Electronic tongues and aptasensors

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13.1 Introduction

Multidisciplinary research within life and material sciences provide novel insights for designing bioinspired materials and devices for medical applications (Choy et al., 2010; Gentile et al., 2011). Bioinspired technologies artificially mimic the physiology sensing capabilities using several types of sensors coupled with chemometric tools (Gutiérrez et al., 2012). These sensor approaches may be used per se or as an integrated part of a chemical or biomedical sensing element, applied in stationary or flow mode and may be categorized according to their electrochemical principles, as potentiometric, amperometric, or voltammetric sensors (Liu, 2000). Sensor-based devices are alternative techniques to retrieve valuable chemical information from a specific matrix, by using appropriate multivariate statistical qualitative/quantitative data processing-modeling techniques, aiming to overcome drawbacks like the presence of nonidealities, interference effects, collinearity between measured variables and noise issues (Gutiérrez et al., 2012). These analytical electrochemistry-based sensors range from chemical applications to biomedical monitoring, from invasive to noninvasive approaches, from off-line to real-time in situ analysis, from in vitro to in vivo use, from single cell to animal and human measurement including clinical screening, diagnosis, and treatment (Mc Caffrey et al., 2015; Wang et al., 2008; Zhang et al., 2008). However, unlike wearable physical sensors for monitoring vital signs, the availability of noninvasive chemical sensors is still scarce (Bandodkar and Wang, 2014). Among them, the use of chemical sensor arrays, a simple and straightforward electrochemical sensing strategy that includes ion selective electrodes (ISE) and low-selective electrodes, such as in electronic tongues (E-tongues) and the development of more elaborate and complex aptasensors, such as RNA- and DNA-sensor based strategies, will be the focus of this chapter.

Despite the great advances of the (bio)electrochemical technologies, eg, sensor arrays/E-tongue and aptasensor devices that are recognized as promising tools for medical and pharmaceutical applications, there are still relevant challenges in the design and applications of electrochemical sensors in order to meet the demands of modern health care. Indeed, there is an urgent need to take advantage of the unique capabilities of these sensors, such as low-cost, miniaturization, portability, short response times,

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minimum, or no sample pretreatment, and wide applicability, already demonstrated at research level, in real-world applications. This chapter will review the state-of-the-art and recent developments of electrochemical sensor approaches, as well as some novel trends on this exciting on-going research field.

13.2 Electrochemical devices: Chemical sensors and aptasensors

13.2.1 General principles and apparatus

Multisensor chemical arrays and aptasensors are generally electrochemical-based sensor devices (eg, potentiometry, amperometry, voltammetry, and impedance spectroscopy).

13.2.1.1 Potentiometry

In potentiometry the potential signal is measured at zero current intensity (Scholz, 2010). The typical potentiometric analytical cell has two electrodes immersed in a solution containing the analyte, whose concentration is to be measured (Fig. 13.1). The reference electrode (RE) has a constant contribution to the signal, independently of the solution matrix. Usually it contains a metal electrode in contact with an insoluble salt of the same metal (second type of electrode) and its potential depends only on the solubility of the salt. The most used RE is the silver/silver chloride (Ag/AgCl). The second electrode is the indicator electrode (IE) that contains a membrane sensitive to the

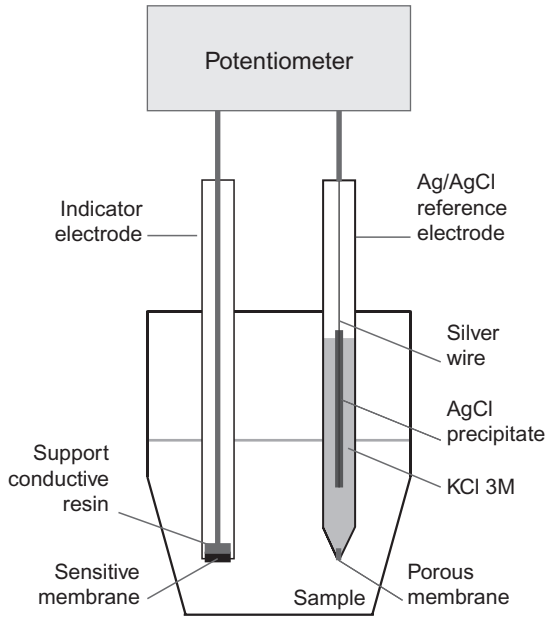

Fig. 13.1 Typical potentiometric analytical system.

compound to be analyzed, thus its potential depends on the compound's concentration. The analyte of the sample solution enters the electrode membrane causing a change in membrane potential due to the modification of the electrochemical properties. A high impedance potentiometer is used to measure the potential between the two electrodes (see Fig. 13.1).

