Recent advances in electronic tongues

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Received 5th May 2010, Accepted 12th July 2010
DOI: 10.1039/c0an00292e

This minireview describes the main developments of electronic tongues (e-tongues) and taste sensors in recent years, with a summary of the principles of detection and materials used in the sensing units. E-tongues are sensor arrays capable of distinguishing very similar liquids employing the concept of global selectivity, where the difference in the electrical response of different materials serves as a fingerprint for the analysed sample. They have been widely used for the analysis of wines, fruit juices, coffee, milk and beverages, in addition to the detection of trace amounts of impurities or pollutants in waters. Among the various principles of detection, electrochemical measurements and impedance spectroscopy are the most prominent. With regard to the materials for the sensing units, in most cases use is made of ultrathin films produced in a layer-by-layer fashion to yield higher sensitivity with the advantage of control of the film molecular architecture. The concept of e-tongues has been extended to biosensing by using sensing units capable of molecular recognition, as in films with immobilized antigens or enzymes with specific recognition for clinical diagnosis. Because the identification of samples is basically a classification task, there has been a trend to use artificial intelligence and information visualization methods to enhance the performance of e-tongues.

1. Introduction

The taste formation is related with an impressive chemical transduction in the papillae with activation of intrinsic and extrinsic neuronal circuits, mediated by regulatory membrane receptors/mediators through a complex network.1–3 Recent results reveal regional differences in taste sensitivity to sweetness and saltiness, suggesting that different transduction mechanisms might occur.4 Considerable efforts have been made in the last decade to determine the mechanisms responsible for taste formation, but there is still no consensus. The Classical Threshold Theory assumes that taste sensations depend on the intensity of the attributes of the stimulus, so that the stimulus is perceived only when its intensity is above a specific level or threshold.4 Taste disturbances are observed under circumstances such as in anxiety or depression, indicating the importance of neurotransmitters in determining taste thresholds in health and disease.4 Scale descriptors and/or geometric mean values are normally used in human tasting panels,2 and it is difficult to...
assign a scale or unit for measuring taste. In addition, even if members of a taste panel are well trained and calibrated, the evaluation still remains subjective, which might be troublesome in some industrial applications. This is why researchers and the food, beverage and pharmaceutical industry have been seeking a reliable, reproducible analytical tool, in addition to the ethical and safety concerns related to the possible toxicity and exposure of human beings to unpleasant formulations.

One alternative that has been increasingly used is the electronic taste sensing for the classification and identification of very similar liquid samples, which are useful for various applications in the food and pharmaceutical industries, for the quality control and monitoring of waters in addition to representing an important challenge in analytical chemistry and in the study of methods to enhance the performance of sensing units. In this context, electronic tongues (e-tongues) have emerged as a powerful tool in the rapid assessment of information of complex liquid systems. The term e-tongue was coined owing to the similarity with the human gustatory system, which is based on the concept of global selectivity. By global selectivity one means the unique ability of the brain in grouping all the information received from the tongue in distinct patterns of response encoding the taste quality. A way of mimicking it is the formation of non-specific sensor arrays able to recognize tastes (sweet, salty, sour, bitter, and umami) and respond to suppression effects resembling its human counterpart. For an artificial taste sensing, the easiest way to mimic the global selectivity concept is using an array of poorly selective materials that are simultaneously sensitive to several components of the samples analysed (cross-sensitivity), without specific interactions.

Selectivity is not a crucial requirement in this sort of application, but artificial taste sensors must have high sensitivity and stability, and a specific tuning might be achieved with specific molecular recognition depending on the application desired. Furthermore, using label-free detection processes is advantageous since uncertainties are removed which are associated with the effects of labels on molecular conformation, blocking of active binding sites, steric hindrance, and the inability to encounter available labels for specific molecules. By removing labels, the experiment is simplified and the cost of the materials is tremendously reduced.

The first reports on the analysis of liquids using a multisensory array appeared in the 1980s, after which several research groups have produced new technologies summarized in various reviews. Desingh et al. described e-tongues and noses for the food industry, including methods of analysis and the main research groups involved. The use of lipid membranes in e-tongues was discussed by Toko and Habara, while Vlasov et al. reviewed e-tongues using potentiometry and De Saja et al. discussed the use of phthalocyanines as sensing units in electrochemical measurements. The latter measurements and flow analysis were the focus of a brief review by Ivarsson et al. The concepts and methods of statistical and computational analysis were reviewed by Huang and Deng.

In this paper we focus on papers published in the last few years. It is organized as follows: Section 2 brings the principles of detection, while the materials used in the sensing units are presented in Section 3. The statistical and information visualization methods for treating the data are described in Section 4 and a summary of the applications of e-tongues is given in Section 5. Final remarks close the paper in Section 6.

