the ISEs corresponding to the ion to be determined (primary ion) are subject to interference, multivariate methods allows for the establishment of a calibration which would not be possible by univariate methods. This has been demonstrated in the simultaneous determination of halide mixtures from data coming from an array of commercial ISEs employing Principal Component Regression (PCR) or PLS [30]; the first significant use of ANNs with an ISE array was demonstrated in the seminal work by Van der Linden [31].

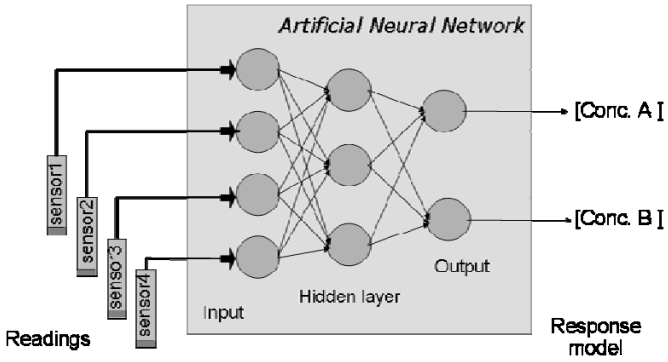

Fig. 2. The electronic tongue concept applied for multidetermination employing an ANN model.

Using an equivalent array of PVC ISEs, somewhat enlarged to nine different membrane formulations, a qualitative application was planned, in which different beverages such as waters, teas or orange juices could be classified [32]. An interesting result shown here was the prediction of the amount of natural orange juice present in orange beverages having a large range of variation. This property can be taken as example of the *software sensor* mentioned before, given it is a complex feature, attainable only with difficulty from standard chemical analyses.

In an equivalent context of use of ISEs with PVC membranes, there is the active group of Prof. Wróblewski in Warsaw University of Technology (Poland), who accomplishes the cross-sensitivity condition of their sensors preparing membranes with mixtures of ionophores, instead of using a conventional membrane of just PVC+plasticizer+ionophore. For example, they normally use a 1-charge cation selective membrane from a valinomycin plus a commercial sodium ionophore, or a mixed anion response sensor incorporating at the same time a fluoride and a dihydrogenphosphate ionophore. multidetermination of ions [33]. Their sensor system was mainly applied to identification of different types and brands of certain families of beverages, such as milk, beer, orange or apple juices, mineral or tonic water, etc. Moreover, they have tested extensively and compared many of the available chemometric procedures, such as PCA, PLS, nearest neighbour and ANN-based identification [13].

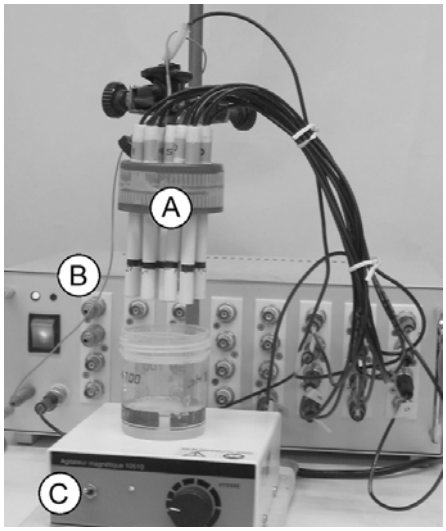

Fig. 3. Array of sensors used in the experimental setup of potentiometric electronic tongues in our laboratory. A, all-solid-state PVC membrane sensors; B, computer controlled high impedance multichannel potentiometer; C, magnetic stirrer.

Precisely commenting on the different variants of the sensors employed in potentiometric sensor arrays, it has to be said that almost all technologies have been used for its construction. The simplest range from the conventional ISE with internal reference

solution [33], or the use of all-solid-state configurations, where the PVC membranes are deposited over a polymeric graphite contact [34], and with the experimental setup shown on the Figure 3. This concept has also been reapplied on miniaturized substrates, derived from Au layered printed circuit board technology, on single devices [35], or integrating several of them in a single piece [36]. The integration, gathering together the different sensing elements facilitates enormously the manipulation and miniaturization, which in turn permits the use of more reduced sample volumes. A widely explored technology for this integration/miniaturization has been the preparation of sensor arrays by screen-printing standard PVC membranes over polyester supports [37]. This technology may be directed easily to the mass production of low-cost disposable electronic tongue microsystems, with arrays of ca. 8-10 sensors [38]. This technology has been employed as well to prepare a sensor array from different metallic inks screen-printed on a ceramic support, working as electrodes of the first kind [39]. The use of solid state supports to form an array of potentiometric sensors has also been attempted with polyurethane membranes formulated with usual electroactive materials [40]. Another special mention is needed for the use of microfabrication technologies, either to prepare solid-state ISEs or ISFET potentiometric sensors. Verrelli et al. prepared an electronic tongue system depositing PVC membranes on platinum over silicon microfabricated structures [41]. Very recently, Ciosek et al. prepared a sensor array employing Low Temperature Cofired Ceramics (LTCC) technology and conventional PVC membranes [42]. Although potentially it is a perfectly viable option for developing electronic tongue systems, there is a single group at the Microelectronic Institute in Barcelona [43] employing an array formed by Ion Selective Field Effect Transistor (ISFET) potentiometric sensors; they use large area ISFET chips, encapsulated in a single device, where photocured polyurethane membranes sensitive to K^+, Na^+, Ca^{2+} and Cl^- are deposited, allowing for a 6-channel device after incorporation of pH and redox potential elements. Work has been presented in the identification of mineral waters, and in the to grape juice and wine sample analysis [44]. More recently, an extra variant of potentiometric sensor, the Light Addressable Potentiometric Sensor (LAPS) has been prepared in an array format and furnished with

differentiated selectivity chalcogenide thin films; its application to heavy metal determination in environmental field has been described [45].

Being the potentiometric sensors more than half of the described works with electronic tongues, they have also been used employing different operating techniques. Particularly, sensor arrays have been integrated frequently in flow systems in order to automate the sample handling and the recording of the multichannel signals. A recent review inspects in detail the use of electronic tongues as detection systems in flow systems, both using the Flow Injection Analysis (FIA) or Sequential Injection Analysis (SIA) principles [46]. While FIA is more used as an automation aid, SIA has proved to be of invaluable help in the training stages of electronic tongues, automatically preparing the hundreds of multiple-species standards needed for developing the calibration models. In the year 2000, Mortensen, in collaboration with the group of Saint Petersburg University, described a FIA system employing an array of seven chalcogenide glass sensors to determine the heavy metals Pb^{2+}, Cu^{2+}, Cd^{2+} plus CrO_4^- and Cl^- [47]; concentrations were calculated from PLS regression models or from ANN approach. In our laboratories we tested a multidetermination case in the analysis of nitrate in river water samples with high levels of chloride interference; for this purpose an array employing three different PVC membrane flow-through ISES sensitive to nitrate plus a flow-through chloride sensor [48]. Nitrate ion could be determined in the low ppm range without the need to eliminate chloride interference up to 1000 ppm, thanks to the use of three nitrate sensors showing different effect by the chloride interferent. The conceptual approach employed in these examples is sketched in Figure 4. Other experimental arrangements exploit rather the sample processing capability of the FIA systems, as demanded by qualitative applications which typically acknowledge a high sample throughput, for example in analysis of beverages [35, 36].