These two electrodes (half-cells) are used separately or are combined into a single body. The most common IEs are sensitive to ion activity and not to ion concentration. The Nernst equation shows that the logarithm of the measured ion activity (a_i) is linearly related with the potential signal (Eq. 13.1), in the ideal case where the electrode only responds to a single analyte (electrodes with high selectivity towards an analyte) (Scholz, 2010):

$$E = E_0 + \frac{2.303 \times R \times T}{z_i \times F} \times \log\left(a_i\right) \tag{13.1}$$

where E is the measured potential between the indicator and reference electrode; E_0 is the temperature-dependent standard potential of the electrode; R is the general gas constant; T is the temperature in Kelvin; z is the ionic charge including the sign; and F is the Faraday constant.

Due to the temperature dependence, the potentiometric measurements must be carried out at a constant temperature. If interferences are relevant, an extended expression of the Nernst equation, the Nikolsky–Eisenman equation, should be used (Scholz, 2010):

$$E = E_0 + \frac{2.303 \times R \times T}{z_i \times F} \times \log\left(a_i + \sum_j K_{ij} \times \left(a_j\right)^{\frac{z_i}{z_j}}\right) \tag{13.2}$$

where K_{ij} is the selectivity coefficient of the indicator electrode for the interfering ion j, a factor giving the influence of the interfering ion in relationship to the analyte; z_i and z_j are the ion charge of the primary and interfering ions, respectively.

This equation predicts the linear dependency between the sensor response and the logarithm of a function of the ions activity in solution measured in the electrode membrane. The substance activity is directly related with the analytical concentration (C) via the activity coefficient (γ). The γ is a function of the solution's ionic force (I), which represents the total electrolyte content. Therefore in quantitative work, solutions should have approximately the same ionic strength, achieved by using a solution containing a high concentration of an inert salt (total ionic strength adjustment buffer solution, TISAB).

Two types of potentiometric electrodes are usually described, the ISE, which was developed to selectively recognize ions and used to analyze ions (anions or cations) in a solution; and the cross-sensitivity electrodes (CSE), used in sensor arrays for solution analysis, which due to their low selectivity gives, as output, a matrix "fingerprint." E-tongue systems (multisensor systems with several low-selective sensors) are an example of this application.

Different ISE systems have been described in the literature, including (1) glass-membrane, with a framework of silicate glass interstitial sites for H^+ and Na^+, being the pH electrode the best example; (2) crystal-membrane, defined by a crystal lattice containing defined gaps for the ion to be measured, eg, the fluoride electrode

that uses a crystal of lanthanum fluoride (LaF_3) doped with europium fluoride (EuF_2) to create lattice gaps; and (3) polymer-membrane—polymeric membrane containing an additive molecule (an ionophore, ie, a high-selective molecule) that binds with high selectivity to the ion to be measured, for instance, the potassium electrode with a membrane of polyvinyl chloride (PVC) impregnated with the macrocyclic antibiotic valinomycin (Mikhelson, 2013; Wang, 2006).

In low-selective electrodes, assembled as sensor arrays, several membranes can be used for potentiometric sensors, namely chalcogenide glasses and lipid polymeric-membranes (Vlasov et al., 2005). These arrays constitute analytical tools whose performance capabilities depend on the selected electrodes but also on the practical purpose. Qualitative, semiquantitative and quantitative analysis are possible by using advanced chemometric techniques.

13.2.1.2 Amperometry and voltammetry

The most common techniques that apply a constant and/or varying potential at an electrode surface, within a three-electrode system, measuring the resulting current intensity in an electrolytic solution are amperometry, cyclic voltammetry (CV), square wave voltammetry (SWV), and differential pulse voltammetry (DPV). These electro-analytical techniques evaluate the redox properties of a single compound or a mixture of compounds. The three-electrode system (Fig. 13.2) comprises an RE, a counter electrode (CE or auxiliary electrode) and a working electrode (WE). The RE contributes with a stable and known potential.