2. Principles of detection

2.1. Electrochemical methods

The most-used methods in e-tongues employ electrochemical measurements, especially potentiometry, amperometry and cyclic voltammetry. A typical configuration for an e-tongue based on cyclic voltammetry is shown in Fig. 1. Briefly, the sensing units are lipid membranes, ion-selective electrodes or

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noble metals functioning as working electrodes, with the voltage difference measured between the sensing materials and a reference electrode. The advantages of the electrochemical methods include simple instrumentation and high sensitivity. On the other hand, these methods are limited because of the requirement of intrinsically electroactive species (materials with low potential redox provide unstable and poorly reproducible behaviour) and the problem of integrating a stable, reliable miniaturized reference system. Various issues have been addressed for enhancing the performance of e-tongues using electrochemical methods. For instance, Holmin et al.\textsuperscript{72} showed that cleaning the metallic electrodes with various aqueous solutions is essential for a rapid activation of the electrodes when complex liquids are analyzed with cyclic voltammetry. This procedure helps to avoid the contamination of the sensing unit that could lead to a decrease in the electric current, and yields higher reproducibility with a smaller drift. The electrochemical reactions were monitored in a calibration procedure that decreased the need of human intervention in e-tongues based on voltammetry.\textsuperscript{73}

Using cyclic voltammetry Parra et al. obtained a high cross-sensitivity for electrodes made with phthalocyanines with different metals in the centre of the ring, and carbon paste in the working electrodes. A sensor array with these sensing units was successful in distinguishing red wines.\textsuperscript{74} The same group combined phthalocyanines with conjugated polymers in sensor arrays to determine organic and ionic substances responsible for the bitter taste in foods and beverages.\textsuperscript{75} Sensing units based entirely on conjugated polymers were used in cyclic voltammetry measurements to evaluate basic tastes, where polypyrrole was proven more appropriate than poly(3-methylthiophene) and polyaniline, as its electrochemical response was more stable and could be enhanced by incorporating relatively large doping ions.\textsuperscript{76} Such incorporation was important because the electrochemical response was usually unstable when small doping ions were used.

Potentiometric measurements were used by several groups, and a typical e-tongue based on potentiometry has its configuration shown in Fig. 2. Nitrate ions could be identified in complex liquids containing variable amounts of chloride with an e-tongue formed by four potentiometric sensors, three nitrate ion-selective electrodes and a chlorine ion-selective electrode, whose data were analyzed employing neural networks.\textsuperscript{24} A discussion on the use of statistical and computational methods to treat the data in electronic tongues is provided in Section 4. Toxic phenolic compounds, including catechol, phenol and m-cresol, were distinguished in amperometric measurements with an automated injection system for flow measurements, with 95% accuracy when neural networks were used.\textsuperscript{77}

2.2. Impedance spectroscopy

In 2002, Riul et al. used impedance spectroscopy for electronic tongues,\textsuperscript{15,79} which is advantageous because the materials of the
sensing units do not need to be electroactive and there is no need of a reference electrode – unlike the electrochemical methods. The high sensitivity obtained led to a number of contributions to be mentioned here. In this method, the complex impedance of the whole system is measured for varying frequencies of the signal applied on interdigitated electrodes (IDEs) covered with ultrathin films of different materials, as illustrated in Fig. 3.

This system can be described by an equivalent electrical circuit, as the one shown in Fig. 4, in which the electrostatic double-layer formed at the electrode/electrolyte interface governs the response at low frequencies, the solution conductance and ultrathin films coating the electrodes rule the total impedance at intermediate frequencies and the geometric capacitance is most relevant at high frequencies. 79,80

2.3. Fluorescence

Though the principle of detection does not involve electrical measurements, one may also classify the sensor array proposed by Kirby et al., 81 as an e-tongue owing to its functionality. Fluorescence measurements were used to detect proteins in biosensors containing aptamers, which may replace antibodies for their functional binding species. The aptamers used were selected from combinatorial oligonucleotide libraries and immobilized on sensor arrays to detect and quantify proteins. 81 In another study, Thete et al. 82 reported on an ‘optochemical tongue’ to recognize alcoholic beverages.

2.4. Flow analysis

The first e-tongues were conceived for static measurements with liquids in a reservoir with no movement. Obviously, for various applications a flow analysis is required, with the advantage of time saving in the measurements. Several injection systems have been devised. Hayama et al. 83 reported a good stability for the electrical potential in the sensing units under a continuous flow of liquid samples, but some drift was observed owing to the high resistance of the porous membrane of the experimental setup. Winquist et al. 23 observed that measurements with a continuous flow are less affected by calibration procedures, with a smaller drift in the electrochemical signal. This drift may be corrected via software in the data treatment, which improves the sensor performance. Furthermore, the washing of electrodes may be adapted for automation of the system. Indeed, devices made with 5 potentiometric sensing units and with Sequential Injection Analysis (SIA) have been automated. 84

The importance of flow analysis for the dairy industry was illustrated in refs 85 and 86 as the microbial growth may be monitored. The continuous flow systems also allow for different substances to be added during the measurements, 87 as was exploited to analyze wine samples. 88 Using polymeric micro-particles functionalized with different biological receptors as transducer materials for the sensing units, Sohn et al. 51 produced an e-tongue that allowed the liquid to be analyzed while passing through a micromachined fluidic structure. The dynamic response of the e-tongues in continuous flow systems may require an even more sophisticated data analysis process. This type of response for detecting metal ions in solution simultaneously was

Fig. 3 Experimental setup usually employed in impedance spectroscopy measurements.

