Review

State of the Art in the Field of Electronic and Bioelectronic Tongues – Towards the Analysis of Wines

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Abstract
This review compares various types of (bio)electronic tongues. The design and principles of potentiometric and voltammetric electronic tongues are discussed together with applications in food and environmental analysis. Different approaches towards bioelectronic tongue are presented. Several methods for evaluation and interpretation of the measured data are described. Finally, the potential of such devices for analysis of wine is discussed.

Keywords: Electronic tongue, Bioelectronic tongue, Sensor array, Biosensor array, Electrochemical sensor, Amperometry, Voltammetry, Potentiometry, Biosensors

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1. Introduction

Wine industry forms a specialized sector dominated by a large number of small producers. The production of the wine is completely dependent on the long-lasting experience of wine makers and quality of input products. For these reasons, it is necessary to monitor the quality of wine. The adequate control requires continuous, simultaneous and selective monitoring of several key compounds that determine the quality and the flavor of wine.

The quality control of wine is still under development. The oldest approach is based on wine tasters. These well-trained and certified persons evaluate flavors, aromas and general characteristics of a wine sample in order to establish properties of wine as complexity and character, potential (suitability for aging or drinking) and possible faults. In other words, human taste and olfaction play an important role in evaluation of the quality of wine [1]. The ability of simultaneous detection of a large spectrum of compounds in one step and providing a comprehensive information on the sample within a few seconds can be considered as a basic model for the design of an artificial analytical systems [2]. These systems can provide useful information not only in wine industry but also in different fields including food production, health care and monitoring of the environment.

At present, the entire analysis of the sample can be realized either through well-established techniques of analytical chemistry, or using a large number of calibrated (bio)sensors. Instrumental methods provide comprehensive information on the sample within a tenths of minutes or several hours, the procedures are influenced by complex sample pretreatment. However, instrumental analysis is usually expensive due to equipment cost and it requires highly sophisticated handling. On the other hand, cheap and simple sensors and biosensors provide only basic information about sample typically limited to a single analyte, hence these individual devices can not be used for comprehensive characterization of the complex sample. The possible way how to monitor the quality of wines or other fluids represent the multisensoric systems. This review describes the technological development of sensor arrays and discusses possible use of the biosensors in this field.

2. Electronic Tongue

The sensor array exhibiting limited individual selectivity together with an appropriate pattern recognition tool was defined as the 'electronic tongue' [3, 4]. In 2005, IUPAC established the international nomenclature for potentiometric analysis of liquids. In this case, the electronic tongue is defined as a multisensor system, which consists of a number of low-selective sensors and uses advanced mathematical procedures for signal processing based on pattern recognition and/or multivariate analysis [5]. This device serves for classification of the sample, the principle is based on the measurement of large numbers of samples and monitoring of their variability through the use of principal component analysis (PCA) and thus obtained data are distributed to specific groups representing particular properties of samples.

The second design of electronic tongue is based on sensor arrays where each element is selective for one analyte or group of similar analytes and provides information about their quantity. The obtained data are often distorted due to overlapping or interference signals, therefore it is necessary

to use multivariate calibration. Furthermore, different levels of analytes in sample can also strongly influence its quality [6].

The developed electronic tongues exploit electrochemical properties of samples. Potentiometry and voltammetry utilizing various modified working electrodes serve as sufficient detection systems providing adequate responses. As alternatives to electrochemistry, systems operating on optical and piezoelectric (Surface Acustic Wave, Quartz Crystal Microbalance) principles were proposed [7, 8].

2.1. Potentiometric Electronic Tongues

The principle of potentiometric sensors is based on the measurement of the difference of potential (voltage) on the interface between the working electrode and the reference electrode. The interface composition determines sensitivity and selectivity of the sensor. The interface mostly consists of different kinds of electrode materials, different types of polymeric membranes or crystalline compositions. The development of potentiometric electronic tongue is based on the combination of various types of membranes with different selectivity, sensitivity and cross-reactivity [9, 10].

The first combination of potentiometric electrodes with lipid membranes was the 'taste sensor' introduced by Hayashi et al. [11]. The membrane was a mixture containing lipid, polyvinyl chloride (PVC) and plasticizer, such arrays combined with PCA were successfully applied for quantitative analysis of drinks including beer [12], coffee [13], mineral water [14] and sake [15].

In 1995, a concept of electronic tongue was proposed [16] as an array consisting of chemical sensors which are non-specific, poorly selective and displaying cross-sensitivity to multiple components in liquids. The electronic tongue including ion selective sensors (based on solid-state and plasticized organic and chalcogenide glass membranes) served for discrimination of different kinds of beer, soft drinks, juice, tea and coffee [16]. Similar systems were used for analysis of mineral water, wine [17, 18], polluted water [19] and inorganic ions in a model ground water [20]. Another modification of this system was proposed for monitoring of bacterial [21] and mold fermentations [22]. The quantifications of tastes provided discrimination between bitter-sweet-salty substances, discrimination between substances eliciting the same taste, quantity and content in pharmaceutical products [23]. Another successful application was the recognition and classification of various spirits including ethanol, vodka, eau-de-vie and cognac [24]. The comparison of the developed electronic tongue with FTIR and HPLC was demonstrated on discrimination apples varieties [25]. The method was also applied for sugars and acids discrimination in different tomatoes cultivars [26] and further compared with the commercial ASTREE device [27]. For identification of mastitic bovine milk, the sensitivity and specificity of the electronic tongue was higher than the predominantly used conductometric assay [28].