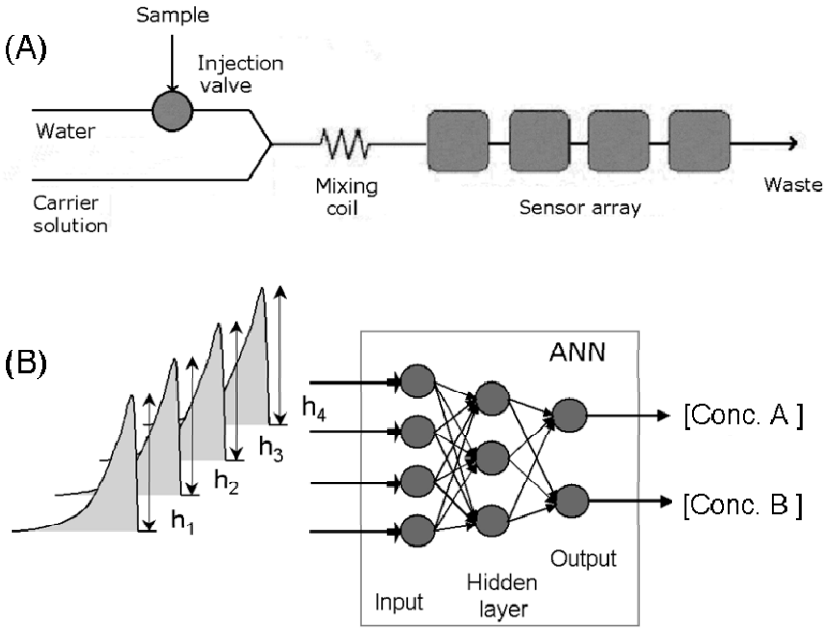

Fig. 4. Schematic illustrating the use of a potentiometric electronic tongue within a FIA system: (A) typical flow manifold to use a sensor array; (B) conceptual approach for a multidetermination application employing ANNs that use as input information the obtained FIA peak heights.

Concerning the use of SIA systems (SIA is the evolution of FIA in which liquids can flow in both direct and reverse direction, and many different liquids can be combined together through the use of selection valves), our laboratory was pioneer in conceiving an intelligent, automatic system based on an array of non-specific-response chemical sensors. As an electronic tongue system in a multidetermination application requires a great amount of information for its correct calibration, we proposed a SIA system to generate it automatically. The SIA technique was then chosen to prepare dozens of standards, by dilution and homogenization of stock solutions, thus enabling the processes of training, calibration, validation and operation to be automated simply, as can be deducted from the manifold depicted in Figure 5. As case study, the simultaneous determination of alkaline-earth cations Ca^{2+}, Mg^{2+} and Ba^{2+}, from an array of five flow-through potentiometric sensors based on PVC membranes of different selectivity and an ANN response model was successfully accomplished [49]. Recently, a different group

was inspired by this SIA system, proposing its use with an array of ISFET sensors and PLS multivariate analysis to automate the multidetermination of analytes [50].

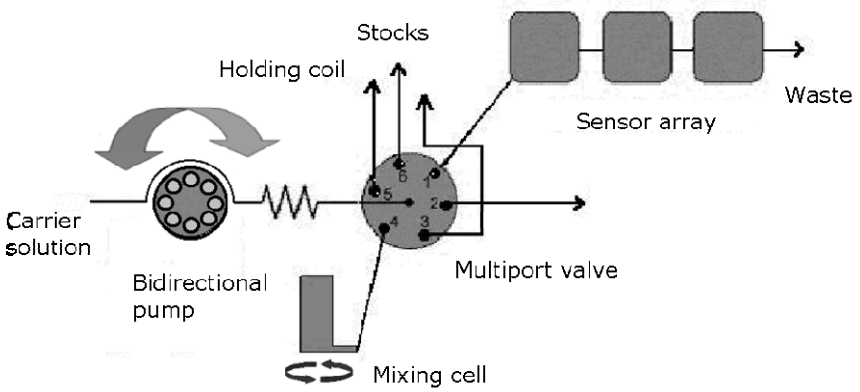

Fig. 5. Manifold of a SIA flow system designed for the automated operation with electronic tongues.

2.1.1 Electronic tongues with transient signals

When electronic tongues are operated using automated flow systems, a new possibility can be exploited that arises from the dynamic nature of the measurements occurring in these systems. If measuring procedures can be adapted to record the transient response corresponding to the introduction of a sample, instead of acquiring the steady-state value, the dynamic components of the signals can be then utilized (Figure 6). The purpose is to improve the modelling ability of electronic tongue systems, thanks to kinetic resolution now added. This can be justified in the potential differences in the sensor's speed of response versus primary or secondary species (interferents), which may be used as extra information for better discriminating or differentiating a sample. This interesting concept, initially proposed with gas sensors and electronic noses [51] was firstly utilized by Legin and colleagues in Saint Petersburg University with an FIA electronic tongue [52]. This additional dimension in information, needs to be extracted somehow, if not, the three-way signal originated is too complex for its processing with conventional chemometric tools; this first work determined mixtures of heavy metals from transient signals

originated in flow-through PVC membrane sensors, employing a multi-way n-PLS regression method.

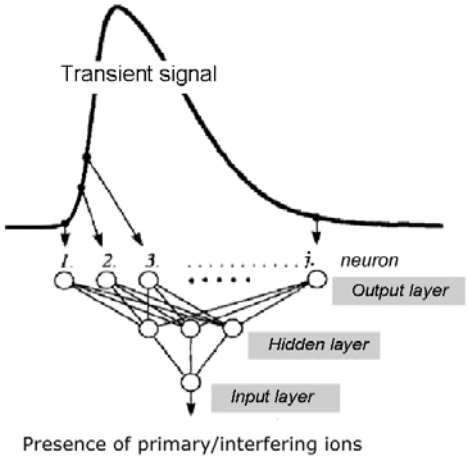

Fig. 6. Conceptual scheme of an electronic tongue employing the dynamic component of a sensor transient signal: information at different times after insertion of the sample is processed to extract chemical information.