Fig. 4 Equivalent electric circuit used to interpret impedance spectroscopy measurements. 15 [Reprinted with permission from ref. 15. Copyright 2002, American Chemical Society.]
treated with multivariate analysis and neural networks. A detailed description of flow injection analysis was performed by Gutès et al., while Fig. 5 illustrates the functioning of the SIA technique with a sensor array.

2.5. Combining sensorial functions (tongue + nose)

Because the olfactory system functions in a similar fashion to the gustatory system, i.e. without specificity, electronic noses have also been developed, which have many features in common with e-tongues, but a major difference is the moisture effect in the measurements. Electronic noses have been used to improve the quality of foodstuff and beverages, for example, by monitoring the odours produced during the fermentation and/or roasting processes and environmental monitoring. They have also been combined with e-tongues for the analysis of wines with sensing units made with metalloporphyrins. The overall performance in predicting qualitatively and quantitatively the flavour parameters and the chemical descriptors of the samples was improved upon combining an electronic nose and an e-tongue. While the error in the chemical descriptors ranged from 0.6 to 52%, the error for the sensorial parameters varied between 2 and 12%, which indicates that the non-specific sensing units capture information that is more related to the global properties of the wines. A similar work was based on amperometric measurements in a Sequential Injection Analysis system for samples of wine from the North of Italy. The data from the electronic nose and tongue were compared to chemical analyses and spectrophotometric evaluation of colour, with a statistical analysis resulting in an accuracy of 98% in distinguishing different wines. That the combination of e-tongue and electronic nose leads to an enhanced distinguishing ability was also demonstrated by Katsube et al. using Principal Component Analysis (PCA) to treat the data, and Kataoka et al. included the evaluation of the palatability of isotonic beverages.

2.6. Electronic tongues in FETs, miniaturized and commercial systems

As the concept of e-tongues was proven useful for many applications, efforts have been made by many groups to fabricate prototypes that could lead to low cost commercial devices. Two important issues in this connection are miniaturization and the integration with existing silicon technology for microelectronics. The design and strategies for integration with field-effect transistors (FETs) were discussed by D’Amico et al. FETs covered with carbon nanotubes modified with DNA were used to identify odours, but the authors did indicate that the ideas could be extended to e-tongues. Similarly, Siqueira Jr. et al. described biosensors obtained with field effect devices whose concepts can now be used in electronic tongues. A miniaturized, automated system was developed by Sehra et al., with surface acoustic wave (SAW) devices used in detecting aqueous solutions representing the basic tastes, namely sucrose, HCl, NaCl and quinine. Further developments in this area were presented by Hossenlop. Devices employing wireless technology, which allows for data transmission from a measuring to a processing unit, were developed by Kim et al. The sensor array comprised 7 ion-selective membranes and potentiometry was the principle of detection. A software tool was integrated into the system, permitting the treatment of the data.
with PCA or fuzzy logic methods, which allowed good distinction of beer and tea samples.

In addition to the extensive research work on e-tongues, there are commercial products in the market. The Alpha_MOS system contains 7 sensing units with silicon FETs coated with organic materials. With a lifetime of one year, it has been applied to evaluate bitter substances used in the pharmaceutical industry. In a study by Zheng and Keeney, this commercial system was used to analyze samples containing 10 mM of quinine, to be compared with a control sample (placebo). Distinct substances were employed to suppress the bitter taste, including NaCl, potassium acetate and artificial sweeteners, and this suppression ability could be captured by the device. This ability did not apply, however, to complex liquids (such as soft drinks). An example of an ion-selective FET (ISFET) is depicted in Fig. 6. With the liquid solution under analysis in contact with the gate, any change in the liquid sample affects the current between source and drain, and then the FET properties.

3. Distinct materials for the sensing units

The choice of materials for the sensing units is crucial for a high performance to be obtained. Even though the e-tongues do not require specific interactions with the analyte, the sensing-uniforming material still needs to respond electrically to small changes in the liquid under analysis. Furthermore, depending on the method of detection, some extent of electrical conductivity and/or electroactivity may be required.

One of the first class of materials used for e-tongues were lipid membranes, in an attempt to mimic the materials of the human tongue. One recalls that a lipid bilayer provides the framework for a cell membrane. Chalcogenide glasses in electrochemical measurements were largely employed whose main advantages are the ease of electrode preparation and cross-sensitivity to extract both quantitative and qualitative information. The electrochemical response of sweeteners was affected by changing the electrical charge density of a lipid membrane, which could also be used to distinguish pungent substances such as capsaicin and piperine. Potentiometric measurements with lipid membranes enable the evaluation of the suppression of the bitter taste from paediatric medicines by incorporation of two amino acids. This system was used to study suppression of the bitter taste in diet foods, and to assess the taste of soft drinks in terms of attributes such as refreshing, sour, fruity, etc.