Metalloporphyrins were introduced as an alternative sensing material. The analysis of red wines was carried out using the electronic tongue with six porphyrin/PVC based electrodes, pH sensor and the electronic nose [29]. The sensitivity of membranes in single-component alcohol solutions decreased in the order ethanol > methanol > isobutyl alcohol, the alcoholic beverages of two different sources, grape and barley, were recognized [30, 31]. The electronic tongue based on platinum coated with cobaltporphyrins/PVC membranes discriminated Italian white wines [31, 32]. Miniaturization version of this device identified wine defects caused by H_2S, SO_2 and acetic acid as markers added during the fermentation process [31, 33].

The improved electronic tongue based on electropolymerization of metalloporphyrins on glassy carbon electrode discriminated classical tastes [34]. This device together with an electronic nose analyzed red wines providing chemical parameters (pH, total alcohol, sugar, volatile acidity, lactic acid and glycerol) and sensorial descriptors (sourness, spiced clove, almond, fresh grass) evaluation [35].

The all-solid state planar potentiometric tongue was based on carbon paste electrode array screen-printed on a polymeric substrate (SCPE). High cross-sensitive solvent polymeric membranes (array of 12 elements) consisted of different matrices (PVC, aromatic polyurethane, polypyrrole) doped with plasticizers and ionophore, the resulting system successfully classified various drinks.[36, 37] Another potentiometric SPE-based tongue contained pastes of RuO_2, Cu, Ag, Pt, Ni, Al and C, this array successfully differentiated rather similar mineral waters [38, 39] and served also for fish freshness analysis [40, 41]. Modification of ion-selective membranes with nanofiltering sieves with different pore diameters was proposed for detection of homologous anionic surfactants in river waters [42 – 44].

The light-addressable potentiometric sensor (LAPS) developed by Hafeman et al. also seems interesting for the electronic tongue concept [45]. The LAPS methodology was derived from ion-selective field effect transistors (ISFET) [46, 47]. The opportunity of simultaneous detection of several compounds provided LAPS as an effective platform for electronic tongue applications. Schöning et al. proposed preparation of the chalcogenide glass materials using the pulsed laser deposition techniques (PLD) [48] and thus developed ion-selective sensor arrays for multi-analyte determination [49, 50]. The methodology of LAPS combined with other electroanalytical techniques (stripping voltammetry) can be advantageously used for heavy metals detection in waste and sea waters[47]. The ISFETs-based array was suitable to distinguish grape types and vintage of wine samples [51].

Calvo et al. [52] introduced fully automated electronic tongue for mineral water analysis. This system was based on 5 potentiometric sensors employing PVC-membranes selective for three ions (Mg^{2+}, Ca^{2+} and Ba^{2+}). The measured data was evaluated by artificial neural network (ANN). Multidimensional calibration sets used for ANN training were randomly arranged by automatic Sequential Injection Analysis (SIA) system.

In the sensor array, both specific and non-specific responding elements play an important role in sample characterization. The sensor array can be characterized by the same parameters as a single sensor. During exposition to the examined sample, the response of each sensor is sum of specific and nonspecific interactions. This cross-sensitivity to different substances has primary importance together with a stable and reproducible response. Currently, there is no theoretical background how to create the electronic tongue with accurately predicted properties. Vlasov et al. tried to propose the algorithm of choosing different sensors for the array by following parameters of cross-sensitivity: the average electrode slope (S, mV/pX), the nonselectivity factor (F), the reproducibility factor (K) [53]. However, these parameters may have different meanings for the sensor array and for the discrete ISEs and it was not possible to utilize these parameters for general design of potentiometric arrays [5, 54].

The cross-sensitivity effects of the nonspecific sensors together with selective part of sensor array can improve the limit of detection and the selectivity. Furthermore, chemometric treatment as multivariate analysis increased the range of measurable concentrations. In addition, the cross-sensitivity effects allow detecting the substances for which each discrete sensor can produce only small output signal, which is hardly valuable separately [55].

2.2. Voltammetric Electronic Tongues

Voltammetry is commonly used in analytical chemistry due to the high sensitivity, versatility, simplicity and robustness. The principle is based on measurement of current flowing between the working and counter electrodes when a pulse of potential is applied between them. Depending on the range of potential and type of the working electrode, electrochemically active compounds become either oxidized or reduced. The disadvantage is low selectivity, because all compounds in sample which are electrochemically active below the applied potential, contribute to the measured signal [56, 57].