In our laboratory we have retaken these ideas, and have incorporated the dynamic component of the information to improve the resolution of our SIA-based automated electronic tongue systems. A first example was the determination of mixtures of Na^+, K^+ and Ca^{2+} cations, from an array of five flow-through PVC membrane sensors [53]; significant information contained in the transient response after a step introduction of sample was extracted employing the Fast Fourier Transform (FFT), from which a number of coefficients were fed into an ANN model, used to perform the quantitative multidetermination. A related work made in our laboratory performed the determination of mixtures of anions (chloride, nitrate and hydrogencarbonate) from an array of five PVC membrane sensors selective to nitrate or chloride [54]; in this case the extraction of kinetic components was performed through fitting of transient data to orthonormal third-degree Legendre polynomials, which coefficients were the input information fed to a backpropagation ANN. Hydrogencarbonate ion could be quantified without using an ISE selective sensor, just deducted from the interference shown by the sensors used. Finally,

one can consider the way the sample is introduced into the system, as one factor of modulation of the obtained signal. Further work was done comparing the use of pulse versus step introduction of sample in an automated SIA system [55] . In this case, a four analyte cation mixture (Na^+, K^+, Ca^{2+} and Mg^{2+}) was resolved from an 8-ISE sensor array, while information was preprocessed using a FFT coupled to an ANN model.

2.2 Electronic tongues employing voltammetric sensors

The voltammetric principle has also been applied to develop electronic tongues since its early years [22]. In principle, voltammetry is better suited to the lower concentration applications, as it has a favourable signal to noise ratio when compared to potentiometry. In these original works, originated at the laboratories of Prof. Winquist in Linköping (Sweden), the sensor array was formed by an array of different metallic disc electrodes, and a scanning voltammetric technique was used to generate the analytical information. Noble metals, such as Au, Pt, Pd, Ir were initially selected for the array, and normal pulse voltammetry (both with large amplitude pulse, LAPV, and DC with superimposed small pulse, SAPV), the electrochemical techniques used. Main information was extracted from the exponential decay after each pulse, and its chemometric processing employing PCA permitted to distinguish liquid solutions as different fruit juices, milk and buffer solutions. Extensive studies were done in order to optimize the electrode materials for the sensor array, demonstrating how the different metals used could originate variation in the originated voltammetric signal [56]. In this way, the voltammetric electronic tongue becomes a very rich source of analytical information, given the original signal is formed by all electroactive components present, and, simultaneously, this is determined by the nature of the working electrode and by the electrochemical technique used. Thus, one can postulate systems employing cyclic voltammetry, linear scan voltammetry, NPV, SWV, DPV, etc., including the use of the stripping variants.

If the analytical signal needed is clearly identified beforehand, simpler instrumental designs can be adopted. For example, a smart arrangement was the use of a number of metallic electrodes, each one polarized at a fixed potential, and with its individual amperometric current recorded as the departure signal, what has been named the amperometric electronic tongue. With this simpler setup, typically 1-2 metallic electrodes and 4-5 potentials per electrode, sufficient information was generated in a very fast way (compatible with a fast FIA system) and with high simplicity, i.e. no scan needed; the system permitted the monitoring of flavour-related phenols (catechols) and the subsequent classification of tea varieties or the assessment of their astringency [57].

In the recent years, particular improvements of the initial concept have been proposed, e.g. the combination of three voltammetric LAPV scans, made at three frequencies (1, 10 and 100 Hz); the setup was illustrated with the discrimination of different distilled spirits according to flavour, and green teas according to geographical source [58].

Apart from the work in electronic tongues employing potentiometric sensors, our group in Barcelona has made also significant contributions with voltammetric electronic tongues. Initially, we combined metallic electrodes with an epoxy-graphite composite electrode, as shown in Figure 7; an Ag/AgCl disc was integrated in the same element as well, and used as a pseudo-reference. With this array, a quantitative multidetermination application of the three analyte mixture ascorbic acid, uric acid and paracetamol was developed [59]. The voltammetric technique used was a multichannel linear scan, equal for the three electrodes, and an ANN the chemometric tool used to resolve the analyte concentrations. In a collaborative study with the Swedish group, a quantitative application determination in the environmental field was developed, in which important process parameters for the paper industry, such as chemical oxygen demand, pH and conductivity were predicted from the multichannel LAPV signal with an ANN model [60].

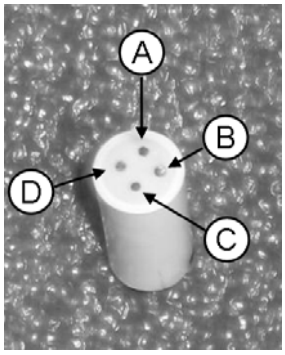

Fig. 7. Image of a voltammetric sensor array formed by four discs of conductors (1 mm diameter each). A, epoxy-graphite composite; B, platinum; C, gold; D, Ag/AgCl element, acting as reference element.

From a conceptual point of view, a voltammetric system employing a single electrode can somehow be considered as an electronic tongue, if we assume that the multidimensional signal comes from the multiple currents recorder in a single voltammogram. This is true if the voltammogram contains multicomponent information, given the dominant point will be the high dimensionality of the signal entered to the data processing stage. In view of the particular electrochemical technique used, and, as before, the information may be slightly different, as we can deal with a simple linear scan, a cyclic voltammetry, a differential pulse voltammetry, a chronoamperometry, etc. In this variant, some of the cases studies have been the resolution of electroactive compounds, departing from an overlapping signal voltammogram (Figure 8).

Per example, with an epoxy-graphite composite electrode, an environmental application was developed in the quantification of mixtures of the pollutants 2-cresol, 4-chlorophenol and 4-chloro-3-methylphenol below the 1 μM level and from their direct oxidation signal. [61]. Bessant made a similar contribution employing an unmodified Pt electrode, in which mixtures of glucose, fructose and ethanol were resolved, again, departing from the pulse voltammogram [62]. This work was later enlarged to a four analyte determination, including ethanol, methanol, fructose and glucose [63].