The analyte is measured (quantification or evaluation of the redox properties) in the WE surface, where the potential varies linearly with time in relation to the constant contribution of the RE. The charge is transferred to and from the analyte at the WE surface, resulting in a current flow to or from the cell, through the auxiliary electrode. The measurements must occur in an electrolytic solution to provide ions to the electrodes during oxidation and reduction. The equipment, a so-called potentiostat, is a device that simultaneously produces an accurate potential and measures the current involved in the system comprised of the WE surface plus the analyte. The WEs are electrodes of metals or metals with its surface chemically modified, allowing high sensitive electrochemical determination of organic molecules, as well as ions (Scholz, 2010; Thomas and Henze, 2001; Zoski, 2007). When a dropping mercury electrode or a static mercury drop electrode is used as the WE, the analytical technique is usually referred to as polarography (Scholz, 2010; Thomas and Henze, 2001; Zoski, 2007).

Amperometry. In this technique, the current is continuously measured as a function of a constant potential applied to the WE. The current intensity signal from the oxidation or reduction of the substance analyzed is proportional to its concentration.

CV. A cyclic voltammogram is obtained by applying a linear sweep potential (the potential increases or decreases linearly with time) at the WE. When a potential change occurs, the current flows through the WE, oxidizing or reducing the compound under analysis. The intensity of this current is proportional to the concentration of the compound in the solution, thus enabling the use of this technique in analytical

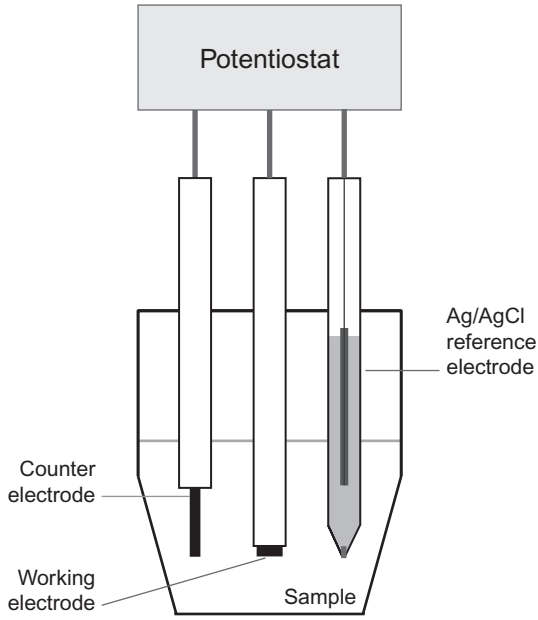

Fig. 13.2 Typical three-electrode voltammetric analytical system.

quantification. Fig. 13.3 shows the information that it is possible to obtain from a cyclic voltammogram during the course of a reversible redox reaction.

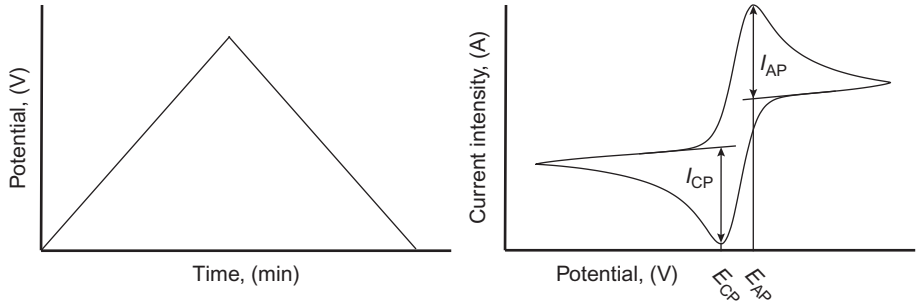

Fig. 13.3 Cyclic potential sweep and typical voltammogram of a reversible reaction using a three-electrode system (I_{AP} and I_{CP}, anode and cathode peak current intensities; E_{AP} and E_{CP}, anode and cathode potentials).

The quantitative information regarding the concentration of electroactive(s) compound(s) can be obtained from the voltammogram using the Randles–Sevcik equation, for the case of a reversible reaction. This equation expresses the relationship between the peak current intensity I_p (either anode or cathode) and the analyte concentration (C) at a given absolute temperature (T, in Kelvin or Rankine) and is given by

$$I_P = kn^{\frac{3}{2}} v^{\frac{1}{2}} D^{\frac{1}{2}} AC \qquad 13.3$$

where k contains all constants at T, n is the number of electrons that are involved in the reaction for the redox pair, v is scan rate, ie, the speed at which the potential is swept (V/s), A is the area of the electrode (cm^2), and D is the diffusion coefficient of the compound to be analyzed (cm^2/s) (Scholz, 2010; Thomas and Henze, 2001; Zoski, 2007).

