

CHEMICAL IMAGES OF LIQUIDS

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Abstract

Following the success of electronic noses in a variety of applications related to many areas such as industrial, medical, environmental, spatial, etc. where the objective was to construct chemical images of volatile compounds including odors, here we introduce another system able to perform chemical images of liquids of different origin, quality, and composition. In line with mammalian senses such as olfaction or taste, recently a strategy which applies the sensors not specific in character for a single analyte but rather broadly sensitive with the only necessary characteristic and prerequisite to be different from each other in terms of their responses, has been developed. The sensors are grouped in a matrix and supported by a suitable pattern recognition analysis in order to generate a global response of the overall array. For liquid phase analysis such a system is called Electronic Tongue. This chapter describes in some detail the operating principle of the sensors forming the electronic tongue multisensor system, the matrix strategy, the most suitable data analysis adopted for the purpose, and a number of chemical images of different liquids including potable and waste waters, soft drinks, alcoholic beverages, clinical samples and some biological objects, taking into account the different components' concentration distributions present in the tested samples.

Keywords:

electrochemical sensors; electronic tongue; pattern recognition.

1. Introduction

An application of sensor arrays for analysis of liquid samples started in the middle eighties, when Otto and Thomas [1] applied the principle of simultaneous measurement with several plasticized polymeric membrane potentiometric sensors to a detection of Mg^{2+} -ions concentration in multicomponent solutions of composition similar to biological liquids (blood plasma and urine). An accurate detection of magnesium ion concentration in biological liquids is important especially due to an absence of highly-selective Mg^{2+} - electrodes which may permit a direct measurement of Mg^{2+} on the high background of calcium. After this pioneering work many applications of multisensor arrays for liquid phase analysis have been reported both for qualitative discrimination and quantitative determination of various components and several comprehensive reviews were published [2, 4]. Multisensor arrays composed of various types of electrochemical sensors and using different detection principles were devised. Thus, an array based on sensors with polymeric lipid membranes was reported by Toko et al [5] from Kyushu University, Japan. Such a system was named a “taste sensor”, since it showed an ability to distinguish 5 basic tastes: sweet, bitter, salty, sour and umami. Later a “taste sensor” with global selectivity similar to a human perception principle [6] and a commercial version of a ‘taste sensor’ [7] were reported. A multisensor array based on potentiometric chalcogenide-glass chemical sensors with membranes doped with various metals has been reported by Legin and colleagues [8] from St. Petersburg University, Russia. As a result of joint work of the same authors with the Chemical Sensors Group of ‘Tor Vergata’ University, Rome, Italy, the term “Electronic tongue” was suggested for a multisensor system composed of an array of chemical sensors and an appropriate data processing tool [9]. This term was chosen based on the similarity of the ‘electronic tongue’ working principle and the human gustatory system. Later the chalcogenide-glass electronic tongue was supplemented by various solvent polymeric membrane electrodes, the suitability of which for electronic tongue applications was specially studied [10, 11]. A potentiometric electronic tongue based on electrochemically polymerized porphyrin- and metalloporphyrins’ polymeric films was reported in [12]. Other types of transducers like ISFET, LAPS (light addressable potentiometric sensors) were recently reported for multisensor arrays applications [13]. An artificial tongue composed of four sensors produced as ultra thin Langmuir-Blodgett films of conducting polymers and their mixture and utilizing AC measurements (impedance spectroscopy) was suggested in [14]. Arrays of ISEs were also reported for qualitative discrimination of several liquid foodstuffs

[15]. The Electronic tongue developed at Linkoping University [16], consisting of an array of different inert metal electrodes and based on pulsed voltammetry, has been used for analysis of liquid samples. An application of potentiometric metallic sensor, so-called first kind electrodes, in electronic tongue systems were recently reported in [17], while a thick film technology application for metallic sensor array development was reported by Martinez-Manez et al [18]. Hybride electronic tongues based on various types of electrochemical sensors and various detection methods [19, 21], using a combination of electronic tongues and electronic noses for liquid phase applications, were also reported [22, 25]. It was found that a fusion of several techniques may significantly improve the analysis results, while the comparison of various methods used in electronic tongues may exhibit the weak and strong sides of such methods, yielding further improvements. An electronic tongue system based on an array of electrochemical sensors may not provide a taste of analyzed samples as does the human tongue, but “chemical images” of target samples received as a result of chemical interaction between samples and sensors may be correlated either with a taste or with other particular features. Such “chemical images” may be used for different analytical purposes, either for discrimination and classification tasks, or for quantitative analysis. That is why multisensor systems, and electronic tongues in particular, have attracted much interest in the last two decades. In this chapter we focus on new results and achievements in the area of electrochemical multisensor array applications for liquid phase analysis.

2. Liquid phase analytical methods

Common methods of liquid analysis, especially for complex multi-component samples, are well established and highly sensitive gas / liquid chromatography (GLC) coupled with specific detection methods like mass-spectrometry (MS) or visible (or ultraviolet) spectroscopic detection, Atomic Absorption and Atomic Emission Spectroscopy (AAS and AES), colorimetry; elemental analysis [26]. Classical titrimetric and precipitation gravimetry are often used for water and wastewater analysis, while an application of immunoassays or other biochemical tests is common for analysis of biological liquids and foodstuffs [27]. Application of electrochemical methods dealing with analysis of complex liquid media by means of electrochemical sensors (electrodes) utilization is a big part of modern analytical chemistry [28]. Electrochemistry deals with the study of chemical reactions that produce electrical effects (oxidation and reduction of particles presenting in solution), and of the chemical phenomena that are caused by the action of currents

and voltages. In contrast to the many analytical measurements that involve homogeneous bulk solutions, electrochemical processes happen on electrode-solution surface. The application principle of electrochemical sensor is a transformation of its' electrical signal to a concentration of analyte based on known theoretical principles of electrode processes. The two main groups of electrochemical methods are dynamic and static methods [29]. In potentiometry, which is a static method the change of electrode potential due to the interactions of electrode sensing membrane with charged analyte particles in solution is registered under open circuit static mode (zero-current conditions). Dynamic electrochemical methods deal mainly with metallic electrodes and observe the situation when the current flows and analyte concentration change as the result of electron-transfer Red-Ox reaction on electrode surface. Minimum two electrodes are required for electrochemical measurements. Dipped in electrolyte solution these electrodes constitute an electrochemical cell. An electrode which responds to a target analyte is an indicator (or working) electrode. In general, a potential of working electrode either in static or in dynamic electrochemical technique has some significant value only being measured against a well defined reference point, an electrode with a stable potential value, independent on analyzed solution composition. This electros is called a reference electrode. The first well known reference electrode, which is rarely used for routine applications now, but still important because it is used to establish standard-state oxidation-reduction potentials of many electroactive particles is a standard hydrogen electrode (SHE). The SHE is composed of porous Pt plate electrode immersed in a HCl solution in which the hydrogen ion activity is 1.00 and in which H_2 gas is bubbled at a pressure of 1 atm, Figure 1.

The standard potential of such electrode is established as equal to $E_0 = 0.00$ V for all temperatures and determined by the reaction: $H^+(aq) + e^- \rightarrow \frac{1}{2} \cdot H_2(g)$.

In practice an application of SHE is difficult due to a complexity of preparation and handling. Other types of reference electrodes, which satisfy to a main demand of reference electrode (a high inertness to analyzed solution composition and stable potential) are used nowadays: saturated calomel (SCE) and silver/silver chloride (Ag/AgCl) electrodes, Figure 2. Both these electrodes are based on the red-ox couple between metal (Hg or Ag) and its insoluble salt (calomel Hg_2Cl_2 or AgCl) and their potentials are determined by the concentration of Cl^- ion used for their preparation. Hence such electrodes can be classified as potentiometric sensor of second kind (see below).

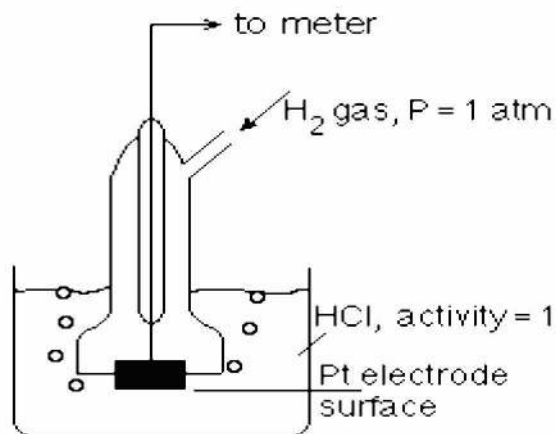


Figure 1. The SHE schematic diagram.