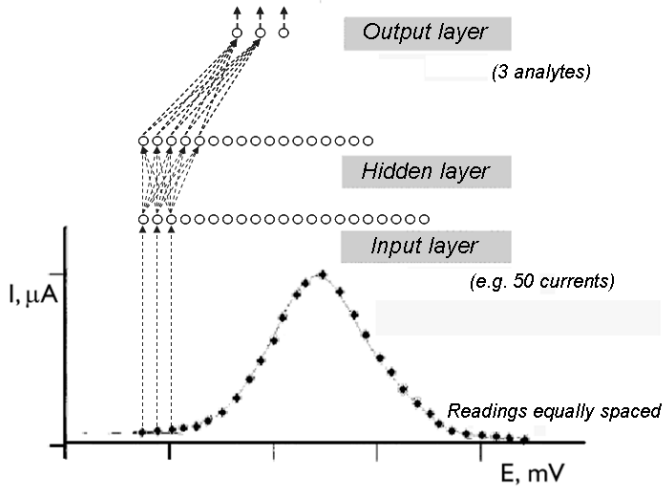

Fig. 8. Conceptual scheme of an ANN-based calibration model departing from the vector-like voltammetric signal.

A very promising variant in the work with electronic tongues is the use of modified electrodes, as the way to generate differentiated signals in the sensor array. Apart of using pure metallic elements, diverse materials have been used as modifiers of electrode surfaces, among others, conducting polymers or catalytic materials, such as metalloporphyrins or phtalocyanine complexes. In this line, a pioneer group was the one of Rodriguez-Mendez in Valladolid (Spain), who used conventional carbon paste electrodes modified with rare-earth bisphthalocyaninate compounds, including the Lu^{3+}, Gd^{3+} and Pr^{3+} complexes [64]. This first array was later completed with electropolymerized polypyrrole layers doped with a range of counterions and carbon paste electrodes modified with perylene imide derivatives [65]. Traditionally, this group has applied its electronic tongue systems to the food field, mainly in the analysis of wines and virgin olive oils. A somehow related work is that of Seeber, in Modena (Italy), who first combined two metallic electrodes made of Au and Pt with a third superficially modified with poly(3,4-ethylenedioxythiophene) conducting polymer (PEDOT); in their work, they first used this voltammetric electronic tongue to discriminate among fruit juices [66]. To end this discussion, a completely new generation of electronic tongues may be commented, in which a sensor array was proposed with four electrodes, one

modified with thioctic acid, and the other three modified with a short peptide (3 to 10 aminoacids). With these, N-rich metal complex-forming surfaces are obtained, whose differentiated properties could be used to resolve a Cu^{2+}, Cd^{2+} and Pb^{2+} heavy metal mixture. The concentration levels attained went down 1 μM, and the four square-wave voltammograms were processed in multiway-mode using NPLS1 algorithm [67].

The use of flow systems with electronic tongues has also been a successful coupling when the sensors considered are of the voltammetric type, both with FIA [57] and SIA [59, 60] techniques. The use of flow techniques offers, as advantages, the intrinsic correction to sensor baseline drift, the reproducibility, speed of operation and automation. As before, the automation capabilities may help in the processing of large number of samples, or also in the unattended preparation of the large number of standards needed to develop the response models.

2.2.1 Data compression in voltammetric electronic tongues

As it has been mentioned, the intrinsically richness of the analytical signal that can be generated with the voltammetric electronic tongue (electroactive components present x number of working electrodes x scanning range) makes this technique potentially applicable to a large number of analytical situations. Unfortunately, this richness can become also a drawback, if taking into account the high dimensionality that is easily engendered and the difficulty in its handling. Easily, a voltammogram may be formed by several hundred of measured currents, and the given application may use 5-6 modified electrodes to form the array. This rather simple sensing system is translated in a highly dimensioned information record per sample (of several thousand numerical values) which may make difficult the second stage in any electronic tongue, that of data processing (Figure 9). One feasible solution, is to use the recent three-way processing methods (samples x potential x electrodes), such as NPLS or PARAFAC, although their complexity and newness limits their application to the groups with best chemometric abilities [67].

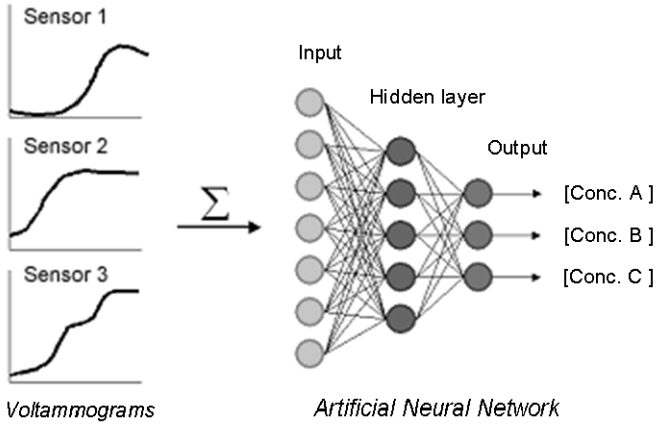

Fig. 9. Fusion of the voltammetric signals originated with a voltammetric sensor array to develop a quantification application of three electroactive compounds employing an ANN model.

A more widely spread option is to simplify somehow the high dimensionality, that in part may be redundant or just be related to noise, into its significant part employing a feature extraction stage. A first attempt by the Swedish group compared different data compression methods to determine their ability to reduce large data sets without loss of information [68]. The study was focused on the qualitative application, i.e. to discriminate different types of samples/beverages. The methods investigated were the wavelet transformation (WT) and hierarchical principal component analysis (HPCA). In HPCA, a large data set is divided into meaningful blocks, which is followed by a PCA to extract score values. These are in turn combined to a new data set and PCA is performed again. Also, a third procedure using a parametric model based on voltammetric theory was developed in order to extract interesting features from the current transients, revealing different information about electroactive species present. From the examined variants, Wavelet transform was the most promising for data reduction, which could be done down to a factor of 100, a very interesting feature to save computation time, to optimize data storage and to denoise.

This aspect of voltammetric electronic tongues has also received attention from our laboratory. In a first work, PCA and Wavelet transform were used and compared as a compression tool stage, in order to extract significant information from the voltammograms and use the reduced information for the modelling [69]. The case studied was the resolution of mixtures of the three oxidizable amino acids tryptophan, cysteine, and tyrosine, and the best response model was obtained with Wavelet transform followed by ANN modelling. Cocchi et al. made shortly before a similar work, where they used the Wavelet transform to extract meaningful data in the resolution of heavy metal mixtures by MLR, PLS and ANN modelling [70]. A compression ratio of ca. 10 demonstrated to be the best choice to deconvolute the voltammograms, in cases with high difficulty, as the signal corresponded to the overlapped individual signal plus noise and the oxidation of containing media [71].

In the more recent years, we have been significantly contributing with a new specific variant for the data processing, namely the Wavelet Neural Network (WNN). This tool has the advantage that it combines in a single stage the feature extraction and the modelling, with the choice of optimized wavelet functions as the transfer functions in an ANN structure. The potentialities of this new tool have been assayed with the resolution of a Pb^{2+}, Cu^{2+} and Cd^{2+} heavy metal mixture, in presence of In^{3+} and Tl^{+} interferents and employing the ASV technique [72].