Analysts with distinct taste properties were found to alter the oscillatory pattern of the electrochemical response of lipid membranes deposited onto oscillators. The time-dependent signals were non-stationary, i.e. chaotic. In a sensor array with 5 lipid membranes as sensing units, the individual electrical signal was not stable, but the combination of all signals could nevertheless be used as a fingerprint of commercial beverages. The sensitivity toward sucrose was low.

The extensive use of electrochemical methods prompted researchers to explore well-known electroactive materials, including phthalocyanines, porphyrins, ruthenium complexes and conducting polymers. The use of porphyrins, for instance, enhanced the sensitivity of a potentiometric e-tongue to detect basic tastes down to 10⁻⁶ M, which was not possible with the electrodes built with chalcogenide glasses previously studied. The data from potentiometric measurements taken with a sensor array comprising 12 electrodes coated with thick films of ruthenium complexes were treated with neural networks to distinguish between 6 brands of mineral waters. Szpakowska et al. used modified membranes of poly(3,4-ethylenedioxythiophene) (PEDOT), a widely used polymer in electroluminescent devices, in potentiometric measurements, to assess the differences in the sour taste of three acids, viz. HCl, citric acid and acetic acid. The incorporation of PEDOT made it possible to eliminate the KCl solution used to stabilize the potentiometric measurements since PEDOT exhibits a more stable redox potential, leading to a stable membrane that could be operated for two weeks of continuous use.

Other materials utilized in electrochemical measurements for e-tongues include cellulose composites, anthracene-modified platinum electrodes and immobilized enzymes for detecting specifically trinitrotoluene (TNT) and nitro-compounds used in land mines at the μM (ppm) concentration level. Thick films of RuO₂, Ag and Cu were used as sensing units in potentiometric measurements to detect several salts and their mixtures. The samples differed by the anionic (SO₄²⁻, Cl⁻, PO₄H₂⁻, CO₃H⁻ and NO₃⁻ ) and cationic (Na⁺ and K⁺ ) species in solution, and could be distinguished by treating the data with Principal Component Analysis.

With the advent of e-tongues based on impedance spectroscopy, several issues appeared with regard to the materials for sensing units. First of all, it has been shown that almost any type of material can be used in the sensing unit, as the interfacial electrical properties are amenable to considerable changes upon small variations in the solution in contact with the electrodes. So much so that e-tongues have been produced with metallic electrodes with no coating (see later on), basically because the cross-sensitivity may be gained by using electrodes with variable morphology. It is clear, nevertheless, that a judicious choice of materials helps enhance the sensitivity of the electronic tongue. Furthermore, it has also been proven that the use of nanostructured films is essential for a high sensitivity to be reached. The metallic electrodes, generally interdigitated gold electrodes, are coated with organic or hybrid films produced either with the Langmuir–Blodgett (LB) or the layer-by-layer (LbL) techniques. Here, it is still an open question of whether a commercial e-tongue system is possible that could distinguish the flavors of commercial beverages.
question whether the importance of being nanostructured lies in the small thickness or in the supramolecular architecture of the LB and LbL films. Many are the materials used in e-tongues based on impedance spectroscopy. They include conducting polymers, lignins, azourethane, ruthenium complexes, enzymes, phospholipids, chitosan and liposomes in LB and LbL films.

The interest in turning the e-tongue technology viable commercially has brought motivation to investigate the fabrication of cheap electrodes. Lvova et al.⁴⁴ produced a relatively cheap configuration of electrodes made with a matrix of poly-(vinyl chloride) (PVC), aromatic polyurethane and polypyrrole with different plasticizers on carbon paste, which were used to evaluate waters, soft drinks and beers. Legin et al.⁴⁴ used 23 sensing units composed of chalcogenide glasses, metallic and plasticized polymer membranes to assess 56 samples of Italian wines, and were able to distinguish the wines with an accuracy ranging from 87 to 92% – in comparison to the assessment by professional sommeliers – when the potentiometric data were treated with neural networks. Sim et al.⁴⁴ fabricated disposable sensing units with standard screen-printing technology using lipid membranes, which were employed in the identification of maturity stages of jackfruit. In a similar work Abdul Rahaman et al.⁴⁸ described a microcontroller-based e-tongue system capable of discriminating between samples containing herbal medicine (Eurycoma longifolia).

Still with regard to cheap electrodes, Borato et al.⁴⁵ have found that e-tongues can be produced with uncoated metallic electrodes. They used a set of 4–5 bare chrome-deposited electrodes that were nominally identical but that differed in morphology, as indicated by atomic force microscopy (AFM) measurements. This led to a cross-sensitivity among the sensing units, which allowed the sensor array to be able to distinguish the basic tastes with a similar performance to other e-tongues containing organic nanostructured films, and detect trace amounts of copper ions in aqueous solutions.⁴⁵

A final comment should be made on the type of material in the sensing units for e-tongues. As already mentioned, because the latter are based on the global selectivity concept, the sensing-unit-material is not required to interact specifically with the analyte. However, this concept can be extended by using one or more sensing units with immobilized molecules capable of molecular recognition toward the analyte.⁴⁶ This idea has indeed been pursued and the concept of an e-tongue extended to biosensing, with antigens and enzymes used in nanostructured films as the sensing units.⁴⁶ Results on this topic will be discussed in Section 5.