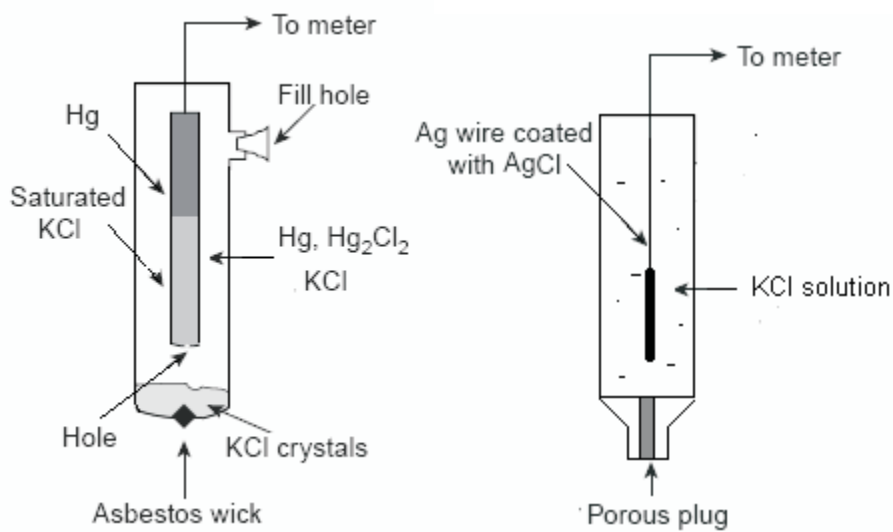


Figure 2. The schemes of SCE (left) and Ag / AgCl (right) reference electrodes. SCE constructed using an aqueous solution saturated with KCl has a potential of +244 mV at 25 C, while Ag/AgCl often prepared with 3.5 M KCl has a potential of +205 mV at 25 C.

3. Theoretical basis behind electrochemical sensors response

Dynamic electrochemical methods

Coulometry

Among the dynamic electrochemical methods coulometry is the most attractive since it doesn't demand any calibration procedure. The method is based on the electrolysis of a solution, when the analyte is quantitatively oxidized or reduced at the working electrode or reacts quantitatively with a reagent generated at the working electrode. The total charge, Q , in coulombs, passed during an electrolysis is related to the absolute amount of analyte by Faraday's law:

$$Q = n \cdot F \cdot N \quad (1)$$

where n is the number of electrons transferred per mole of analyte, F is Faraday's constant (96487 C mol^{-1}), and N represents the number of moles of analyte. A coulomb is also equivalent to an amper per second (A*s), thus, for a constant current i the charge is given as:

$$Q = i \cdot t_e \quad (2)$$

where t_e is the electrolysis time. If current varies with time, as it does in controlled-potential coulometry, then the total charge is given by

$$Q = \int_{t=0}^{t=t_e} i(t) \cdot dt \quad (3)$$

Voltammetry

The voltammetric method is based on determination of ion concentration in dilute solutions from current flow as a function of voltage when ion polarization (depletion of concentration caused by electrolysis) occurs around the electrode. In general, working electrode potential is used to derive an electron-transfer reaction:



where O and R are oxidized and reduced forms of RedOx couple, and resulting current is measured with a help of auxiliary (counter) electrode. For system in a thermodynamic equilibrium the potential of electrode can be expressed by the Nernst law, resulting from the expressions of free Gibbs energy (ΔG) and electrochemical potential change:

$$E = E^O + \frac{2.3 \cdot R \cdot T}{n \cdot F} \ln \frac{[O]}{[R]} \quad (5)$$

where E^O is a standard potential of RedOx reaction 1, R is the universal gas constant, T is the absolute temperature in Kelvins, n is a number of electrons transferred in reaction, F is Faraday constant and [O] and [R] are the concentrations of electroactive particles on electrode surface. The current resulting from the oxidation state of electroactive particles is a Faradic current, since it obeys Faradays law 1 - 3. Nevertheless, the total current flowing on electrode surface is the result of several processes and a sum of faradic currents for the analyte and blank solution as far as nonfaradic charging currents [28]. Often a plenty of several steps are involved in electrochemical reaction, the simplest steps are: mass transfer of electroactive particles to the electrode surface, electron transfer along the electrode surface and the back transfer of resulting product back to bulk solution. The resulting current of reaction is limited by the slowest step. For mass transport-limited reactions (when the overall electrode reaction is only controlled by the rate at which electroactive particles reach the surface) the particles flux J to electrode is expressed by Nernst-Planck equation. The overall current i is proportional to the particles flux (diffusion):

$$i = n \cdot F \cdot A \cdot J \quad (6)$$

and according to the first Ficks' law the rate of diffusion is directly proportional to the slope of concentration gradient:

$$J(x, t) = -D \cdot \frac{\partial C(x, t)}{\partial x} \quad (7)$$

where t is a time and x is a position of particle relatively to the electrode surface, D is a diffusion coefficient. By combination of 6 and 7 a general equation for current response limited by diffusion (diffusion current) may be expressed as follows:

$$i = -n \cdot F \cdot A \cdot D \cdot \frac{\partial C(x, t)}{\partial x} \quad (8)$$

The time and position dependence of diffusion flux (and hence, diffusion current) may be evolved then, using Ficks' second law. The voltammetry resulting diagram (voltammogram) represents the current signal (Y axis) versus variation of applied potential (X axis). Voltammogram may be used either for qualitative analysis: measure a half-wave potential and detect electroactive species which undergo electrode discharge at characteristic potentials, or quantitative detection of concentration of

species in the test solution by means of diffusion current. In a latter case a calibration with standards and a preparation the linear standard curve of diffusion current vs concentration is first required. There are several various modifications of voltammetric technique, depending on form of applied potential (Pulse Voltammetry, amperometry which uses a constant applied potential, etc) and measure procedure (for instance, stripping voltammetry). Several types of biosensors use voltammetry as detection method [30].

Conductimetry

In this method the conductance of a solution is measured, using inert (third kind metallic electrodes), alternating current and electrical zero-circuit. By this no net current flow through electrochemical cell and no electrolysis happens. The concentration of ions in the solution is estimated from the conductance value.

Potentiometry

As noticed before, potentiometry is a static electro chemical method, in which the potential of an electrochemical cell is measured under static conditions (zero current). The electro motive force (EMF) of electrochemical cell, E , may be expressed as:

$$E_{cell} = E_{ind} - E_{ref} + E_{lj} \quad (9)$$

where E_{ind} is a potential of indicator electrode, E_{ref} is a potential of reference electrode, E_{lj} is a liquid junction potential which develops at the interface between two ionic solutions that differ in composition and for which the mobility of the ions differs. Liquid junction potential can be almost eliminated by using a salty bridge filled with electrolyte solution of salt formed with ions with very similar mobility (KCl for instance). The potential of indicator electrode is expressed by the Nernst law 5, thus, for discussed earlier SHE:

$$E = E^O + 0.05916 \cdot \log \frac{[H^+]}{[H_2]^{\frac{1}{2}}} = 0.05916 \cdot \log[H^+] \quad (10)$$

There are two main classes of indicator electrodes used in potentiometry: metallic electrodes, and ion-selective electrodes (ISEs).

Metallic potentiometric sensors

Potentiometric metallic electrodes can be divided on 3 groups. **First** kind metallic electrodes are wires of active metal immersed in solution, contained the ions of this metal, thus, for instance, copper wire dipped

in solution of copper chloride is a metallic electrode of first kind. The potential of such electrode is a function of concentration of metal in solution:

$$E = 0.3419 + \frac{0.05916}{2} \cdot \log[Cu^{2+}] \quad (11)$$

Where $E^0 = 0.3419$ V is a standard potential of $Cu^{2+}(aq) + 2e^- \rightleftharpoons Cu(s)$ reaction determined versus SHE. **Second** kind metallic electrodes are the electrodes involving an M^{n+}/M RedOx couple and respond to the concentration of another species if that species is in equilibrium with M^{n+} . SCE and silver/silver chloride electrodes discussed earlier are the second type potentiometric electrodes. Their potentials are a function of chloride in solution:

$$E_{SCE} = 0.268 - 0.05916 \cdot \log[Cl^-] E_{Ag/AgCl} = 0.2223 - 0.05916 \cdot \log[Cl^-] \quad (12)$$

Inert metallic electrodes (namely Pt, Au, etc) which simply serve as a source of, or a sink for, electrons in other red-ox reactions are the third kind potentiometric metallic electrodes, or red-ox electrodes. These electrode do not participate in electrochemical reaction.