2.3 Electronic tongues employing impedimetric sensors

The third main group of electronic tongues is the one that employs impedance changes as the departure signal for the application. In this, again a full scan of different AC frequencies may be used, or just a selected number of discrete values used, of conductivity or capacitance, in a simpler conception of this analytical system.

In a comprehensive study, Pioggia conceived an array of five sensors depositing, over microfabricated electrode structures, different mixtures of carbon nanotubes or carbon black dispersed in polymeric matrices and doped with polythiophenes. Each

electrode was interrogated at a frequency of 150 Hz to develop an artificial taste emulation, showing nature and stability of the response after several months of usage for solutions of sodium chloride, citric acid, glucose, glutamic acid and sodium dehydrocholate [73, 74].

The most active laboratory in this subtype of system is that of Mattoso and Riul in São Carlos/São Paulo (Brazil). For example, a first system employed an array of electrodes modified with Langmuir-Blodgett (LB) films of pure and composite nanostructured films of conducting polymers, like polyaniline and polypyrrole. These were interrogated at different frequencies, allowing the detection of trace amounts of basic taste solutions and inorganic contaminants in liquid systems, as visualized in PCA plots [75]. A more application-related work described an array formed by LB films of conducting polymers and lipids and layer-by-layer (LBL) films from chitosan deposited onto gold interdigitated electrodes. The system was applied to the classification of red wines, where the possibility to identify the vintage, vineyard and brands of the red wine with a properly trained ANN was shown [76]

Recently, Olivati and co-workers employed an array of impedimetric sensors formed by different LB films of conducting polymers and phtalocyanines to detect trace levels of phenolic compounds and chlorophenols in water [77]. Samples could be distinguished by PCA analysis of the capacitance signal at a discrete frequency of 1 kHz. The instrumentation details of such system were also described recently [78]. The most interesting variant is the use of electropolymerized materials [79], that may be nanostructured to specific analytes by insertion of specific dopants during electrolysis [80].

In an original contribution from our laboratory, we employed an ionophore-based membrane to develop an ion sensing electronic tongue, departing from the different frequency information. The dibenzo-18-crown-6 generic ionophore was entrapped by electropolymerization of a polypyrrole layer on a microfabricated structure. A 43-frequency impedance spectrum (in the 60 Hz-1 MHz range) was modelled employing ANN, whose abilities permitted the quantitative determination of mixtures containing K^+, Na^+ and NH_4^+ [81], as sketched on Figure 10.

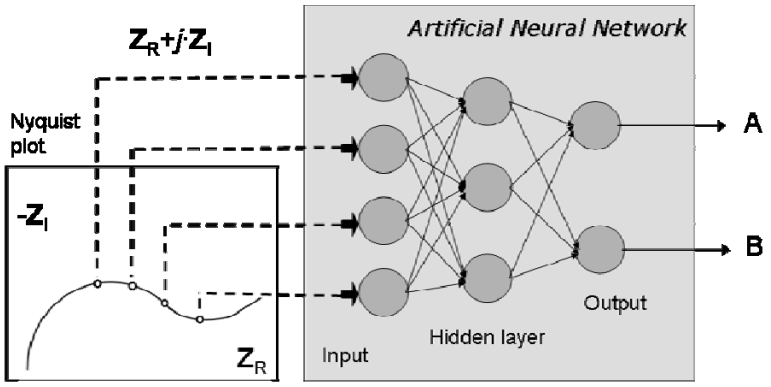

Fig. 10. Illustration of the operation concept of an electronic tongue which employs the impedance spectra of an impedimetric sensor and an ANN as classification/identification tool.

2.4 Other variants of electrochemical electronic tongues

An additional possibility exists to configure an electronic tongue, which is to use sensors of different nature to form the sensor array, i.e. to combine potentiometric, voltammetric and conductimetric sensors. These hybrid electronic tongues were proposed since the beginning, and complemented the performance of classical ones. For example, a hybrid electronic tongue was designed combining pH, CO_2 and Cl^- ISEs, with six metallic electrodes working with the voltammetric technique and a conductivity detector. The system was used to classify of six different types of fermented milk, such as yoghourt or kefir, for which a multivariate data processing based on principal components analysis and an artificial neural net was employed [82]. In a similar application, the groups of Prof Winquist (Sweden) and Prof. Toko (Japan) presented a collaborative work combining 8 potentiometric sensors and 4 voltammetric metallic electrodes to differentiate classes of two types of samples, teas and detergents for automatic washing machine [83].

The combination of electronic noses and electronic tongues to improve detection or identification capabilities, in a sensor fusion approach has also been proposed [84, 85]. This approach has been typically applied to the classification/identification of wines. Per

example, six porphyrin/PVC based ISEs were combined with a pH sensor and an electronic nose to identify wine varieties [85]. A simpler system, employing discrete amperometric signals from a glassy carbon electrode at 4 potentials and an Au electrode, plus an electronic nose formed by 10 MOS gas sensors permitted also the Characterization and classification of Italian Barbera wines [86]. An even more complex sensor fusion variant was attempted, combining three sensory modalities: an array of gas sensors, an array of electrochemical liquid sensors, in this case carbon paste electrodes modified with conducting polymers and rare earth metal phtalocyanines, and an optical system equipped with 11 LED as light sources to measure colour by means of CIElab coordinates. This new analytical tool was called “electronic panel” and was successfully applied in the identification of geographical origin of the same variety of Spanish red wine [87].

Scarcely a few applications are found in the literature that employ piezoelectric or SAW sensors [88]. A remarkable recent work by Sun et al. used a sensor array prepared by depositing Molecularly Imprinted Polymers (as derived from methacrylic acid polymerization) for saccharine, quinine plus a blank over piezoelectric quartz crystals. The setup was end up with a FIA flow cell, and permitted the challenging determination of quinine and saccharine at the mg/L level in bitter beverages [89].

2.5 Electronic tongues employing biosensors

The first analytical system employing an array of biosensors was described by Magalhaes in an application to determine creatinine in urine samples [90]. The approach used two identical creatinine potentiometric biosensors employing the enzyme creatinine iminohydrolase and four ISEs for K^+, Na^+, NH_4^+ and Ca^{2+}, intended to compensate any interference or matrix effect. The setup was calibrated employing a multivariate response model based on PLS, and the results validated against the standard Jaffé method. The same goal was recently attained by the simple use of an array of potentiometric sensors, formed by 8 different pure metal and metal alloy electrodes plus 5 PVC membrane ISEs.