Winquist et al. introduced the first voltammetric tongue based on two metal electrodes (gold and platinum) [3]. The recorded voltammograms were based on large (LAPV) and small amplitude pulse voltammetry (SAPV). The LAPV method resembles the normal pulse voltammetry, whereas SAPV is similar to the square-wave voltammetry. Fruit juices and milk were characterized, the collected data evaluated by PCA showed good separation of samples. Furthermore, the working electrodes included metals like copper, gold, iridium, nickel, palladium, platinum, rhenium, rhodium, silver, tin, titanium and zirconium together with glassy carbon, sensing arrays were designed as either three, four or six working electrodes [58–61]. Different techniques including LAPV, SAPV and staircase voltammetry (resembling cyclic voltammetry) were used for characterization of tea [58], detergents [61], juices [3] and milk [62]. Various species of molds growing in liquid media [63], different microbial species and microbial contamination were detected [64] and quality of drinking water was monitored [60]. In 2007, the upgrade of LAPV was published as multifrequency large amplitude pulse voltammetry (MLAPV), this was a combination of three waveforms (segments) of LAPV with different potential pulse steps in lengths of 1 s, 0.1 s and 0.01 s, respectively. This electronic tongue incorporated several metallic working electrodes (platinum, gold, palladium, titanium, nickel, etc.) and six Chinese distilled spirits and seven Longjing teas were successfully classified in different frequency segments [65].

The chemically modified electrodes (CME), which can be built by either deposition of electroactive compounds over the conductive surface or using chemically modified carbon paste electrodes (CPE) appeared. The modification of CPE by deposition of metallophthalocyanines was an early approach; three bis(phthalocyanines) with praseodymium, gadolinium and lutetium as central metal ions were used. These 'double–decker' derivatives have two phthalocyanine macrocycles coordinated to the central metal ion, which allows several oxidation states, hence these compounds exhibit extremely rich electrochemical properties. In addition, substituents in the periphery of the phthalocyanine rings further increase the variability. The resulting sensor array discriminated six Spanish red wines prepared from the same variety of grape but from different origin denominations and ageing stages [66]. The poly(3,4-ethylenedioxythiophene)-modified electrode was used in cyclic voltammetry or differential pulse voltammetry followed by PCA for determination of nine different Italian red wines [67].

The hybrid electronic tongue represents further technological improvement of CME arrays, it consisted of three families of CMEs, including polypyrrole sensors doped with a range of counterions (decane-1-sulfonate, hexacyanoferrate, sulfate), CPEs modified with metallophthalocyanines and CPEs modified with perylene imide derivatives. The hybrid voltammetric electronic tongue monitored ageing of red wines in oak barrels [68]. Additionally, the electronic tongue based on the same principle was able to distinguish red wine with altered organoleptic characteristics [69].

In another study, the array based on three rare-earth bis(phtalocyanines) and three perylenes was constructed. Both families of compounds possess different chemical reactivities leading to increased capability of discrimination towards the sensor arrays containing only one group of modifiers. This voltammetric tongue was able to distinguish each member among white wines elaborated with different varieties of grapes [70].

Automatization of measurements with electronic tongues is a further step desired for practical use. The array consisting of three metallic working electrodes (gold, platinum and rhonium) was incorporated into the flow injection analysis (FIA) system and it analyzed waste waters from the paper mill industry. In this case, the results served for classification of samples by their origin using PCA. The obtained data correlated to chemical oxygen demand

(COD), conductivity and pH as quantitative chemometric parameters [71, 72].

The differences in results for the same samples measured repeatedly in time using the voltammetric tongue were attributed to fouling of the electrode surface that in turn caused drift of signal and eventually loss of activity [73]. Drift was mainly observed in measurements of complex liquids as milk and waste water. Accumulation of different molecules such as halide ions, organics and sulfur compounds on the surface of the working electrode was reported. In addition, a higher anodic potential applied can form a thin layers of metal oxides and hydroxyl radicals. These modifications may at least influence or completely inhibit electrochemical reactions of interest. Hence a simple and quick activation of electrodes is essential when the electronic tongue is used in complex liquids and for on-line assays; electrochemical cleaning [74] and mechanical polishing [75] were commonly used.

2.3. Other Types of Electronic Tongues

The impedimetric electronic tongue employed measurement of impedance either at one fixed frequency [76 – 78] or a broader spectrum was probed using impedance spectroscopy [79]. Impedimetric tongues were introduced for diverse analytes such as cations (potassium, sodium, ammonium).[80] In another study, small molecules including sodium chloride, citric acid, glucose, glutamic acid and sodium dehydrocholate as representatives of tastes, salty, sour, sweet, umami and bitter were monitored [79]. The sensitivity of the reported devices differs also very much from semiquantitative preliminary measurements to electronic tongues possessing high sensitivity and ability to precisely characterize samples. The impedimetric tongues presented by Riul et. al. were able to distinguish among different kinds of red wine with excellent sensitivity; together with the subsequent data treatment using PCA and ANN, even the wine storage conditions could be determined [78].