ISEs

A big group of potentiometric sensors which have enhanced selectivity to the only one analyte in solution are Ion-selective electrodes. ISEs function by using a membrane that reacts selectively with a single primary ion. ISEs differ depending on a material of sensing membrane which can be made of glass, crystal, chalcogenide-glass, polymeric. Schematic presentation of ISE with plasticized polymeric membrane is given on Figure 3.

The properties of ISE membrane are dependent on its composition and the velocity of permeation to the various ions. When the ISE is placed in a solution containing the particular ion (to which electrode is reversible), a small number of ions (to which the membrane is selective) pass from the solution of higher concentration through the membrane and result in a membrane potential. One side of the membrane is in contact with an internal solution containing a fixed concentration of analyte, while the other side of the membrane is in contact with the sample. Current is carried through the membrane by the movement of either the analyte or an ion already present in the membrane's matrix. The membrane potential is given by a Nernst-like equation:

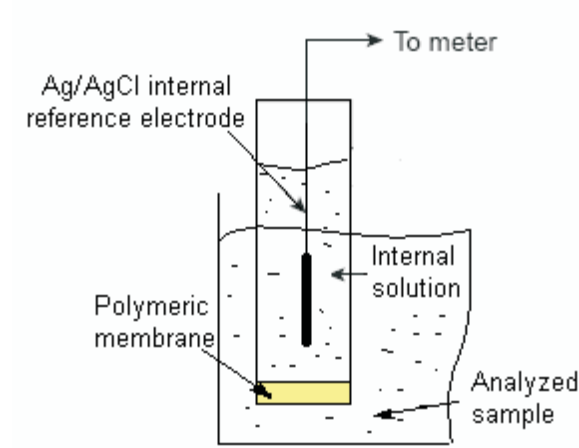


Figure 3. Schematic presentation of ISE with plasticized polymeric membrane.

$$E_{mem} = E_{Asym} - \frac{RT}{nF} \cdot \ln \frac{[A]_{int}}{[A]_{sam}} \quad (13)$$

where $[A]_{sam}$ and $[A]_{int}$ are the concentrations of analyte in the sample and the internal solution, respectively, and n is the analyte's charge. Ideally, E_{mem} should be zero when the concentrations of analyte on both sides of the membrane are equal. The term E_{asym} , which is called an asymmetry potential, accounts for the fact that the membrane potential is usually not zero under these conditions. Membrane potentials result from a chemical interaction between the analyte and active sites on the membrane's surface. Because the signal depends on a chemical process, most membranes are not selective toward a single analyte. Instead, the membrane potential is proportional to the concentration of all ions in the sample solution capable of interacting at the membrane's active sites. The Nikolskiy equation includes the contribution of an interferer :

$$E = E^0 + \frac{0.05916}{n_A} \cdot \log([A] + K_{A,I}[I]^{\frac{n_A}{n_I}}) \quad (14)$$

where n_A and n_I are the charges of the analyte and interferer, and $K_{A,I}$ is a selectivity coefficient accounting for the relative response of the interferer. The selectivity coefficient is defined as:

$$K_{A,I} = \frac{[A]_E^{\frac{n_A}{n_I}}}{[I]_E} \quad (15)$$

where $[A]_E$ and $[I]_E$ are the concentrations of analyte and interferer yielding identical cell potentials. The selectivity is a main characteris-

tics of ISEs. Selectivity is an ability of sensor to respond only to primary ion in presence of other ions in analyte. By the other words, an ideal ISE will only respond to changers of primary ion. Selectivity of ISE is determined by the composition of sensor membrane. Membrane may be made of glass (composition of approximately $22Na_2O$, $6pcCaO$, and $72pcSiO_2$ for pH -sensitive glass electrode), crystalline (Ag_2S , $Ag_2S/AgCl$, LaF_3 doped by Eu), polymeric. The polymeric membrane of ISE is based on some polymeric material (polyvinyl chloride, polyurethane, silicon rubber, etc) dissolved in a water-immiscible solvent-plasticizer (the esters of organic acids). This mixture is doped by Membrane Active Components (MAC), the active ionic or/and neutral sites which interact specifically to ISE primary ion. A plenty of MAC has been studied in the last four decades when the application of ISEs for different purposes was extremely important and useful in many areas [31][32].

4. The Electronic tongue strategy

In the traditional approach, the ideal chemical sensor should be highly selective, which means that the device is able to detect a single analyte in a complex mixture [33]. In this case the response of the sensor can be directly correlated to the concentration of the target analyte, after a calibration procedure. The high selectivity of the chemical sensor can be achieved in two different strategies:

a) chemical approach: synthesis of a molecular receptor designed to bind only the target analyte in the complex mixture, thanks to a strong interaction (but this can be detrimental of the device reversibility), or by multiple weak interaction operating by groups geometrically arranged in an optimized way [2].

b) physical approach: selection of the transduction mechanism, in which only the target analyte is able to induce the physical property variation then transformed in a readable signal by the chosen transducer [2].

Both of these approaches can be useful for the development of a high selective sensor, although the progress of supramolecular chemistry concepts resulted in a great popularity of the first chemical approach. In this concept of chemical sensor, all the chemical species present in the analyzed environment other than the target analyte are considered as interfering species, which should be ignored as much as possible (ideally in a complete way) by the sensor, because their interaction induce an error in the sensor response, so reducing the reliability of the device. This kind of chemical sensors has been exploited in different fields of application, where it is important to know the exact concentration of a chemical species, such as for example tracking a metabolite in clinical

analyses. However this high selectivity of chemical sensors in several cases is not possible or even not useful, mainly for two reasons:

a) it is difficult to reduce the interaction with interfering species and the design and preparation of the selective molecular receptor is in some cases a Tantalus effort;

b) the chemical environment to analyze is often particularly complex, with hundred or thousand of different compounds, each of them contributing to the characterization of the analyzed matrix. In this case is not possible to individuate a single compound able to characterize the whole chemical environment. A classical example is a food aroma: the complex chemical image cannot be described with a single pixel of the whole picture, because it induces a significant loss of the necessary information [2].

Because of these difficulties a different approach has been proposed, taking inspiration from the working mechanism of the “chemical” mammalian senses and in particular from the olfaction. In these senses Nature does not use selective receptors, as it is the case of enzymes; receptors are not selective towards single chemical species, but they can interact with a large family of chemicals, although with different intensity, and it is the brain that it is in charge to extract the information derived from the senses, classifying and discriminating among the different odors or tastes. When applied to sensor field, these concepts led to a different idea of chemical sensors, where it is no more necessary the high selectivity of the device, but it should be able to interact with almost all the analytes present in the environment, although with different sensitivities. In this case interfering species cooperate to the sensor responses, giving important chemical information and being no more considered in a simple, negative way. In this case the sensor response loses the direct relationship with the concentration of a single chemical species, because of the action of interfering molecules. As a result, the sensor cannot be used stand-alone, because of this response ambiguity, but it should be integrated in a sensor array. Each sensor contributes in giving the multidimensional response of the array, which can be considered the chemical image of the analyzed sample. Data analysis techniques are in charge to extract and decode the chemical information contained, as the definition of a single color in a multicolored picture. It is worth mentioning that in this approach the rational design of the molecular receptor is not abandoned, because it is necessary that the sensor responses in the array should not be correlated each other and the selectivity towards a molecule, or class of molecules cannot completely be lost. The sensor array should be cross-reactive, or cross-selective, but they should be different to preserve the content of information present in the chemical image of

the analyzed environment [34]. This approach is now quite popular for the characterization of gaseous environment and several examples of electronic noses have been reported in the literature and are now available in the market [35], while quite surprisingly similar devices operating in the liquid phase are far less studied. The attempt to mimic the natural taste sense has been the starting point of the “electronic tongue” application. This concept has been later widened to the development of devices able to give multicolored chemical images of different liquid environments and not just the discrimination of the basic tastes. Thus, Electronic tongues devices have been exploited in different field of application, as detailed in the next sections of this review.