Although without using creatinine specific enzyme, this metabolite was correctly predicted and, moreover, diagnosis was attempted of urinary system dysfunctions [91].

Our laboratory also made a motivating contribution with an array formed by two urea biosensors based on the enzyme urease, plus ISEs for H^+, K^+, Na^+, NH_4^+ and cation generic response [92], in what it was named a bioelectronic tongue. Response models were built employing PLS and ANN, achieving a correct prediction of urea in urine when comparing to standard clinical methods, and without the necessity of eliminating the alkaline interferences or compensating endogenous ammonium. In the same progress line, the system was enlarged with an additional potentiometric biosensor employing creatinine iminohydrolase to determine urea, creatinine and alkaline interferents [93]. Such a bioelectronic tongue is ready at the moment for its clinical application in studies of renal function or the monitoring of haemodialysis processes.

Also in a significant contribution from our laboratory, Gutes prepared a voltammetric bioelectronic tongue grouping different glucose biosensors, formed by epoxy-graphite biocomposites with glucose oxidase enzyme and different metallic catalysts (Pt, Pd and Au-Pd) to promote a differentiated response [94]. The system was successfully applied to the simultaneous determination of glucose and ascorbic acid in an attempt to determine simultaneously glucose plus its typical interferents in biological fluids without any separation stage or barrier membrane. In a very interesting study case [95], Solna and Skladal attempted the determination of various phenolic compounds combining the responses of a multiple screen-printed amperometric enzyme biosensor employing laccase, tyrosinase and peroxidase enzymes. The amperometric flow-injection determination of phenolic compounds at discrete applied potentials and using the multichannel biosensor was demonstrated at the nM level, although the multivariate approach with combination of the multiple responses was not fully developed. A contribution more in consonance with the bioelectronic tongue approach was the determination of mixtures of phenolic compounds (phenol, catechol and 3-cresol) from the overlapping voltammogram obtained with the tyrosinase enzyme biosensor [96].

ANN was the tool used to resolve the mixture at the μM level. Torrecilla and co-workers derived afterwards a similar work to determine phenolic compounds in olive oil mill wastewater, in which the main difference was the use of enzyme laccase for the biosensor [97].

A biosensor array was constructed, in a collaborative work intervening our laboratory, employing acetylcholinesterase enzymes from different nature in order to obtain differentiated inhibition by different pesticides [98]. The array employed the wild enzyme type from electric eel (EE) and two different genetically modified enzymes (B1 and B394). In this way, an inhibition bioelectronic tongue was constructed to resolve dichlorvos and carbofuran mixtures at the nM level. The signal employed was the % of signal inhibited for the different assays, done with equivalent response, disposable screen-printed amperometric biosensors, given the pesticide inhibition is irreversible. The idea of resolving a mixture of pesticides from the differentiated responses of enzymes with different characteristics was initially proposed by the works of Bachmann [99] and Crew [100], although our cited work resolved a mixture containing dichlorvos, a more difficult analytical case. Recently, a similar problem was attempted similarly but employing an automated FIA system, in which a dichlorvos and methylparaoxon pesticide mixture was resolved [101]. Ni and co-workers approached this case by resolving the strongly overlapping DPSV voltammograms of direct oxidation of ternary pesticide mixtures formed by methylparathion, fenitrothion and parathion [102], a variant without the specificity that can be obtained with enzymes.

A fascinating idea is the extension of the electronic tongue principles to the multiplex detection with arrays of biosensors based on antibodies or DNA [20, 103, 104]. Polsky developed an electrically addressable array to selectively functionalize biologically spots on a microfabricated array using aryl diazonium chemistry [105]. In this way, arrays of DNA, antibodies, enzymes, and peptides can be selectively immobilized, aiming to different multiplex biosensing schemes. For examples, a protein and a DNA fragment can perform as a double confirmation test for presence of

bioterrorism threats; or the double detection of a gene mutation and a tumour protein marker may confirm a specific malignant growth [106]. Similarly, the combined response of an array of Surface Plasmon Resonance biosensors with different antibodies was employed for the detection of the bacteria Salmonella Typhimurium in meat products [107]. The multiplex biosensing concept was implemented with an impedimetric DNA biosensing scheme [108]. Two DNA probes were immobilized on an electrode surface and hybridization experiments were monitored by following its electrochemical impedance spectrum. The direct observation did not allow discerning if the first, second or both DNA targets were present in the sample. The application of the electronic tongue concept, where an ANN response model was trained with the complex impedance spectra, permitted the correct identification of all the training cases in a leave-one-out scheme.

3. Illustrative applications

Significant applications of electronic tongues furnished with different sensor types have been grouped from the application point of view, in order to illustrate the rich scene defined in the last years by their use. The selected topics are: applications in agricultural and related areas, the wine field, pharmaceutical industry and novel analytical applications.

Water analysis is a classical application field for chemical sensors, which normally receives a lot of studies [29, 61], normally of detection of pollutants. In contrast, much greater impact is received by the contributions aimed to the agricultural field. Being this an important economic sector for many countries, any productivity increase, as such monitored or favoured by the use of information provided by electronic tongue systems, may represent important improvements in their exploitation. Per example, our group contributed with studies to monitor NH_4^+, K^+, NO_3^- nutrients and Na^+, Cl^- contaminants in fertigation solution for greenhouse cultivation [109]. The

system employed eight PVC membrane ISEs and complex data processing by ANNs, counterbalancing any temperature effect on the concentrations found. A recent contribution used a four sensor array and an expert system based on Case-based reasoning to detect deviations in the composition of equivalent fertigation solutions [110]. A curious work by Ciosek and co-workers optimized an electronic tongue for the recognition and classification of plant samples [111]. Leaf material from the plants was extracted in aqueous solution and measured with an array formed by 8 PVC membrane ISEs, allowing to differentiate between plant (maize and guinea grass) and to deduct cultivation conditions of the plant, such as light conditions and cultivation time, features hard to investigate by classical analytical techniques. [27]

Rudnitskaya and collaborators completed an study to recognize apple varieties [112]. The sensor array was formed by 15 ISEs devised for organic acids, which permitted also to quantify galacturonic, malic, citric, succinic and tartaric acids. A similar work from the same laboratory [113] presented the analysis of tomato cultivation variants according to the recorded taste profile from two different electronic tongues, one based on PVC membrane and chalcogenide potentiometric sensors, and other based on ISFET sensors, which showed some degree of complementarity. The electronic tongue was also applied to classify varieties and to correlate with sensory analysis of apricots during harvest and storage [114]. An original contribution [115] used an electronic tongue with 20 ISE sensors, and applied it to the classification of different types of honey, whose grouping correlated well with biological properties such as their pollen profiles.