The amperometric electronic tongue was reported, too. A system was based on the 8-electrode array where the different potentials 0.9, 0.7, 0.6 and 0.5 V were applied on the Pt and Au bare working electrodes. The antioxidant levels monitored in various teas were evaluated as astrigenicity factor.[81]

3. Bioelectronic Tongue

Electronic tongue imitates the signal processing in natural tongue, although the elementary steps of signal transduction and identification of chemical patterns differ from those realized in the biological tongue. The signal transduction in biological system involves a cascade of biochemical reactions leading to the transport of electrons, ions or molecules while the result is the complex information - taste. Otherwise, the concept of biosensors can be considered as a simple

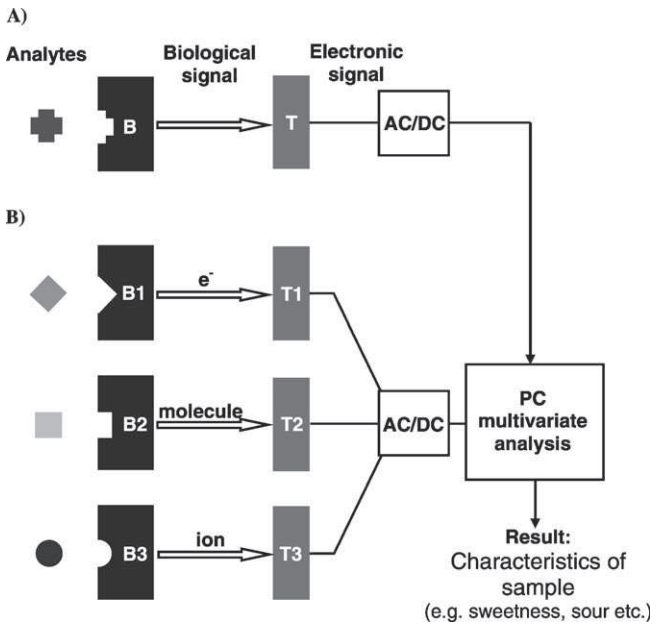

Fig. 1. Schematic model of bioelectronic tongue. A) general scheme, B) general array of biosensors and biological signal variability which can be used for readable communication of biorecognition element with transducer. Bn: biorecognition elements, Tn: transducer elements, AC/DC signal converters.

draft of bioelectronic tongue (see Fig. 1A). If one creates a field of biosensors, where each biosensor recognizes one or group of analytes and the quantity of analyte in the sample, then with the help of advanced statistical methods, very accurate characterization of the sample can be obtained (Fig 1B). Thus, this biosensor array can be seen as a bioelectronic tongue.

Several designs of bioelectronic tongues were proposed. First, the principle of voltammetric electronic tongue based on three biocomposite electrodes containing glucose oxidase and different metal catalysts (Pt, Pd and Au-Pd) was used for simultaneous determination of glucose and ascorbic acid. The metal catalysts improve the biosensor response by decreasing the oxidation potential for hydrogen peroxide from the enzymatic reaction. This electronic tongue was applied to measure juice samples. However, the responses were influenced by the presence of other interferents occurring in the samples and provided increased results [82]. Another system utilized the combination of the developed potentiometric sensor array (electronic tongue for Na^+, K^+ and NH_4^+ [83, 84]) and enzyme urease covalently attached to carboxylated PVC [85]. The extended version with creatinine deiminase was applied for the analysis of urea and creatinine in urine without necessity to eliminate the alkaline interferences and compensation of their effects.[86]

The second approach based on amperometric biosensor arrays was proposed for detection of pesticides (organophosphates and carbamates) and phenols. In this case, cholinesterases (ChEs), tyrosinase, peroxidases (soybean and horseradish, HRP) and cellobiose dehydrogenase

(CDH) were combined on the same array consisting of one Ag/AgCl reference electrode surrounded by eight radially distributed working electrodes from either carbon or platinum. The substrates for the enzymes were acetylthiocholine for ChEs, cellobiose for CDH and hydrogen peroxide for peroxidases. The detection limits for pesticides and phenols were in the nanomolar and micromolar ranges, respectively. The developed biosensor array was evaluated on wastewater samples. To simplify interpretation of results, the measured data were treated with multivariate analysis [87]. The modified system of bioelectronic tongue was applied for discrimination of four different wastewater samples (untreated, alarm, alert and normal) from chemithermo-mechanical pulp mill. HRP-modified and bare platinum sensors provided most significant signals [88].

The amperometric bioelectronic tongue able to resolve pesticide mixtures of dichlorvos and methylparaoxon was based on the screen printed system with three acetylcholinesterases: the wild type from *Electric eel* and two recombinant mutants B1 and B394 originating from *Drosophila melanogaster* [89].

The olfactory and taste receptors are other biorecognition alternative to more common enzymes. The olfactory receptor I7 was immobilized on a gold electrode by multilayer bioengineering based on a mixed self-assembled monolayer and biotin/avidin system, which allowed for a well-controlled immobilization of the bioreceptor within its lipid environment. The odorant detection was realized using electrochemical impedance spectroscopy (EIS) [90].

The living cells were employed in the systems described by Lobanov et al., whole microbial cells (*Gluconobacer oxydans* and *Pichia methanolica*) enabled selective determination of ethanol and glucose in mixtures. The cells of *G. oxydans* were sensitive to both substrates, while *P. methanolica* oxidized only ethanol. Information from two microbial sensors was resolved with the help of ANN [91]. Alternatively, olfactory and taste living cells grown on the surface of LAPS chips really mimicked bioelectronic nose and tongue technology, which can detect odors and taste, the recorded action potentials represented odorants and taste factors interacting with receptors embedded in the cellular membrane. The system was used repeatedly for up to 6 hours; prolonged operational lifetime is expected by providing a microenvironment supporting the cell culture [92, 93].

The presented principles point to a very diverse approaches of research groups to realization of bioelectronic tongues. The key to the further development of these systems and their possible commercialization are stability of the biocomponent and sufficient reproducibility of the measured data.