4. Methods of data analysis

The main task of an e-tongue is that of classifying the samples under analysis. Since the number of samples may be very large and many measurements are needed to distinguish between very similar samples – whose variability may also be considerable for complex liquids such as wines, juices, etc. – the amount of data generated is tremendous. Therefore, resorting to chemometric or pattern recognition methods is inevitable.

The most used process in the literature for e-tongues is Principal Component Analysis. PCA is widely used in statistical analysis to display the data. Briefly, samples are presented as a matrix whose rows represent the number of experiments and the columns the number of sensing units used. By using PCA, multivariate data can be explored, reducing their noise, without loss of information, besides the possibility of assessing the significance of individual components.⁵⁵–⁶⁵ PCA plots can be obtained in 2 or 3 dimensions (2D or 3D), depending on the need to distinguish the samples. Fig. 7 shows a 3D plot in which different types of vinegar are identified.

When the number of classes of samples is very large, for instance if one wishes to distinguish between a set of dozens of wines, the many points placed lead to overcrowding of a 2D or 3D PCA plot. Hence, other methods to treat the data are required. In this context, methods involving artificial neural networks (ANNs) are widely used. ANNs are distributed computing systems composed of processing units connected by weighted links that can be assembled in one or more layers, simulating the structure and functioning of the human brain. One of main advantages of using ANNs is their ability to learn from data, through training algorithms.⁵⁵,⁶⁵ The procedure inherent in treating voltammetric data with ANNs is illustrated in Fig. 8.

Neural network methods have been used to treat potentiometric data for identification of nitrate ions in liquids containing variable quantities of chloride⁶⁴ and to treat amperometric data for determining toxic phenolic compounds in waters with 95% accuracy.⁷⁷ The distinction among samples of various brands of mineral water was obtained with 91% accuracy by processing the potentiometric data from a 12-unit sensor array.⁹ Using neural networks has allowed Ishihara et al.⁶⁶ to assess quantitatively the intensity of the basic tastes (sour, salty, bitter, sweet and umami) in 30 types of beverages, whose results were compared to the perception of a panel of 51 human beings, for which a deviation of only 7% was found in the classification of the beverages.

Fig. 7  PCA classification of diluted and pure vinegars. Note that when the percentages of variance information conveyed in the 3 axes are added, one obtains 100%, which indicates that the PCA analysis is very reliable.⁶⁵ [Reprinted with permission from ref. 153. Copyright 2006, Elsevier.]
Artificial neural networks have also been used for pattern recognition \(^{157}\) and in distinguishing homologue ionic surfactants and phenols. \(^{158} - {160}\)

Methods combining neural networks and other approaches have been exploited by del Valle et al.: artificial neural networks and deconvolution techniques were used to identify amino acids, \(^{162}\) phenolic compounds, \(^{77,163}\) various types of ions and fertilizers, \(^{29}\) and substances used in medicines (ascorbic acid, aminophenol and paracetamol). \(^{16,164,165}\) Enhanced performance was obtained when the input data for the neural network method were pre-processed with reduction via orthogonal Legendre functions, which allowed an e-tongue to discriminate the ions Cl\(^-\)/Cl\(^0\), NO\(_3^-\)/NO\(_3^0\) and HCO\(_3^-\)/HCO\(_3^0\) to an accuracy of 93%, with no need to eliminate interfering species from the samples under analysis. \(^{166}\)

A modified method, referred to as Wavelet Neural Networks, \(^{161}\) was used for analysing voltammetric data for ascorbic acid, 4-aminophenol and paracetamol, \(^{161}\) uric acid and ascorbic acid, \(^{167}\) whose cyclic voltammograms exhibit overlapping.

In addition to leading to higher performance of e-tongues, statistical and computational methods may also serve for screening materials and experimental conditions for the measurements. For example, the correlation among samples was investigated with PCA in order to reduce the number of required sensing units for the array. \(^{168}\) Furthermore, machine learning methods may be used to correlate the electrical response of the sensing units with the human taste. Sadrieh et al. \(^{169}\) made a comparative study with a commercial e-tongue whose electrochemical results were correlated with the assessment of a panel of human tasters for three pharmaceutical drugs, namely antibiotics against infections caused by Bacillus anthracis (anthrax) and other bacteria. The three antibiotics were dissolved in water, milk, chocolate-based drinks, yogurts and juices to mask the strong bitter taste of the drugs. The study indicated that the palatability of the drugs increased and the classification of the drugs differed somewhat from that produced by the human tasters. Using multivariate regression, Scampicchio et al. \(^{67}\) were able to correlate the astringency feature of tea samples with the response from the e-tongue based on amperometric measurements. A strong correlation was observed between the response from an e-tongue with impedance spectroscopy measurements and the scores assigned by professional tasters for several samples of coffee. \(^{170}\)