Similarly, the livestock activity has received the help of analytical monitoring by electronic tongue systems. Abu-Khalaf and Iversen [116] presented an automated electronic system where livestock odours sampled from bioscrubbers could be classified according to key odorants present, such as n-butyrate, ammonium and phenolate; the classification system employed was a backpropagation ANN. The dairy sector has also received significant attention from the different laboratories developing electronic tongues. First attempts, done with a voltammetric array of metal electrodes, were directed to the classification of raw milk sources, the monitoring of clean conditions

[117], and also the estimation of milk freshness [118]. Electronic tongues employing potentiometric sensors were applied in this field as well [13, 36]. General taste assessing was attempted with the artificial taste system developed by Toko [25]. In another work, milk samples were classified according to producer and to their fat content; the approach employed an array formed by 5 PVC membrane ISEs plus a glass pH electrode and Support vector machines [119]. A more specific study was intended to improve detection of clinical mastitis in infected cows from their fresh milk, an important detection target for automatic robotic milking [120]. Dias et al. [121] presented an interesting work that showed the identification of adulteration of goat milk with bovine milk. For this, an array of 36 potentiometric sensors and linear discrimination analysis were used. To end with the farming activities, a recent work was dedicated to the monitoring of water quality in fish farms employing a screen-printed electronic tongue formed by 8 potentiometric sensors [122]. In a quantitative derivation, $NO_3^-$and NH_4^+ ions were monitored along 8 days period in tilapia and catfish tank water.

The application of electronic tongues in food analysis is also an important topic with frequent contributions [23, 32, 104]. Among these, a common topic is that of wine identification, classification or analysis. Typically, the array used is formed by ISEs and many studies have been attempted. Some works from the laboratory in St. Petersburg in collaboration with other universities have been the prevention of wine defects as originated from non-compliant corks, from evaluation of phenolic contents in their ethanol-water solution extracts [123], the prediction of Port wine aging (between 2 and 70 years old) [124] or the effect of new elaboration technologies, such the use of micro-oxygenation or wood chip maceration to improve wine characteristics [125]. From Rome, Verrelli et al. developed a microfabricated sensor system equipped with potentiometric PVC membranes formulated employing metallic porphyrins and corroles [41]. The miniaturized system was applied on real white wine samples for the detection of some analytes mimicking wine defects, namely H2S, SO2 and acetic acid. One of the scarce electronic tongues made with ISFET sensors was applied to the recognition and classification of wines according to the used grape variety and the vintage year [44].

After building a PLS regression model, several parameters of interest in wine production were shown to be estimated, such as pH, alcohol content or volatile and total acidity.

Also, voltammetric electronic tongues have been carefully applied to wine analysis. The cyclic voltammetric signals obtained from copper electrodes, which present rich electrochemical signals with sugars and other hydroxyl compounds in alkaline pH, were processed by PCA, permitting classification of Chinese yellow wines[126]. The group of Rodriguez-Mendez has extensively studied the use of voltammetric electronic tongues to the wine field [64, 126]. Some of the applications have been the classification according to grape variety used in elaboration or the geographic origin, the monitoring of wine aging, the identification of the nature of barrels used for the wine aging, etc. [65]. A recent work updates the work of this group in the wine field, who has systematically applied a voltammetric sensor array formed by different Carbon paste electrodes modified with phtalocyanines or conducting polymers [127]. The work demonstrates a rich variety of signals related to acids, bases and antioxidants presents in wine, such as tannic acid or citric acid, that can be exploited to discriminate wines produced employing different grape varieties.

A group from Modena (Italy) has made recently interesting contributions in the field of wine analysis. Seeber and co-workers use electrodes modified with the conducting polymer poly (3, 4-ethylenedioxythiophene), PEDOT, to prepare a voltammetric array, for example with PEDOT, and PEDOT-Au or PEDOT-Pt composites. A first application described the discrimination of white Italian and French wines from the voltammetric signals of the array [128]. Later, a similar system was used to classify different types of Italian red wines [129], combining both cyclic voltammetric and differential pulse voltammetric signals for a better performance. An interesting study is that already commented of the Brazilian group, who used an impedimetric electronic tongue formed by LB and LBL films onto gold interdigitated electrodes to identify six classes of red wine from different producers, grape varieties and vintage [75, 76].

A recurrent option to improve results obtained by the electronic tongue in the analysis of wine, has been to combine different sensing strategies, that is electronic noses and electronic tongues [85], or even with additional transduction principles [87], as

described in Section 2.4. Buratti described the identification of Italian Barbera wines combining data from an electronic nose and an amperometric electronic tongue that employed two working electrodes, a glassy carbon and a gold electrode [86] Later, a very similar hybrid system was applied to assessment of geographical origin of extra virgin olive oils; the tools used were an electronic nose, an amperometric electronic tongue and ANNs as the processing tool [130]. Analogously, the group of Rodriguez-Mendez applied their experience with wine analysis also to the evaluate the polyphenolic content and bitterness index of extra virgin olive oils and to use this information to discriminate between them [131]. The work of Torrecilla was applied to the assessment of the environmental impact, as pollution with phenolic wastes, derived from the production of these oils [97].

In connection to wine applications, there is also the recurrent application of electronic tongues to beer sensing [21, 25, 35, 37]. Recently, a very ambitious work was initiated, in which the response obtained by an electronic tongue made of an array of potentiometric sensors was applied to identify Belgian beers and correlated with success with a large number of attributes from a standardized human sensory panel [132].

An application field related to the industrial sector is the assessment of the organoleptic aspects of pharmaceutical formulations of drugs. The use of electronic tongues to characterize the taste and acceptability of pharmaceutical formulations represents an objective measure of the perceptions raised during administration of drugs, in contrast to subjective impressions of volunteers participating in clinical studies [133]. In addition, the electronic tongue can be used in situations of toxicity or poisoning, without endangering volunteers. Such an analytical system may furnish an automated alternative, or even replace a sensory panel in pharmaceutical development and production. A currently followed practice in drug formulation is the masking of the active ingredient (normally bitter) by the addition of sweeteners and flavours. For example, a study from the group of Toko characterized the bitterness suppression by using phospholipids, such as phosphatidic acid, while other taste components were unaffected [134]. Results obtained with the artificial taste system were in agreement with

the human sensory panel in use. A similar study dealt with the suppression of saltiness by the use of monosodium glutamate, the standard compound for the umami perception [135]. The interest of these studies is clearly to make more palatable the administered drug and help in the marketing and acceptation of new formulations. Another interesting study was presented recently, in which different variants to prepare famotidine tablets were compared employing the commercial Astree (AlphaMOS, France) electronic tongue with the goal of reducing its bitterness intensity [136]. Also of interest for the pharmaceutical industry is the analytical verification after synthesis or isolation of active pharmaceutical ingredient, per example when extracted from specific medicinal plants [27]. A family of compounds with special role also in the pharmaceutical industry is that of surfactants, widely used to solubilise, to form emulsions and to favour wetting properties of active ingredients. The group of Kulapina in Saratov (Russia) has participated with several contributions for the resolution of surfactant mixtures employing arrays of solid-contact ISEs with cross-sensitivity for the separate detection of surfactant homologues [137]. Their mixtures were also determined automatically with a specially devised electronic tongue employing an a SIA system [138]. Recently, also nonionic surfactant mixtures were determined in a similar way [139].