3.1. How to Design Bioelectronic Tongue for Analysis of Wines?

The current trends in design of biosensor and multibiosensor systems are mostly based on the unification of the mechanism of reaction or detection which simplifies the construction of whole biosensing devices. In this way, different oxidases, NAD^+-dependent dehydrogenases and PQQ-dehydrogenases are incorporated to the biosensing systems as modules functioning in a similar manner. Another trend is using the same working potential for various products of enzymatic reactions in individual modules. For example, the biosensor array based on cholinesterase, peroxidase and phenol oxidase can be combined as shown Solna et al. [87]. The use of various enzymes and working potentials complicates the development of biosensor equipment.

This study is focused on the development of bioelectronic systems for monitoring the quality of the wines. First it is necessary to define the parameters (analytes) for analysis. Wine is quite complex fluid, consisting of a mixture of acids, sugars, alcohols, polyphenols and other compounds. Mostly, biosensors used for the analysis of wine focused on determination of compounds contained in ripe grapes (glucose, fructose, malate and polyphenols), products of fermentation (ethanol, glycerol, acetate, lactate) and added preservatives (sulfite). These compounds either influence the taste of produced wine like sweetness, bitterness, sourness or are characteristic compounds for faults of wine.

For determination of the above mentioned molecules, mostly enzymes as oxidases and dehydrogenases are chosen. Biosensing systems based on oxidases utilize oxygen which serves as acceptor of electrons providing either hydrogen peroxide or water. Such enzymatic reaction can be monitored by measuring consumption of oxygen or production of hydrogen peroxide at rather high working potentials of -650 mV and $+650$ mV, respectively. Unfortunately, wine contains many compounds electrochemically interfering at the positive potential; hence procedures reducing interference are required.

Interference can be reduced or completely removed using different approaches. The permselective barriers probably represent the first choice. In fact, the common oxygen electrodes employs the Teflon membrane, through which only gases can permeate [94–96]. In the case of hydrogen peroxide, the selective non-conductive electropolymerized films formed on the working electrode are useful. Carelli et al. [97] used poly-*o*-phenylenediamine and overoxidized non-conducting polypyrrole films for biosensor for alcohol determination. The mixture of alcohol oxidase and bovine serum albumine (BSA) was immobilized over the polymer modified surface using glutaraldehyde as crosslinker. Other modification were based on immobilization of glucose oxidase inside electrogenerated poly-*o*-phenylenediamine film [98]. However, these systems often exhibit limited stability due to high potential forming passivating metaloxide layers.

The alternative approach reflects efforts to reduce the working potential using either artificial redox mediators or bi-enzymatic system based on the oxidase-peroxidase cascade. The latter principle profits from the large substrate variability of HRP allowing to use different mediators transferring electron from electrode to the enzyme, the reported examples include ferrocyanide [99, 100], ferrocene

[101 – 103], and reagentless system based on osmium redox centers incorporated to the polymer backbone [104].

Artificial systems imitating peroxidase function were published. Biosensing systems based on alcohol oxidase immobilized on the top of poly neutral red (PNR) modified electrode were introduced. [105 – 107]. Further modification used neutral red as soluble mediator which became reduced on the bismuth film electrode [108]. Another group of mediators utilized in oxidase based biosensors are metal hexacyanoferrates, Prussian blue (ferric ferrocyanide) being the best known [109, 110]. Besides, electrochemically deposited cobalt hexacyanoferrate was successfully used for glucose biosensor [111]. Additionally, manganese dioxide served as mediator for glucose biosensors [112, 113].

Dehydrogenases dependent on NAD^+, $NADP^+$ and pyroloquinolinoquinone (PQQ) are enzymes suitable for conversion of several analytes in wine. The redox cofactors serve as electron acceptors which can be recycled on the electrode surface at high working potential. The oxidation of NADH can be achieved with diaphorase or using artificial mediators. Diaphorase mimics the peroxidase function in the oxidase-peroxidase cascade. Ferricyanide was commonly used as an acceptor of electrons from NADH. After reduction, ferrocyanide was electrochemically recycled at +200 mV [114 – 116]. Tetrathiafulvalene (TTF) served as very interesting alternative to ferricyanide, TTF communicated with both oxidase / peroxidase and NAD^+-dehydrogenase/diaphorase systems [117]. Similar function was realized using polymerized osmium-containing complexes [118]. The classic artificial systems for NADH reoxidation use Meldola blue [119, 120] and its Reinecke salt [110, 119, 120]. Tetracyanoquinodimethane (TNCQ) [119] and TTF [121] were introduced later. Numerous compounds can be used, e.g. poly(ethyleneglycole) diglycidyl ether film containing Brilliant Cresyl blue was described recently [122].

The second group of dehydrogenases contains covalently bound cofactor PQQ. Ubiquinones and cytochromes function as natural electron acceptors for PQQ-dehydrogenases, through many artificial mediators regenerating reduced PQQ were introduced. Ferrocenes [123], osmium complexes [124], heterocyclic compounds such as phenazine methosulfate [125, 126] and redox polymers [127] all serve as mediators between PQQ and the electrode surface. PQQ-dependent dehydrogenases for glucose, ethanol and glycerol were successfully employed for analysis of the corresponding compounds in wine [124]. The quinone-dependent alcohol dehydrogenase containing hem was used for monitoring ethanol during wine fermentation. The enzyme was immobilized to the osmium complex modified redox polymer using poly(ethyleneglycol) diglycidyl ether as crosslinker [128].