There is now a trend to using more sophisticated methods combining data mining, machine-learning approaches and information visualization techniques. \(^{171}\) Albeit not directly related to electronic tongues, the data from biosensors using nanostructured films and electrochemical measurements have already been treated with information visualization methods, more specifically with data projection techniques with great

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**Fig. 8** Schematic diagram of a data treatment procedure using an ANN in voltammetric e-tongues. The cyclic voltammograms, as the one shown on the left of the figure, are analysed with ANNs represented in the central part of the figure to allow for distinction of ascorbic acid, 4-aminophenol and paracetamol. \(^{161}\) [Reprinted with permission from ref. 161. Copyright 2006, Elsevier.]

**Fig. 9** The plot brings the visualization of the electrical impedance data in which the colour represents the different samples of phytic acid, in addition to the buffer. The projection technique used in this figure was Sammon Mapping with data standardization. \(^{172}\) [Reprinted with permission from ref. 172. Copyright 2010, American Chemical Society.]
success. These projection techniques have been used for the first time in an e-tongue for detecting phytic acid, and Fig. 9 shows the distinction achieved for the various concentrations.

5. Summary of the applications

Throughout the text in the various sections, many applications of e-tongues were mentioned in connection with the principle of detection, the materials in the sensing units or the methods for data analysis. In this section we provide a summary of the various applications, which can be visualized in Table 1. It is worth mentioning the difficulties to address some of the parameters involved (detection limit, calibration/linear range, stability, reproducibility, technique used, liquid sample analysed,...) in a table format due to the massive diversity of information offered in the literature, considering the natural diversity of samples (heavy metal ions, beverages, wines, pharmaceuticals...) and techniques used in data analysis (PCA, PLS, Fuzzy ARTMAP, ANN, non-linear least squares, self-organising map...).

The first test for an e-tongue consists of verifying whether the sensor array is capable of distinguishing aqueous solutions representing the basic tastes (sweet, sour, salty, bitter and umami) with small concentrations. In several of the papers published in the literature, demonstrations were provided that the e-tongues could distinguish basic tastes below the human threshold. A related issue is to check whether the tongue is able to detect suppression, which is important for some applications, as in reducing the bitter taste in antibiotics. It has been proposed that the suppression of the bitter taste is associated with a phospholipid in the membrane which blocks the adsorption of quinine in the transducing membrane.

5.1. Dairy and food industries

E-tongues have been used to monitor the fermentation process in the fabrication of diet cheese, as the devices could detect various organic acids relevant to the fermentation with an accuracy ranging from 87 to 95%. Fermentation was also monitored by Kim et al., including the bacterial growth made by Turner et al. The changes in the taste of cow milk from several

Table 1

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<td>Biosensing and bioapplications</td>
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<td>Chalcogenide glasses, enzymes, lipid membranes, dendrimers, phospholipids</td>
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<td>voltammetry, impedance,</td>
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sources were studied with e-tongues based on electrochemical measurements, which also served to identify bacteria in the fermentation process. The bitter taste in olive oils was assessed with e-tongues obtained with sensing units of carbon paste. The cyclic voltammetry data were correlated with the bitterness in 9 olive oil samples using PCA and the Partial Least Squares (PLS) method. Phenolic compounds responsible for the bitter taste were identified in olive oil samples using amperometric enzyme-based biosensors in a Flow Injection Analysis system. The discrimination of various vegetable oils and quality olive oils was obtained with electrochemical measurements, in addition to the identification of antioxidants.

Industrialized products containing tomatoes were assessed with an amperometric biosensor, in which an enzyme was immobilized in the sensing unit to recognize glutamic acid and monosodium glutamate, as these substances are responsible for the umami taste. The astrignency in green tea was evaluated with a potentiometric e-tongue. Soft drinks were assessed with a colorimetric sensor consisting of a hydrophobic membrane on which 25 dyes were imprinted. As the membrane was immersed into a given drink, a coloured pattern was formed, which was taken as a fingerprint for the drink. The statistical methods PCA and Hierarchical Clustering Analysis (HCA) were used to classify the drinks to an accuracy of 98%.

Using sensing units made with ion-selective polymer membranes coating gold electrodes, Ciosek et al. evaluated the fat contents in milk samples, where the amount of fat could be obtained from a classification of the samples using neural network methods that allowed an accuracy of 97%. In a similar work, orange juices, milk from distinct sources and tonic waters were distinguished, while the quality of two cereals used in the food industry was studied, particularly with regard to the influence from nutritional factors. The importance of the materials coating the electrodes was also addressed in the analysis of juices, beers and milk. Also of interest for the food industry is the control of stored products, which may be monitored by detection of strong odours – such as scatol, ammonia and cresolate. The latter may be detected even in small concentrations (μM) to an accuracy of 80% using artificial neural networks. Mineral waters could be analyzed with a potentiometric sensor array with 12 sensing units made with thick films, and an accuracy of 91% was obtained in the distinction of the various samples with neural networks.