5. Experimental routine

Since the target of Electronic tongues application is to provide an alternative to common analytical methods (which are often enough complex) therefore the strategy of experimental approach is to reach the simplest possible experimental routine. As a consequence, no probe pretreatment such as any dilution, pre-concentration, addition of special co-reagents and sample exposure is normally used. In some specific cases a minimal pretreatment like, for instance, a sample buffering is needed, but in a majority of cases sample analyzed as it is. A simple rinsing of sensors working surface with distilled or tap water is needed for e-tongue sensors cleaning. Sometimes a short exposition (about 3-5 min) of sensors in conditioning solution which composition is determined by application task, or peculiarly analyzed sample is required in order to certain a sensor stable response before a signal acquisition. In comparison to the other types of sensors (glass or solvent polymeric membrane sensors) the problem of metallic sensors’ surface contamination due to possible chemo- or physical sorption, oxidation or complexes formation causes a serious sensor response drift and, may cause a serious problems in multisensor system functionality. Hence a special attention must be paid to the metallic sensors drift diminishing. One possibility to solve a problem is an application of mathematical transform for drift adjustment, while the other possibility is to apply either electrochemical cleaning [36] or mechanical buffing, which can be performed either automatically [37]. Depending on application and mainly on properties of analyzed liquid sample, an initial procedure of sensor array composition evaluation is required. There is no well defined method for this, but one of the most often used strategies is to evolve the electrochemical response of discrete sensors in individual solutions of various compounds known as components of target analyzed sample. Then those sensors, which

showed the highest response as far as better reproducibility towards one or several analytes are incorporated in electronic tongue array. Basing on the same strategy an approach providing a set of 3 empirical 'cross-sensitivity' parameters: average slope S , formal sensitivity (signal-to-noise ratio) factor K and non-selectivity factor F was reported in [38]. The higher all three cross-sensitivity parameters, the more favorable the application of sensor in electronic tongue array. It is important to notice here, that utility of ISEs in multisensor arrays application is evident [39], but restricted by the existence of highly selective sensors towards many analytes under interest. The reproducibility of sensors in array should be checked time-to-time in specific calibration solutions to ensure sensors stable response with minimal drift. Multisensor array may be applied either for a qualitative identification (imaging) of analyzed samples, or for quantitative determination of several compounds in target probe. In the latter case a procedure of multicomponent calibration of sensor array in multicomponent solutions mimicking a composition of a target analyzed sample is demanded [40]. During the calibration process the correlation between the matrix of sensor array responses and known content of calibration solution components is evolved. The content of all analytes under interest is varied in calibration solutions in a concentration range they appear in target probe. Obviously, the multivariate calibration is a most time consuming step of multisensor system application, nevertheless, being once calibrated with set of calibration solutions with components of known content such system can be used repeatedly for determination of these components. Moreover, several automated modifications of electronic tongue systems (including sensors calibration step), have been reported [41, 42]. Miniaturized electronic tongue systems are required for applications where only a small amount of sample was available for measurement [43]. Various substrates may be utilized for fabrication of microelectrodes for e-tongue systems: silicon, alumina, or plastic materials. Working electrodes are fabricated on substrate surface by multi-step procedures like silicon etching, screen-printing or thick-film technologies. After fabrication working microelectrodes (mainly metallic, metal-oxide or black carbon) may be either used as independent sensors in e-tongue system [44] or be covered with specific sensing material (membrane), mainly polymeric one. Electrochemical polymerization of monomer from aqueous or organic solution (in case of material ability to electropolymerisation) [45], photocurable polymerization [46] or solvent casting of dissolved in volatile solvent polymeric membrane cocktail [47] are the most often used methods of membrane deposition on working electrode surface. A possibility to function in FIA- (Flow Injection Analysis) [48, 49] mode is a favorable

feature of multisensor systems especially for on-line processes monitoring. Moreover, together with sensor equilibrium potential and FIA peak height, the shape of the FIA curve “potential over time” can be used as additional parameter for multivariate calibration of multiple-sensor systems [50].

6. Data analysis

The electronic tongue produces as measurements results a multidimensional data that can be analyzed with the multivariate data analysis techniques. In the following examples of electronic tongue application techniques like Principal Component Analysis, SIMCA and Partial Least Square are utilized. In this section a brief introduction to these data analysis method is given.

Principal Component Analysis (PCA)

PCA is an unsupervised technique used to describe a dataset often in the two- or tri-dimensional graphical representation carrying most of the data variance [51]. The axes of the new representation space are given by a linear combination of the original axes and they are requested to be uncorrelated and orthogonal. The first axis, called first “Principal Component”, is chosen in the direction of the largest variance, the second axis, called second “Principal Component”, is taken along the orthogonal direction to the first PC with largest variance, and so on.

This technique allows to visualize multi-dimensional datasets for preliminary data exploration and study of the intrinsic capability of the system to discriminate the data in clusters [52]. PCA is one of a wide set of linear transformations that may be described as:

$$S = W \cdot X \quad (16)$$

where \mathbf{X} is the original data set, \mathbf{W} is the transformation matrix, and \mathbf{S} are the data in the representation space. The peculiarity of the Principal Component Analysis (PCA) is the faithful representation of the data set onto a sub-space of reduced dimensionality. The term faithful here means preserving the statistical properties of the original data set. Chemical sensors always exhibit a certain degree of correlation among them, and then their data set may be represented in a sub-space of reduced dimensionality. In order to preserve the statistical properties of a data set it is necessary to make assumption on the statistical distribution of the data. In PCA the Gaussian distribution of the data is assumed. Indeed, PCA is calculated considering only the second momentum of the probability distribution of the data (covariance matrix).

Only for normally distributed data the covariance matrix ($X^T X$) describes completely the data once they are zero-centered. Geometrically, the covariance matrix describes a hyper-ellipsoid in the N dimensional space, and the PCA rotates the coordinates to represent this hyper-ellipsoid in canonical form. The calculation procedure of PCA is the following: let us consider a matrix \mathbf{X} of data, let $C = X^T X$ be the covariance matrix of \mathbf{X} . The i -th principal component of \mathbf{X} is $X^T \lambda(i)$, where $\lambda(i)$ is the i -th normalized eigenvector of C corresponding to the i -th largest Eigen value. Given a matrix of data PCA provides three kinds of information: scores, loadings, and Eigen values of the covariance matrix. The scores are concerned with the measurements; they are defined as the coordinates of each vector measurement (a row of matrix \mathbf{X}) in the principal components base. The loadings are concerned with the sensors. They measure the contribution of each sensor to the array. A high loading, for a sensor, means that the principal component is aligned along the sensor direction. The Eigen values are directly proportional to the variance explained by their correspondent eigenvector, so that looking at the relative Eigen values $\lambda(i)$ it is possible to confine the representation to those components carrying most of the information.

SIMCA

The Soft Independent Modeling of Class Analogy technique, simply called SIMCA, is a supervised technique that calculates the performance of one PCA for each of the classes under study, in order to investigate whether or not new sample belong to one of the classes. The class identification is realized through the calculation of the distance between the sample under study to each of the built PCA class models. This technique is based on the assumption that the data distribution is Gaussian and that the estimations error of the of the mean and variance of the distribution goes to zero increasing the cluster samples.

Partial Least Square (PLS)

Partial Least Square (PLS) regression is a data analysis technique that combines characteristics from principal component analysis and multiple regression. It is mostly useful when there is a need to predict a set of dependent variables from a large set of independent variables (predictors). Let us consider M observations described by K dependent variables which are stored in a $M \times K$ matrix denoted Y , and that the values of J predictors collected on these M observations are collected in the $M \times J$ matrix X . The goal of PLS regression is to predict Y from X and to describe their common structure. When Y is a vector and X is full rank,

this goal is accomplished using ordinary multiple regression [53]. When the number of predictors is large compared to the number of observations, X can be singular and the regression approach is not feasible (i.e., because of multi-collinearity). Several approaches have been developed to resolve this problem. One approach is to eliminate some predictors (trying to avoid the multicollinearity). Another approach, called principal component regression, is to perform a principal component analysis (PCA) of the X matrix and then use the PCs of X as regressors on Y . The orthogonality of the principal components eliminates the multicollinearity problem. But, the problem of choosing an optimum subset of predictors remains. As an example, the choice of the first PCs of X can not be relevant to find a correlation with the variables Y . Instead, PLS regression finds components from X that are relevant for Y . In particular, PLS regression searches for a set of components, called latent vectors, that achieves a simultaneous decomposition of X and Y with the restriction that these components explain as much as possible of the covariance between X and Y . Then PLS algorithm searches the subset of X that shows the maximum correlation with Y . The prediction error of the model is strongly dependent on the number of variables (features) and by the number of measurements. In the case of a small dataset, in order to have a realistic error estimation a cross validation technique is applied. Then, Latent Variables that minimize the prediction error in the validation phase are used to build a model for the further test phase [54].