Phenolic compounds in wine play an important role in human nutrition with respect to their protective properties - antioxidant activity. Phenolics serve as free-radical scavangers and chelators of transition metals such as iron and copper [129]. These compounds are potential substrates for phenol oxidizing enzymes including tyrosinase [119, 129 – 133], laccase [132, 134] and peroxidase [129, 135]. Determination of total phenolics is often preferred as this value corresponds to the total antioxidant capacity of the analyzed sample. Laccase and tyrosinase belong to a small group of copper-containing oxidases. Laccase exhibits low substrate specificity and is able to catalyze oxidation of various aromatic substances. In the opposite, tyrosinase catalyzes *o*-hydroxylation of monophenols to *o*-diphenols and subsequently oxidizes *o*-diphenols to *o*-quinones.[132] Laccase based biosensor can detect polyphenolic compounds in red wines [134], while tyrosinase was employed for biosensing of phenols in wine [130, 133] and tea [136] as well. For operation of these biosensors, either consumption of oxygen [131] or reduction of quinone products [134] was employed. Horseradish peroxidase is another enzyme suitable for determination of total phenolic compounds. Fernandes et al. utilized a green bean homogenate as a source of peroxidase in a chemically cross-linked chitin matrix incorporated in CPE for determination of caffeic acid in white wines, the long term stability of the biosensor was 300 days [137]. Horseradish peroxidase was entrapped on a semipermeable membrane which covered the ferrocene modified CPE. The decrease of hydrogen peroxide was monitored; it corresponded to the oxidized amount of phenolic compounds in wine and tea [135]. Co-immobilization of laccase and tyrosinase on the Sonogel-Carbon electrode using glutaraldehyde as a crosslinker and Nafion-ion exchanger as a protective additive provided the biosensor which summed the responses of both enzymes to five individual polyphenols and estimated the total phenol index in the beer samples with high correlation factor of 0.99 in comparison with the Folin-Ciocalteau reagent [138].

Wine contains many compounds which can be easily metabolized by various microorganisms. To prevent these unwanted degradation processes, the addition of SO_2 into wine is widely used. On the other hand, higher concentration of thus generated sulfite anion lead to the degradation of the quality and may even be deleterious [139, 140]. Currently, the common techniques for the detection of SO_2 include titration of sulfuric acid generated by the transformation of sulfite, high performance ion chromatography with conductivity detection, size exclusion chromatography, capillary electrophoresis and gas chromatography [139, 140]. Alternatively, the use of sulfite oxidase (SOX) seems to be promising. SOX catalyzes reaction in which two electrons from sulfite are transferred in two one-electron steps to acceptor; depending on the origin of enzyme, either hydrogen peroxide is produced (plants) or cytochrome *c* becomes reduced (cyt *c*, mammals). In the case of the plant SOX, the generated hydrogen peroxide becomes partially consumed in the chemical reaction with sulfite, thus oxygen electrode is a better transducer [139]; alternatively, difference in the SO_3^{2-} concentration before and after the enzymatic reaction should be evaluated [140]. In this case, two electrodes functioning as predictor and detector, and one enzyme layer in between, were used for evaluating difference of sulfite caused by the reaction of SOX. The advantage of such set-up was simultaneous differential elimination of interferences. The utilization of mammal SOX for wine analysis was

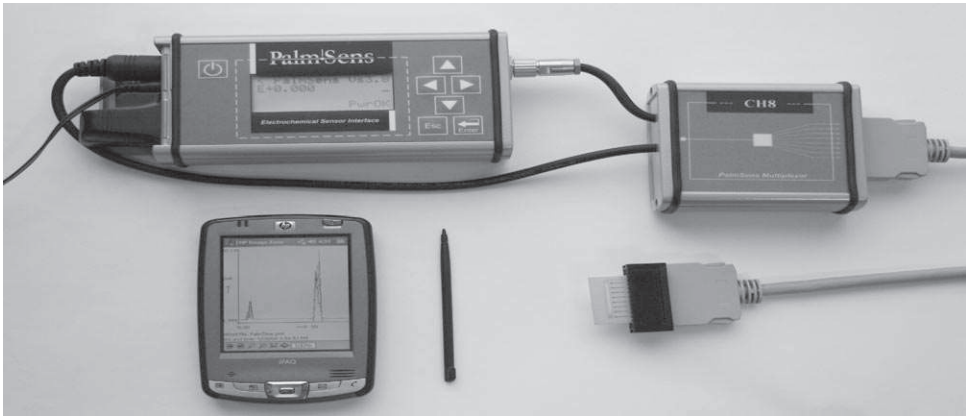

Fig. 2. Multichannel biosensor set up. Commercially available potentiostat (left top) provided with multiplexer (right top) collecting data from 8-channel screen printed electrode (right bottom). Data are recorded through wireless bluetooth dongler and evaluated by PDA (left bottom).

proposed by Spricigo et al. [141] in the interesting biosensor where SOX and cyt *c* were co-immobilized by means of electrostatic interaction with polyaniline sulfonate (PASA). The multilayer biosensor was formed by consequent application of SOX, cyt *c* solution, thus obtained positively charged layer was followed by PASA solution forming negative charged layer. The electrons taken away from sulfite by the molybden cofactor of SOX were transferred by cyt *c* in the 'wired' layers down to the electrode.