5.2. Pharmaceutical industry

There have been various research projects dealing with the distinction and monitoring of pharmaceutical products. Legin et al. screened 41 substances, which could be classified into three classes, namely sweet, salty and bitter. They were able to detect trace amounts of bitter substances in binary mixtures, consistent with predictions made by a panel of human tasters. With only 8 sensing units, it was possible to identify independently ammonia, oxalate and citrate, during the fermentation of Aspergillus niger used in the production of α-amylases. The amount of ammonia was found to lie within 0.4–14 mM, while for citrate the contents were 0.5–5.5 mM and for oxalate 2.6–62.2 mM. Using neural networks, such concentrations during fermentation could be predicted to an accuracy of 92%.

The identification and suppression of the bitter taste in medicines is a crucial commercial need. Potentiometric e-tongues have been used to assess the intensity of the bitter taste in medicines incorporated in water and jelly. It was found that the suppression of the bitter taste could be made by altering the pH of the jelly. Though the intensity of the bitter taste could be deduced with the e-tongue, the bitterness of the drugs could not be correlated with their physicochemical properties in aqueous solutions. The prediction of the bitterness intensity shown in Fig. 10 shows how an e-tongue may be used to assess how bitterness can be suppressed by adding other flavours and water. The suppression of amino acids in natural and supplementary food was also evaluated. Phytotherapeutic products and the detection of homologous anionic and non-ionic surfactants in multicomponent model mixtures, natural waters and drugs were assessed with e-tongues.

Woertz et al. used a commercially available e-tongue based on lipid membranes to obtain a reliable response of how and to what extent an artificial taste sensor can be used as an analytical tool for the characterization of pharmaceutical formulations.

5.3. Wines and vodka

Wine samples stored under different conditions could be recognised with 100% accuracy with regard to vintage, vineyard and brands with artificial neural networks and an e-tongue. The procedure adopted in this analysis is depicted in Fig. 11. Italian wines varying according to the type of grape, acidity, bitterness, colour, astringency and flavour were assessed with a combination of electronic nose and amperometric e-tongue. By using Genetic Algorithms excellent prediction with a good accuracy could be achieved in some sensorial parameters of the overall quality of dry red wines. PCA was applied in the data from cyclic voltammograms of copper electrodes to classify Chinese wines. The importance of fabrication parameters for the wine industry, such as ageing in oak barrels, was investigated with an e-tongue using polypyrrole and perylene derivatives in the sensing units. In another study, the degradation of the lignin from the cork used in the wine bottles was held responsible for the deterioration
of the wine flavour owing to the larger amount of phenol derivatives in the cork attacked by fungi.\textsuperscript{203} Vodkas of distinct brands and quality were distinguished in a study using potentiometric e-tongues.\textsuperscript{204}

5.4. Coffee

Tastes of different kinds of coffees were analysed by taste sensors exploring potentiometric measurements and distinct arrays of sensing materials, such as lipid membranes\textsuperscript{28,55} and ion-selective sensors.\textsuperscript{147} Similarly, conducting polymers and impedance studies were successfully employed in distinguishing different brands of coffee.\textsuperscript{79}

5.5. Contamination in waters

Because the high sensitivity of e-tongues allows one to detect trace amounts of impurities in liquid samples, an obvious application is the monitoring of water quality for various purposes. Of special relevance for the studies with e-tongues has been the detection of heavy ions in waters owing to the importance of environmental pollution. For instance, Men \textit{et al.}\textsuperscript{18} detected metallic ions in solution down to $10^{-6}$ M, while Fou \textit{et al.}\textsuperscript{205} detected ppb concentrations of Zn$^{2+}$, Cd$^{2+}$, Pb$^{2+}$, Cu$^{2+}$, Fe$^{3+}$ and Cr$^{3+}$ in water. Rare earths and divalent cations were also identified with e-tongues in waste water from nuclear power plants.\textsuperscript{206} The presence of chloroform in waters was detected with an e-tongue based on impedance spectroscopy\textsuperscript{207} and used waters from washing machines were studied by Ivarsso\textit{m et al.}\textsuperscript{208} while the same principle was applied to detect a zoonosis (Pasteurellosis) with sensing units made with LbL films of the antigen that could recognize the specific antibody.\textsuperscript{134} Aoki \textit{et al.} explored LbL films of phospholipids as biological membrane mimetic systems, cardiolipin (CLP) and dipalmityl phosphatidyl glycerol (DPPG) in the detection of methylene blue (MB) down to $10^{-11}$ M concentrations.\textsuperscript{131}