To end with the selected applications, some innovative selected works, more connected with research will be commented. In a first place, recently, electronic tongues were postulated to be used in titrations, employing an ISE array as detection system and complex data processing to extract results. The initial proposal described a fast titration procedure consisting in a reduced number of fixed titrant additions to the sample and the recording of the potentials of an array of ISEs (Figure 11). The obtained data matrix was then entered to an ANN model, previously trained to furnish concentrations of a two-component mixture of Ca^{2+} and Mg^{2+} [140]. The proposal opened a procedural methodology for multideterminations, in which the old masking chemistry may be replaced by the electronic tongue approach, and where a very simple analytical procedure, the titration, would yield multicomponent information. The idea was latter extended to a 3-ions mixture, Ca^{2+}, Mg^{2+} and Sr^{2+}, that were titrated with a few fixed

additions of EDTA; the generated data (number of sensors × number of additions) were used as input data to an ANN response model previously trained with standards [141]. The procedure offered an extreme simple operation, just a pH adjustment and a series of automated, fixed volume titrant additions, with appreciable precision. Curiously, in the more than one-decade existence of electronic tongues, no other similar contribution was conceived. The only related work was that of Shamsipur, in which Ag^{+}, Hg^{2+} and Cu^{2+} were resolved after SCN^{-} titration, followed with a carbon paste electrode and the recorded information entered to an ANN model [142]. Although the combination of potentiometric titration and chemometric tools is evident, it is not a case of application of a sensor array. In essence, the proposal follows current trends in modern analytical chemistry, where the complexity is shifted from methodological stages or instrumental complexity to the software field.

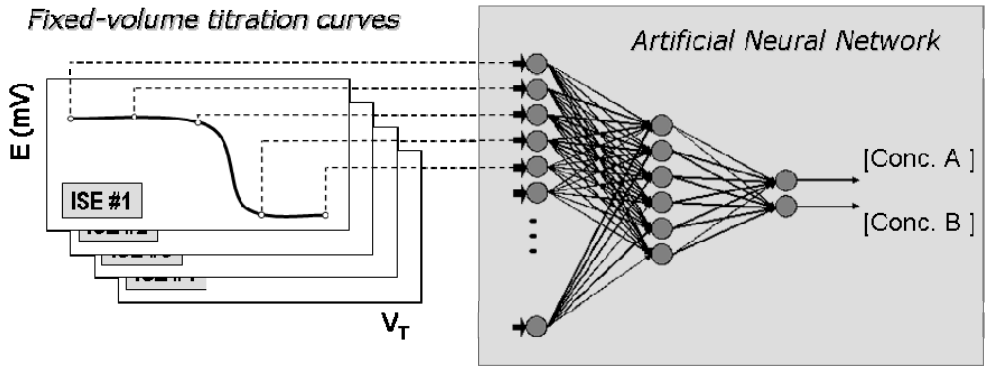

Fig. 11. Conceptual scheme of the titration procedure with the use of the electronic tongue.

Finally, a very exciting work is that developed by Kounaves and co-workers at the University of Tufts for the NASA agency. In their studies, an array made of polymeric PVC ISEs were optimized for the purpose of performing an in-situ analysis of the soluble ionic species in Martian soil [143]. It used 27 solid-state ISEs, being the Li^{+} sensor used as the reference element, in front of the difficulty of deploying a (semi)liquid Ag/AgCl or Calomel reference element during a space travel. Complementing the potentiometric detection system, a voltammetric array was also included, in this case, to

check hazardousness of the Martian regolith for the presence of heavy metals [144]. The project, part of the Phoenix mission [145], was intended for the characterization of Mars environment and for detection of any perceptible bacterial growth following hydration. The system completed his mission successfully in the summer of 2008, after the incident of the Mars surveyor lander in 2001: the unmanned mission allowed confirmation of the presence of essential nutrients for agricultural activity in our neighbour planet. As such, these results may be highlighted as an important milestone in electroanalysis: it represents the most far away place where an electroanalytical technique has been applied so far.

4. Conclusions

The growing demand for (bio)chemical information in the different economic sectors or in the environment and the public concern on health and quality is clearly driving for new trends in analytical methodologies. Electrochemical sensors are a clear option for a fast, simple and cheap gathering of information, although sensors alone cannot solve all the existing situations. In the last decade, a new trend in the sensor field has appeared, which is to couple multidimensional sensor information with advanced computer processing strategies; this approach, known as electronic tongue, can take profit of electrochemical sensors and biosensors from the potentiometric, voltammetric and impedimetric families, among others. In perspective, electronic tongues can be one of the clearest benefits of the application of chemometrics in (bio)sensor research [146]. Examining the progress trend, the field seems to be now mature, and perhaps a more complete commercial dissemination is the only missing requirement. Against this, the important limitation of the drift in sensor signals and their compensation in electronic tongue response models (very hard to build) is one of the weak points still to solve [147]. Sensor non-idealities such as ageing, poisoning and temperature effects may be the reason for the drifts; if the causes are identified, specific correction measures may be periodically taken, and recalibration strategies performed – but this important (and hard to complete) research is still pending. If correction is not possible, the analytical know-

how from the last century also indicates what the solution can be: modulation, to be performed in many ways.

But there are also voices who claim against these new sensor systems [148]. One should not take these multi-determination approaches as the panacea for all applications: a good, well used sensor, with any interference effect kept under control may never be overcome by the array approach. Notwithstanding, there are still many examples where reasonably good sensors need to be used with tedious pretreatment stages to get rid of interfering or sample matrix effects – the sensor array approach may be a good alternative here, especially for automated screening or monitoring purposes. Other unique choice may be when an advanced information is sought, such as a perception, or the confirmation of a situation for which no appropriate single sensor exists. In summary, and in positive, perspectives for electronic tongues seem promising; once consolidated in research, now it is the time to fulfil many niche applications, specially were complex information is needed. Surely the next years will show the demonstration for this.

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6. References

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