This method allows the reduction potential of an analyte and its electrochemical reactivity to be assessed, and is considered nondestructive since only a very small amount of the analyte is consumed at the surface of the WE.

DPV. DPV is a pulse technique that is more sensitive towards oxidation or reduction currents (faradaic currents) than conventional voltammetry since it permits discriminating charging (capacitance) current. In this technique, the currents before the end of the pulse (I_1) and just before pulse application (I_2) are measured, using pulses superimposed on a ramp of potential changing linearly with time (applied with amplitudes between 10 and 100 mV, for several milliseconds). The difference between the two currents ($\Delta I = I_2 - I_1$) is plotted against the staircase potential and leads to a voltammogram with a peak-shaped waveform. Fig. 13.4 shows a typical voltammogram of the DPV analysis.

The peak's position (E_P) and the shape on the potential axis are dependent of the type of analyte under study and, on the other hand, its height (I_P, faradaic response current peak) is directly proportional to the concentrations of the electroactive compounds (Scholz, 2010; Thomas and Henze, 2001; Zoski, 2007). DPV allows a faster analysis and lower consumption of electroactive compounds at the electrode surface (lower adsorption).

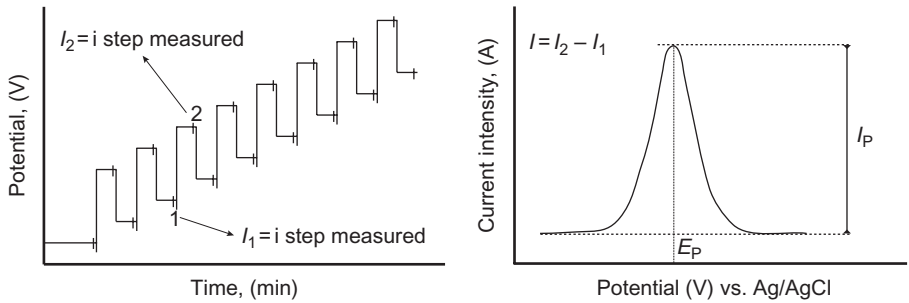

Fig. 13.4 Potential sweep and typical voltammogram of the DPV analysis for a reversible reaction (I_P, maximum peak current intensity; E_P, potential at which I_P was recorded).

SWV. The SWV is also a pulse voltammetry technique and exhibits a greater capacity to discriminate the influence of capacitive current. An SWP analysis is carried out by measuring the current in the WE, while the potential is swept in time over a range of set values by using a voltage ramp with a staircase-shaped between the WE and RE. In the square-wave cycle (potential waveform sweep), the current is measured at the end of the positive direction towards oxidation (forward pulse, I_{fwd}) and negative direction towards reduction (reverse pulse, I_{rev}) generating a peak for each of the processes, which gives information concerning the oxidation or reduction of the electroactive species at the electrode surface.

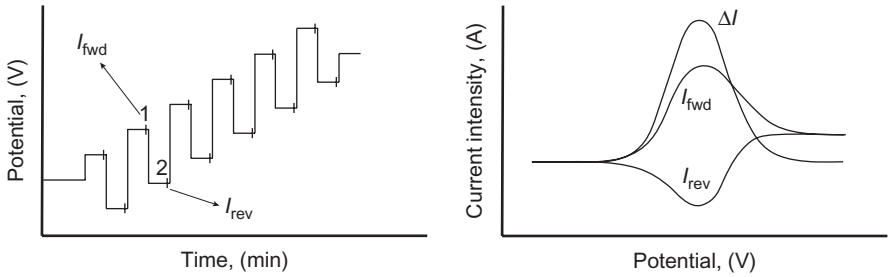

Fig. 13.5 Potential sweep and typical voltammogram of the SWV analysis for a reversible reaction (I_{fwd} and I_{rev}: forward and reverse mode peak current intensities; $\Delta I = I_{fwd} - I_{rev}$).

This double measurement minimizes the capacitive current contribution on the total current reading. The difference between these two currents (response waveform, $\Delta I = I_{fwd} - I_{rev}$) is plotted versus the sweep potential obtaining a peak-shaped voltammogram display (Fig. 13.5), where the peak height in this plot is proportional to the concentrations of the various redox active species (Scholz, 2010; Thomas and Henze, 2001; Zoski, 2007).