7. Electronic tongue electronics

Electronic tongue electronics The data acquisition device for measurements with an array of potentiometric electrodes has been designed on Department of Electronic Engineering of “Tor Vergata” university, Rome, Italy. The measured quantities are then expressed according to the classical relationship:

$$\Delta V_{1,\dots,8} = V_{WE1,\dots,WE8} - V_{REF} \quad (17)$$

where the voltage of each working electrode can be referred to the fixed potential of a reference electrode. The work was aimed at an overall methodological optimization, and the result is a system with the following main hardware characteristics:

- multi-channel input: 8 working electrodes (**WE**), 1 reference electrode (**REF**);
- high input impedance;

- low noise;
- low drift;
- simple connection: RS232 to PC;
- small dimensions;
- low weight.

The device is also equipped with control software, whose simple interface provides the user with many functionalities:

- a simple parameter editor for measurement set-up;
- stacked and overlaid
- plots for the sensors array;
- graphical utilities;
- text format log files, easily importable in data processing tools.

The hardware interface is suited for an 8-elements potentiometric sensors array, as shown in Figure 4, it includes:

- an analog section, which provides adequate buffering for the electrodes and multiplexing to the A/D converter;
- a digital section, based on a RISC microcontroller which manages the correct measurement flow: multiplexer's port addressing, A/D control, data storage and communication with the control software.

The device is also provided with an internal Power Supply module (not shown in the picture). Each input channel ensures an input impedance $> 10^{15}/0.2(\Omega/pF)$, with an input bias current as low as $I_B = 3$ fA. The core of the Electronic Tongue is an ad-hoc programmed 8-bit RISC microcontroller (AT90LS8535), which is responsible for managing the measurement flow:

- control of the multiplexer's line switching;
- control of the A/D's functionalities;
- communication with PC using an RS232 line. Data A/D conversion is performed using a 12 bit (plus polarity) converter, whose functionalities are managed by the controller.

An external low-noise, precision voltage reference with extremely low temperature coefficient (0.5 ppm/C) has been used.

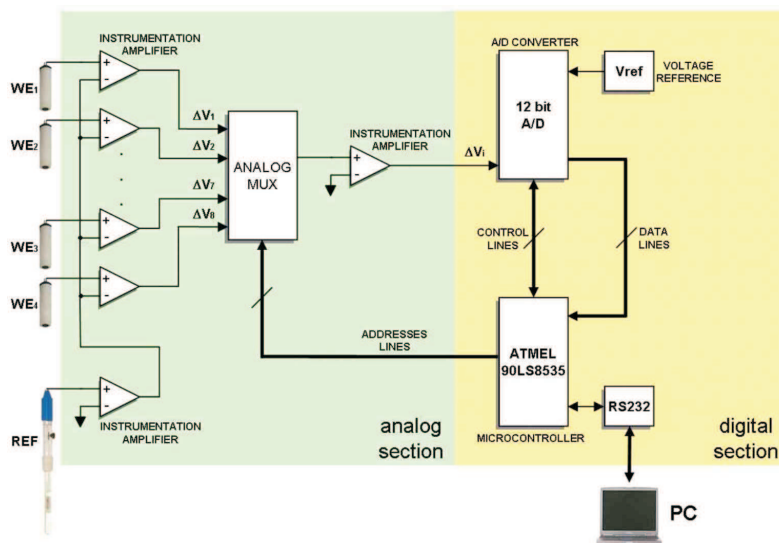


Figure 4. Simplified schematic of the Electronic Tongue electronics.

8. “Chemical images of liquids” - selected applications

A brief review of selected applications of electrochemical multisensor arrays is presented in this section and several examples of Electronic tongue applications are discussed in details. Evaluation of the ability to distinguish compounds responsible for basic tastes and by detection such compounds in analyzed sample to evolve the sample “taste” were performed in several early works of Toko et al [55, 56] and other authors [57, 58]. Besides a plenty of works related to the qualitative discrimination of waters, this target is still attract many researchers, since a monitoring of water quality is an important task for food safety and environmental control [60, 62]. Common methods permit a separate determination of several standard water parameters like pH, COD (chemical oxygen demand), BOD (biological oxygen demand), water hardness expressed in Ca^{2+} and Mg^{2+} content, conductivity, turbidity, content of several inorganic anions, etc. Nevertheless some these methods are enough complicated (for instance, COD and BOD detection demand sample pre-exposition and harmful dichromate oxidation). Often an integral overview is preferable for classification of water samples, especially in ecological monitoring tasks for alarm-like purposes [63]. An application of multisensor systems can provide such type of information. “Images” of water samples provided by multisensor systems may

be classified according to a membership to defined class (like pure standard water or polluted water). Moreover, several quantitative parameters can be evolved. An interesting application of potentiometric sensor array based on thick film technology and composed of RuO_2 , C, Ag, Ni, Cu, Au, Pt and Al micro-electrodes printed on to alumina substrate for analysis of 6 Spanish natural mineral waters was reported in [64]. Basing on possibility to extract chemical information from spontaneous polarization processes on the electrode surface, authors could perform not only an identification of mineral water, but also quantitative analysis of HCO_3^- , SO_4^{2-} , Ca^{2+} and Mg^{2+} ions content. The calibration PLS models were built on the base of known content of mentioned ions and potential readings of 12-element sensor array. Received correlation coefficients were 0.954, 0.913, 0.980 and 0.902 for bicarbonate, sulphate, calcium and magnesium ions correspondingly. Quantitative determinations of copper, zinc, lead and cadmium activity at low ppb and ppt levels in mixed artificial solution mimicking marine water (with salinity about 30pc) was reported in [65]. Average relative error in prediction was 20-30pc, which is encouraging result for ultra low activity. Wine production is a one of the biggest branches of modern food industry and a control of wine production, aging and storage processes as far as taste properties is demanded. Many applications of Electronic tongue systems were reported for recognition of red wines from different vineyards [66, 67]. Results of wine analysis were correlated with taste panel and the standard chemical analysis data. At the same time a few applications were reported for white wine analysis. Either if white wine has a shorted life time (3-5 years in comparison to 5-20 years for red wines) it occupies a considerable part of the wine market. In [68] a potentiometric electronic tongue with PVC plasticized membrane sensors doped with several porphyrins (H_2TPP , Co(II, III) and Pt(II, IV) porphyrinates) deposited on glassy carbon working electrodes were evaluated for analysis of Italian 'Verdicchio' dry white wines. Porphyrin-based "Electronic tongue" system discriminated between artificial and real wines and simultaneously distinguished wine samples from different cantinas and production year, Figure 5. The more old wines have a negative scores alone PC3 component, while a clear discrimination between real artificial wine is directed alone PC1.