5.6. Biosensors

The idea of extending the concept of an e-tongue with sensing units capable of molecular recognition was already present in seminal contributions, and was put into practice in recent years. Indeed, a bioelectronic tongue employing platinum electrodes coated with enzymes was used in amperometric measurements to analyze waste waters.\textsuperscript{19} Pauliukaite \textit{et al.}\textsuperscript{143} produced an amperometric biosensor with an enzyme to recognize the substances responsible for the umami taste. Trace amounts of catechol were detected by Zucolotto \textit{et al.}\textsuperscript{134,135} using impedance spectroscopy and a sensor array containing a sensing unit with the immobilized Cl$_2$-catechol 1,2-dioxygenase (CCD)$^{135}$ (Fig. 12), while the same principle was applied to detect a zoonosis (Pasteurellosis) with sensing units made with LbL films of the antigen that could recognize the specific antibody.\textsuperscript{134} Aoki \textit{et al.} explored LbL films of phospholipids as biological membrane mimetic systems, cardiolipin (CLP) and dipalmityl phosphatidyl glycerol (DPPG) in the detection of methylene blue (MB) down to $10^{-11}$ M concentrations.\textsuperscript{131}

6. Conclusions and perspectives

The prominence of the topic e-tongues has been reflected in scientometry data, with growing numbers of papers and research groups in the last few years. A survey in the Web of Science using the keywords ‘taste sensor∗’ or ‘electronic tongue∗’ led to the results shown in Fig. 13, which points to large increase in activity and in the impact of the work on e-tongues.

The two classes of methods most used in electronic tongues are electrochemical and impedance spectroscopy, with very good results in several instances. It is therefore difficult to recommend which is the most suitable for a given application as such a decision will depend on the type of analyte to be detected and on the experimental conditions under which the experiments are to be performed. In terms of sensitivity, both electrochemistry
and impedance spectroscopy are capable of leading to highly sensitive sensor arrays. The use of ultrathin films coupled with impedance spectroscopy has enabled the lowest detection limits in the literature (down to pM concentrations of analytes in water).\textsuperscript{199} As for the cost of the device, again, cheap systems can be obtained with both methods.

Having proved that e-tongues can be applied to many types of industry, the main challenge now lies in reaching the market. There are two electronic tongue systems commercially available: (i) SA402B, Atsugi-chi (Japan), based on lipid membranes; and (ii) ASTREE e-tongue, Alpha M.O.S (France), based on chemical field effect transistor (ChemFET), but in most studies only prototypes were produced and device engineering needs to be better performed in order to achieve robust, reliable sensors. Up to date, the main challenge in taste measurements is the production of sensor arrays with repeatable electrical or electrochemical properties, minor ageing and temperature effects, as well as the irreversible binding of substances on the materials used as sensing units in some applications. Therefore, whenever the units of a sensor array need to be replaced, the whole system has to be re-calibrated via software, as the electrical response of the new sensing unit will differ from the previous one. Perhaps this is the main drawback of the e-tongues, which have prevented them from being widely used in the market. The information visualization methods are good candidates for this assignment and, incidentally, these latter methods are also essential for demanding tasks of identifying differences in very similar complex liquids, as is the case of clinical diagnosis – for which sensing units with materials capable of molecular recognition are already being used.

A better comprehension of interfacial effects, one of the main reasons for the high distinguinability of e-tongues, is essential to improve the inherent capability of these sensors to detect trace amounts of analytes due to small changes in the electrical properties of the ultrathin films when the latter are in contact with the liquid under analysis. The electrical behaviour of the materials is normally analyzed using equivalent electric circuits, with the system comprising the sensing unit and the sample being represented by capacitors and resistors,\textsuperscript{90,210} usually made due to the difficulty of identifying precisely the molecular-level interactions occurring in each arrangement studied.

There are various factors determining the electrical properties of the sensing units, such as: (i) the capacitance of the inter-digitated electrode; (ii) the conductance at the liquid/film/electrode interface; (iii) the ability of charge injection from the electrode; (iv) the capability of the film to store charges and (v) interactions between the liquid and the film forming the sensing unit. The latter factor is critical for the e-tongue measurements, as it involves not only possible adsorption of analyte molecules on the film surface, but also rearrangement of film-forming molecules induced by the interaction. Most importantly, the water molecules are always structured at an interface, and this degree of structuring depends on the interface, as it has become clear with characterization using surface-sensitive methods such as sum-frequency generation spectroscopy.\textsuperscript{211,212} The structuring of water is also affected by changes in the liquid sample itself, as it occurs when the concentration of a given analyte is varied.

Some efforts should be directed toward taking advantage of the weak-molecular surface interactions in these systems, trying to figure out how the ultrathin films can recognize subtle differences in the liquid samples. Exploring interfacial effects in this field is as important as the application of different technologies for data analysis and acquisition, well explored in the last couple of years, since both are fundamental for future developments of e-tongues. If this can be reached, the accuracy of the sensors might be improved, facilitating its commercial use in quality control, stability testing, formulation of ingredients in foodstuff, beverages and pharmaceuticals, besides the environmental and clinical diagnosis.

**Acknowledgements**

This work was supported by FAPESP, CNPq, Capes, INEO and nBioNet (Brazil).

**References**