Bioelectronic tongue for wine should combine the above mentioned enzymes and corresponding detection mechanisms. The individual enzyme electrodes bring individual requirements on co-reactants and surface fouling complications to the complex system. The techniques employed for the electronic tongues [74, 75] can not be applied in construction of such multibiosensoric systems because of enzyme layers. The solution can be in the already mentioned polymer films beneath the enzyme layer excluding interferents from the sample and lowering the working potential using different intersteps such as enzyme or artificial redox mediators, possibly common to several elements of the enzyme array.

3.2. How Could the Bioelectronic Tongue Look Like?

Multibiosensor systems do not have to be build up from the scratch. Different portable multichannel potentiostats are available on the market. At Figure 2, the well known PalmSens device is depicted which was originally single channel but after including the multiplexing unit it becomes very well suited for multichannel amperometric and voltammetric techniques common for (bio)electronic tongues. The further advantage of this system is control through the Pocket PC device even using a wireless bluetooth modul. Various designs of commercially available 8 channel screen-printed electrodes are depicted in Figure 3. The advantage of SPE is simple and mass production, even the custom design can be easily created by researcher and finalized sensors are available in few weeks.

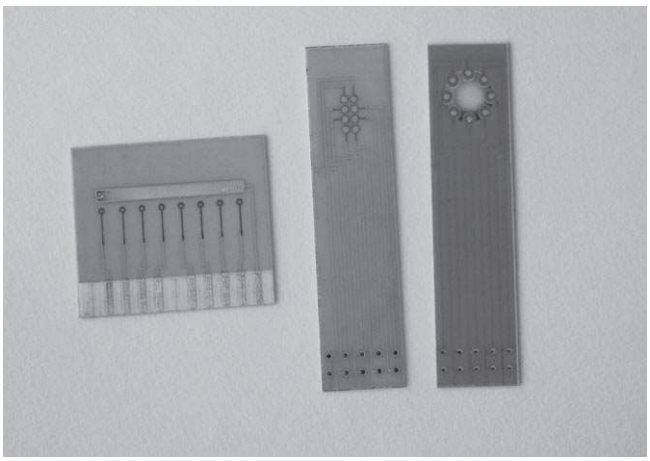

Fig. 3. Multichannel screen printed electrodes produced by BVT Technologies.

4. Chemometric Evaluation of Results

The (bio)electronic tongues which consist of number of individual (bio)sensors produce a number of complex data or analytical signals providing description of the measured sample. However, these data have to be mathematically processed to make them meaningful. Several mathematical methods successfully used for electronic tongue data analysis as well as relevant software tools will be further mentioned. Instead of comprehensive explanation of multivariate data analysis tools just terminology and common features are described. Detail description of multivariate data analysis methods was reviewed elsewhere [142 – 145].

The analytical signals from the sensors of the electronic tongue can be interpreted in two ways. The first is the estimation of concentrations of analytes in the sample using multivariate calibration methods [142]. A number of

Table 1. The list of methods used for analysis of electronic tongue data.

	Method	References
Multivariate calibration	Principal components regression	[34, 142, 146]
	Partial least square	[85, 142, 143]
	Artificial neural network	[19, 146, 147]
Supervised pattern recognition	*k*-Nearest neighbor	[144, 148, 149]
	Principal components analysis	[3, 58, 78, 88]
	Partial least squares	[18, 148]
	Linear discrimination analysis	[77, 150, 151]
	Classification trees	[151]
	Artificial neural networks	[78, 152, 153]

analytes is estimated from the set of analytical signals. The suitable mathematical model is assembled during calibration. This model conducts relationship between the measured variables (analytical signals) and the properties of interest (sample composition). Building of the appropriate model consist of several phases [142]:

a) choosing the type of model
b) estimation of its parameters
c) determination of the predictions performance.

The list of mathematical model types for multivariate calibration methods which were used for electronic tongue is presented in the Table 1.

The second interpretation of electronic tongue experiments provides classification of the analyzed samples into predefined categories. This process is known as supervised pattern recognition [144]. For example, this can be used for estimation what variety of wine (predefined samples categories) corresponds to the unknown samples of wines (classified objects). The supervised pattern recognition is a process consisting of several steps:

a) Selection of a training set consisting of already classified (i. e., set of different varieties of wines for which analytical signals are recorded).
b) Selection of variables which are meaningful for the classification.
c) Derivation of a classification rule or building of mathematical model using the training set.
d) Verification of the derived classification rule using independent validation set. This set consists of known samples, which were not been used for the derivation of the classification rule.

Several types of classification rules exist, which differ in their classification principle and complexity of their computer implementation. As can be seen in the Table 1, several mathematically very different principles for data analysis [142, 144, 148] can be quickly obtained through available software packages. The user has to decide, which method is the best for his particular system. The best way of taking this decision is application of all possible methods on recorded data and selection of the best performing method (based on either analytes concentrations or samples classification).