Several attempts of qualitative ethanol determination with artificial taste systems have been undertake. Thus, in [69] along with the discrimination of four kinds of sake, the concentration of ethanol in analytes was determined by means of taste sensor. Electronic tongue array comprised of 8 potentiometric solid state sensors with polymeric plastisized membranes based on metallo-porphyrins was applied for "chemical imaging"

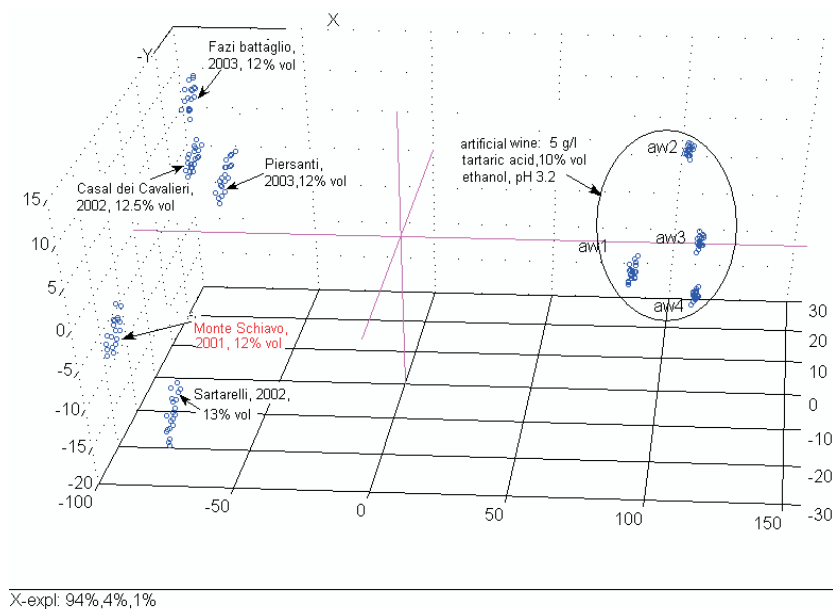


Figure 5. 'Verdicchio' wines identification.

of alcoholic beverages made of two different source materials grape and barley and quantitative determination of alcoholic degree of these beverages [70]. Prior to real samples analysis, multivariate calibration of Electronic tongue was performed using 36 calibration solutions containing 4 alcohols in various concentrations. Model was trained with PLS and good correlation of real and predicted ethanol content ($R = 0.952$) has been received. The alcoholic strength of real alcoholic beverages was evaluated according to the evolved one dimensional scale of 'alcoholic degree' of beverages in ethanol concentration, Figure 6. Except outlying samples of beer "Ceres" and Ballantine's whiskey, a good correlation of predicted using electronic tongue alcohol content and labeled by manufacturers was found. The possibility of porphyrin-based electronic tongue system to distinguish alcoholic beverages made of different source material: grapes and barley was also shown, Figure 7. Two areas, corresponding to the alcoholic drinks made of grape and barley (areas I and II along the PC1 axis on Figure 6 correspondingly) may be evolved.

Classification of spirits such as ethanol, vodka, eau-de-vie and cognac has been performed by means of Electronic tongue system based on array of potentiometric sensors in [71]. The system distinguished different samples of cognac from each other, and from any eau-de-vie, synthetic

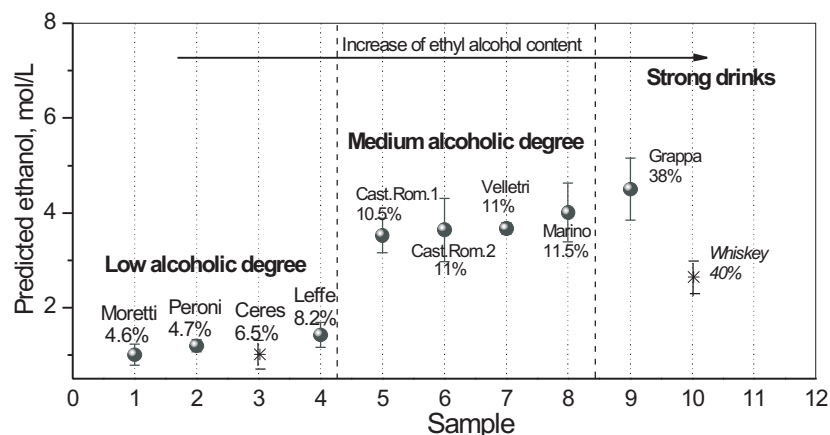


Figure 6. Evaluation of beverages alcoholic degree using porphyrin-based Electronic tongue.

and alimentary ethanol and various sorts of spirit differing in ethanol quality, and presence of contaminants in vodka. Samples of different brands of beer produced in different countries were analyzed with lipid taste sensor [72]. In [43] several beers were distinguished from each other by means of all-solid-state potentiometric Electronic tongue, correlation between beer taste characteristics and multisensor systems output has been found and a “beer taste map” was evolved, Figure 8.

An impressive example of chemical imaging of port wines according to the wine age was demonstrated in [73]. The electronic tongue comprised of 28 potentiometric sensors were applied for analysis of 2 sets (22 and 170 samples correspondingly) of port wines aged in oak casks for 10, 20, 30 and 40 years, Vintage, LV and Harvest wines (2 set) of 2 to 70 years old. The resulted PLS precision of port wine age prediction with Electronic tongue system was 8pc, i.e. one year. Several quantitative parameters such as pH (1pc), total (8pc), volatile (21pc) and fixed (10pc) acidity as well as content of organic acids (tartaric -10pc, malic -15pc), sulphate (9pc) and sulphur dioxide (24pc) were evaluated in port wines by means of preliminary calibration of Electronic tongue system and further prediction of unknown samples with adequate accuracy. Other application closely related to the wine analysis is vinegars identification. Vinegar production is related with a wine industry (wine utilization for vinegar acidification) and identification of vinegars is important due to the effort to achieve an adequate quality of production, to ensure uniformity within a brand, to avoid falsifications (dilutions). An electronic tongue system based on the array of six metallic potentiometric sensors

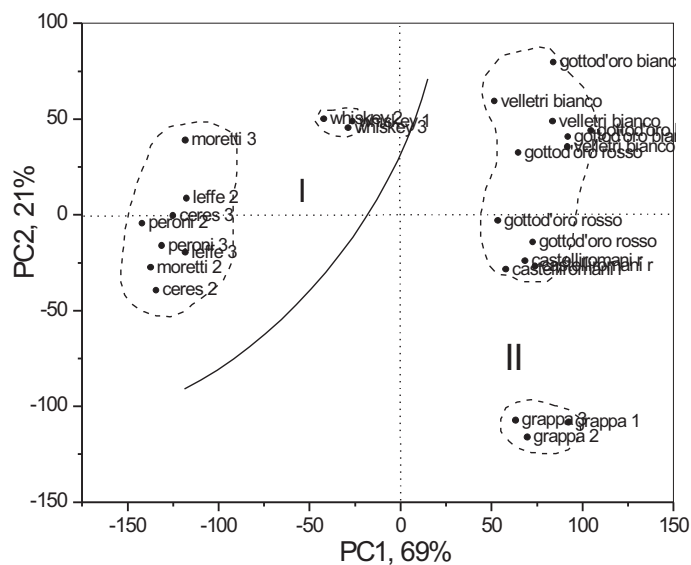


Figure 7. Imaging of grape and barley made alcoholic drinks by means of porphyrin-based Electronic tongue system. All the analyzed beverages were available in local stores in Rome, Italy. Grape-made alcoholic drinks were as follows: 2 kinds of white dry wines (“Velletri superiore” by Le Contrade, Italy, 11.5 vol, 2001; “Marino” by Gotto D’Oro, Italy, 11 vol, 2002); 2 kinds of red dry wines (“Castelli Romani” by Le Contrade, Italy, 11 vol, 2002; “Castelli Romani” by Gotto D’Oro, Italy, 10.5 1.5 vol, 2003) and grappa “Colli del Frascati” by “IlahCoral” s.r.l., Italy, 38 vol, 2003. All the wines were of DOC quality i.e. denominated to controlled origin. Beverages made of barley were: 2 lager beers (“Moretti” 4.6 vol and “Peroni” 4.7 vol both from Italian manufactures); red beer “Ceres”, 6.5 vol, Denmark; amber strong beer “Leffe”, 8.2 vol, Belgium and Ballantine’s scotch whiskey by G. Ballantine and son LTD, Scotland, 40 vol, 2003.

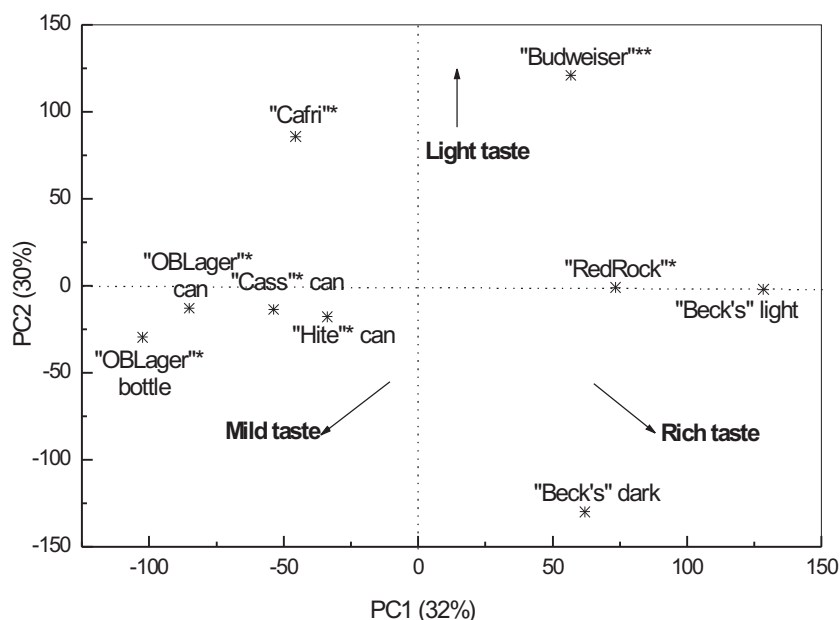


Figure 8. A taste map (PCA score plot) of several beers available in Korea obtained from the all-solid-state electronic tongue chips.

(Cu, Sn, Fe, Al, brass and stainless steel wires) was developed and utilized for discrimination of foodstuffs: several types of vinegar and fruit juices. The result of “imaging” of vinegars prepared from white and red wines, balsamic vinegar and diluted vinegars samples, is given on Figure 10. According to the first two principal components (PC1, 86pc and PC2, 13pc), two main areas can be indicated on the plot, one of them includes all the pure vinegar samples, while the other embraces tap water and diluted vinegar samples. Simultaneously with qualitative discrimination, metallic electronic tongue system was able to observe the direction of quantitative dilution of vinegars.

Fruit juices, dairy products [74], various drinks (black and green teas, coffee) and soft drinks are popular objects of electronic tongues application. A multicomponent analysis of Korean green tea samples was performed in [75] by all-solid-state ‘electronic tongue’ microsystem comprised of polymeric sensors of different types based both on PVC and aromatic polyurethane (ArPU) matrices doped with various membrane active components, electrochemically deposited conductive films of polypyrrole (PPy) and polyaniline (PAn) and potentiometric glucose biosensors. The system successfully discriminated different kinds of teas (black and green) and natural coffee, Figure 10. The output of elec-

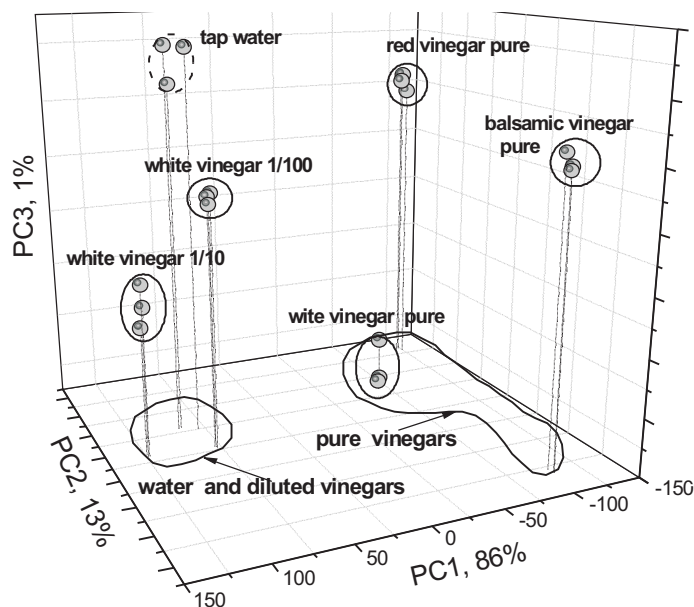


Figure 9. PCA classification of diluted and pure vinegars.

tronic tongue in green teas correlated well with the manufacture's specifications for (-)-EGC concentration, total catechines and sugars content, L-arginine concentration which was determined with enzyme destruction method and then concentrations of mentioned components were determined in green tea samples with unknown manufacturer specifications. Clinical analysis of biological liquids is another area of electronic tongue systems application. In [76] a multisensor system comprising an array of 30 sensors with solvent polymeric membranes doped with various MAC and back-propagation artificial neural network as data processing tool has been applied for multicomponent analysis of solutions close in composition to biological liquids (blood plasma). It has been found that such an approach allows to determine Mg^{2+} , Ca^{2+} , pH , HCO_3^- , HPO_4^{2-} in typical ranges with average precision 24pc that allows to suggest the method as a perspective one for clinic analysis. Six potentiometric metallic sensors: Co, 99.9pc pure, brass (alloy of Cu and 20 wt pc Zn), two alloys of silver (Ag42-Cu17-Zn16-Cd25, Ag60-Cu26-Sn14, where numbers in wt pc), two component alloy Sn40-Pb60, and Cu-P (copper doped with 5 wt pc of phosphorous) and two PVC based electrodes doped with 3 wt pc of monensin and 5 wt pc of TpCIPBK were utilized for clinical analysis of human urines. A PCA discrimination score plot of 36 solutions mimicking human urine composition (points 1-131) and 14 real

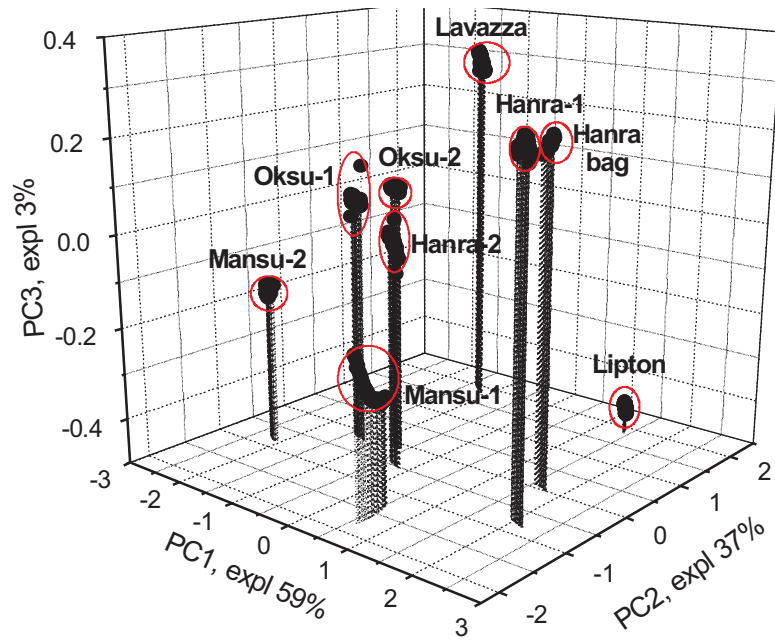


Figure 10. Imaging of Lavazza coffee, black tear Lipton and several sorts of Korean green tea.

human urine samples (points 132-173) are shown on Figure 11. Simultaneously with discrimination between artificial and real urine samples, the groups corresponding to healthy and persons affected by pathologies have been evolved on PCA classification step. It is interesting to notice here, that samples NN 91, 92 has provided by a person, following a strict vegetarian diet. Moreover, a blind sample (points 103-105) has been classified as a non urine sample (according to the legend sample appeared a standard black tea preparation).

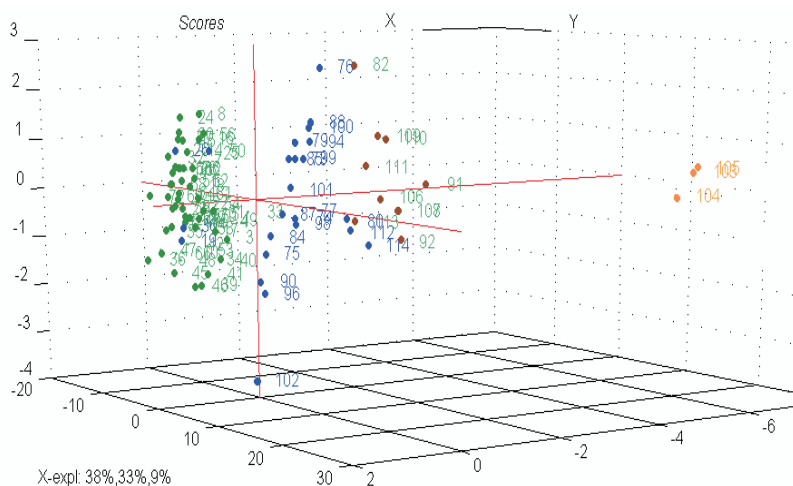


Figure 11. Human urine classified by potentiometric Electronic tongue based on metallic sensors.