Validation of multivariate calibration model is based on two different data sets. The calibration or training set is used for model building. The second set known as validation or test set estimates suitability of the model. This is done by computing prediction error sum of squares (*PRESS*) and root mean square of the prediction error *RMSPE*.

$$PRESS = \Sigma(A_i - A_{i,\text{predicted}})^2$$

$$RMSPE = (PRESS/n_{\text{test set}})^{1/2}$$

Where A_i is known concentration of *i*-th analyte, $A_{i,\text{predicted}}$ is concentration of the *i*-th analyte as predicted from build model, $n_{\text{test set}}$ is number of samples in the test set. The model with the lowest PRESS is considered as the best and *RMSPE* serves for its error estimation [142].

During the supervised pattern recognition procedure, different models are build on the training data set and their quality is again tested on the test set. Recognition and prediction ability is computed for the model testing. Recognition ability is the percentage of members of the training set that are correctly classified. The prediction ability is determined by percentage of the members of the test set correctly classified by the model and this parameter serves for the best model selection. When the prediction and recognition abilities are substantially different, the model is not stable and selected solution should be discarded [144]. A scheme of models building and selection is depicted on Figure 4.

As soon as new model is build and successfully tested, it can be used for unknown samples responses analysis. Number of software packages can be used for fast generation of such models, even without precise knowledge of the mathematical background. Well known software packages are MATLAB with additional toolboxes (MathWorks, USA), STATISTICA (StatSoft Czech Rep.) and Unscrambler (Camo, Norway). An interesting software package realized under GNU General Public License is WEKA [154], which can be used for analysis of miscellaneous data types. This package provides graphical user interface and Java classes, which can be used by programmers for development of specialized software written in the Java programming language.

5. Conclusions

Increasing public demand on comprehensive evaluation of food products, health conditions and environmental situation stimulates development of both standard instrumental analytical methods and novel multisensoric systems combined with chemometric evaluation and simplified presentation of results. This review was focused on the development and applications of electrochemical sensor and biosensor arrays in the field of artificial 'tongue – like' devices, with special attention on the analysis of wine. The mechanism of human taste serves as a basic pattern for development of electronic or bioelectronic tongues. The

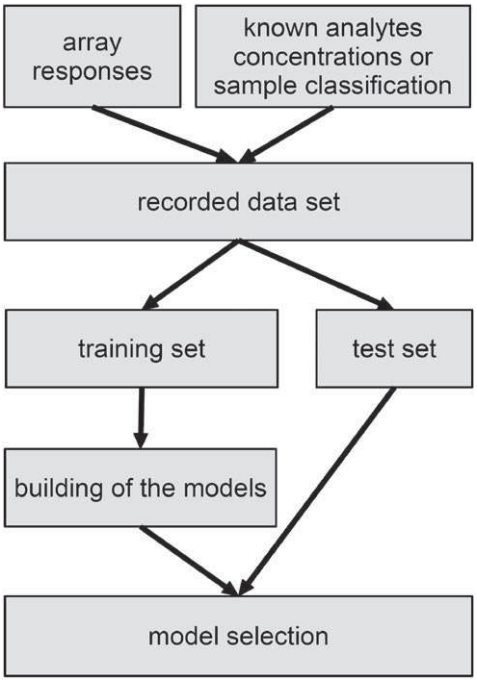

Fig. 4. The scheme of models building and testing. Multidimensional data are produced by electronic tongue and combined with known sample information. These data are split into training set and testing set. Training set is used for model building by appropriate software package. Testing set is used for best model selection as described in the text.

array of sensors which substitutes the taste buds provides partially specific and nonspecific response depending on the selectivity of individual sensors in the array. The responses are subsequently evaluated using multivariate analysis. The combination of recognition elements working together in the sensoric array, the appropriate electronics providing data to the subsequent software realizing multivariate analysis is defined as an electronic tongue. The variability of the above described electronic tongues consists in methods used for sample monitoring e.g. cyclic voltammetry, amperometry, potentiometry, and transducers which are used for building these sensor arrays e.g., metal electrodes, ISE, selective membranes or chalcogenide glasses. Further step forward in the field of electronic tongues is the addition of biorecognition elements with electrochemical transducers based on amperometric, potentiometric and voltammetric detection. These bioelectronic tongues represent biosensor arrays which allow to monitor non-electroactive analytes which are consumed or somehow recognized by the biorecognition element and provide electrochemically perceivable response. Besides, bioelectronic tongues based on immobilization of whole taste cells and recombinant human taste receptors were mentioned.

Since our main interest was focused on the bioelectronic tongue for the analysis of wine, various biosensors for typical wine components as sugars, alcohols, phenols and sulfur compounds were described. The design of such bioelectronic tongue is based on the unification of the responses of individual biosensors. As a potential signal transferring mediator molecules mimicking taste cells signal pathway, the following compounds were proposed: oxygen/hydrogen peroxide, NAD^+/NADH, ferri/ferrocyanate, ferri/ferrocytochrome *c*. The biochemical part of bioelectronic tongue has to be completed by suitable chemometric method translating the obtained signal into correct and simple output. The simplicity, reliability, accuracy, stable response and elimination of interferents are the basic conditions for the introduction of any bioelectronic tongue into practice.

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