In closing several examples of untypical applications of electronic tongue systems should be reviewed. First of all, a several impressive examples of imaging of bacteria, molds and yeasts species [77, 78] as far as for fermentation process with *Aspergillus niger* observation [79] and mold growth [80] were recently reported. The application of potentiometric metallic sensor array composed of 6 metallic sensors of first kind included Fe, Al, Cu, Sn, brass and stainless steel for discrimination of water soluble humic substance (HS) preparations, organic fertilizers and various soil type aqueous extracts was performed. Such a classification may be a first step of qualitative agricultural analysis of soils according to their actual fertility and fertilizers according to their effectiveness since fertility is strictly connected to the amount of water soluble humic substanses. Classification was performed basing on HS complexation affinity with transition metals. A PCA score plot for discrimination of 5 organic fertilizers, humic acid preparation and 2 soils (Halpoc Cher-

nozem and Albic Luvisol) is shown on Figure 12. Aqueous extracts of both soil samples are far separated from the group of all organic fertilizers along PC1 axis, which may be attributed to the lower content and nature of HS in soil extracts. HC and AL soils are well distinguished also along the PC2, which may be correlated to the pH of the samples as far as to the HS lability, since HC contains less amount of labile fulvic acids.

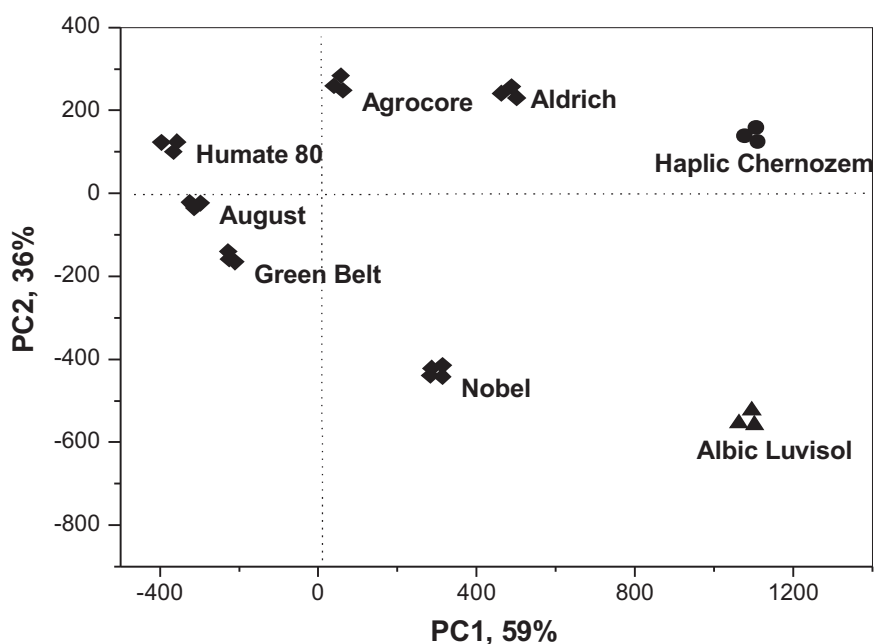


Figure 12. Identification of organic fertilizers and soils aqueous extracts with metallic sensors array.

An interesting application of vegetable oils imaging were reported by Apetrei et al in [81]. Vegetables oils used as electroactive binder material of carbon paste electrodes. The electrochemical response of such electrodes immersed in a variety of electrolytic aqueous solutions has been exploited to discriminate the oils. The features observed in the voltammograms are a reflect of the Red-Ox properties of the electroactive compounds (mainly antioxidants) present in the oils inside of the carbon paste matrix. The different content in polyphenols allows olive oils to be easily discriminated from sunflower oil or corn oil. These parameters together with voltammograms pH influence were on used as the input variable of PCA. The results indicated that suggested method allows a clear discrimination among oils from different vegetal origins and also

allows olive oils of different quality (extra virgin, virgin, lampante and refined olive oil) to be discriminated. An application of the electronic tongue multisensor system (ET) composed of 28 potentiometric chemical sensors both chalcogenide glass and plasticized polymeric membranes was used for discrimination of standard and Mancha Amarela (Yellow spot) cork [82]. Extracts of cork in 10% ethanol water solutions were analyzed. Two sets of cork samples that included both standard (S) and Mancha Amarela (MA) cork samples from two different factories were studied. It was found that ET could reliably distinguish extracts made from S and MA cork regardless samples' origin. ET could predict total phenols' content with average precision of 9% when calibrated using reference data obtained by Folin-Ciocalteu method. Composition of S and MA cork was studied by ET. The largest difference in concentration between two types of cork extracts was found for content of two acids: gallic and protocatechuic.

9. Non electrochemical Electronic Tongues

Although the majority of the electronic tongues reported in the literature are based on electrochemical transduction principles, in the last few years some examples of electronic tongues based on different sensing mechanisms. In this scenario, optical transduction has been exploited by the group working at the University of Austin, Texas [83]. The electronic tongue developed by this group was based on polyethylene glycol-polystyrene resin beads functionalized with different dyes. These beads are positioned within silicon microcavities in order to mimic the natural taste buds, Figure 13.

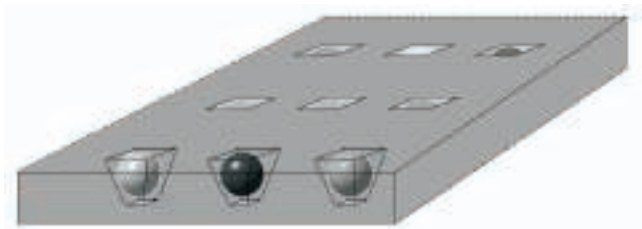


Figure 13. Schematic presentation of optical Electronic tongue based on polyethylene glycol- polystyrene resin beads functionalized with different dyes.

Both colorimetric and fluorescence properties variation induced by the interaction of the immobilized dyes with target analytes can be exploited for the sensing mechanism. These changes are recorded by a CCD camera to obtain the chemical images of the analyzed environments. The analysis of the RGB patterns will allow the identification and potentially

the quantification of the analytes present in the sample analyzed. More recently the electronic tongue has been implemented with a micromachined fluidic structure for the introduction of the liquid samples [84]. As an extension of his work on the “Smell Seeing” approach, Suslick has recently reported a colorimetric array for the detection of organic compound in water [85]. The array was prepared by printing metalloporphyrins, solvatochromic and pH indicator dye spots on a hydrophobic surface. The array was saturated with a reference solution and imaged by an ordinary flatbed scanner to have the starting image. The interaction with target analytes induce color change recorded by the scanner; subtraction of the images with the original reference provides the color change profile, which will be the fingerprint of each analyte. In this configuration the array is not influenced by salts or hydrophilic compounds and for this reason the authors noted that for this reason the array is not really an electronic tongue. Other than optical arrays, nanogravimetric sensors have also been exploited for the development of electronic tongues. The first report was done by Hauptmann and co-workers, with the use of Quartz Crystal Microbalance (QCM) in liquids [86]. QCMs are more difficult to use in liquid phase, because the high damping of the oscillator requires more electronics and care in the measurements, although the advantages of QCMs in terms of miniaturization and integration with a wide range of different sensing materials are preserved also in the liquid phase. While the use of QCM has not been further developed, more recently Gardner and co-workers have reported the use of a dual shear horizontal surface acoustic wave (SH-SAW) device for a realization of a miniaturized electronic tongue [87]. The main advantage claimed by the authors lies in the fact that this device is based on physical and not chemical or electrochemical transduction principles and for this reason it is more robust and durable. The sensing mechanism is based on the measurement of both mechanical (physico-acoustic) properties, and electrical (electro-acoustic) parameters of the analyzed liquid. The selectivity of the system can however improved, if necessary, by the deposition of a selective membrane onto the sensing area of the device. The system has been tested toward the classification of model analytes, representing the basic human taste and exploited also for the discrimination of real samples, such as cow milk.

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