Since the SWV technique removes the capacitive current from the measurements, it allows a fast analysis with a wide dynamic range of electroactive species, thus resulting in higher sensitivity compared to CV technique. Moreover, this electrochemical technique does not require the exclusion of oxygen from the solution, except when it interferes with the electrode reaction in the analysis, as it has no time to diffuse to the electrode surface.

13.2.1.3 Electrochemical impedance spectroscopy

The electrochemical impedance spectroscopy (EIS) consists of varying the excitation frequency f of the applied potential E, over a range of frequencies, while the impedance (both resistance and reactance) is measured. The electrical impedance (Z) is defined as the ratio of the change of the voltage-time function $E(t)$ and the resulting change of the current-time function $I(t)$ and can be calculated by

$$Z = \frac{E(t)}{I(t)} = \frac{1}{Y} = \frac{E_0 \sin(2\pi ft)}{I_0 \sin(2\pi ft + \varphi)} \tag{13.4}$$

where E_0 and I_0 are the maximum voltage and current signals, f is the frequency, t is time, φ is the phase shift between the voltage-time and current-time functions, and Y is the complex conductance (Wang et al., 2012).

This technique enables the study of any material property or specific process, such as the resistive characterization, recombination, and corrosion. It is a useful tool in the development and analysis of new materials for biosensors, since it allows monitoring changes at the surfaces of the modified electrodes (Grieshaber et al., 2008; Wang et al., 2012).

13.2.2 E-tongues and sensor-arrays: design, development, and applications

Multisensor systems for liquid analysis based on chemical sensor arrays that attempt to mimic the human chemical senses coupled with pattern recognition methods are

widely known as E-tongues (Legin et al., 2002). These devices (Fig. 13.6) allow correlating electrochemical signals with the human's ability to measure and compare taste intensities (Gutiérrez et al., 2012; Ramamoorthy et al., 2014). The human tongue contains sensors, in the form of 2000–10,000 taste buds of 50–100 taste cells each (Roper, 1995), for sweet, acid, bitter, salty and umami. So the task of designing an artificial sensing device is very complicated, since the mechanisms underlying natural or chemical perception are not fully known (Ciosek and Wróblewski, 2007).

The E-tongue uses an array of several nonspecific, low-selective chemical sensors with partial specificity (cross-sensitivity) to different compounds in solution, such as salts, acids, sugars, bitter and astringent compounds, among others (Wardencki et al., 2013). These sensors are mainly developed for the classification of foodstuffs, although the results are not necessarily comparable to those of a trained sensory panel (Ciosek and Wróblewski, 2007). The E-tongue's sensing capability is derived from its ability to detect multiple organic and inorganic compounds, from a signal spectrum-profile recorded, which contains complementary information from each sensor, generating a unique fingerprint of each matrix under evaluation, rather than information about the nature of the studied compounds (Gutiérrez et al., 2012; Ramamoorthy et al., 2014).

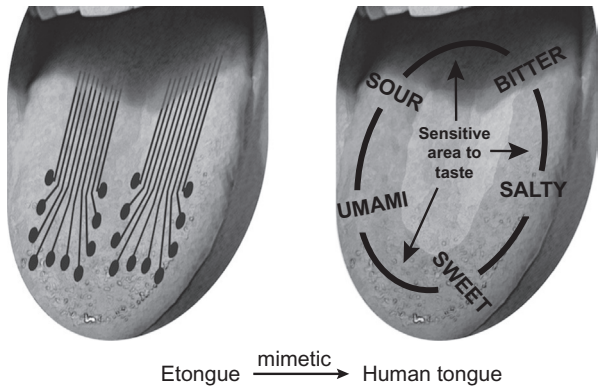

Fig. 13.6 E-tongue: mimicking the human chemical senses aiming to evaluate basic taste intensities.

E-tongue systems (Fig. 13.7) are available in different versions, from desktop to portable devices, static or flow analysis operation modes, consisting of (Baldwin et al., 2011; Wardencki et al., 2013):

- A sensor array including a RE.
- A multichannel electronic interface device (eg, data-logger) that reads the sensor output, converts it to a digital signal and sends it to a computer.
- A computer for data acquisition, storage and processing.

A variety of sensors, from optical to mass spectrometry-based, have been employed in the design of E-tongues, but the electrochemical devices are still the most common (Bagnasco et al., 2014), in particular potentiometric- and voltammetric-based devices.

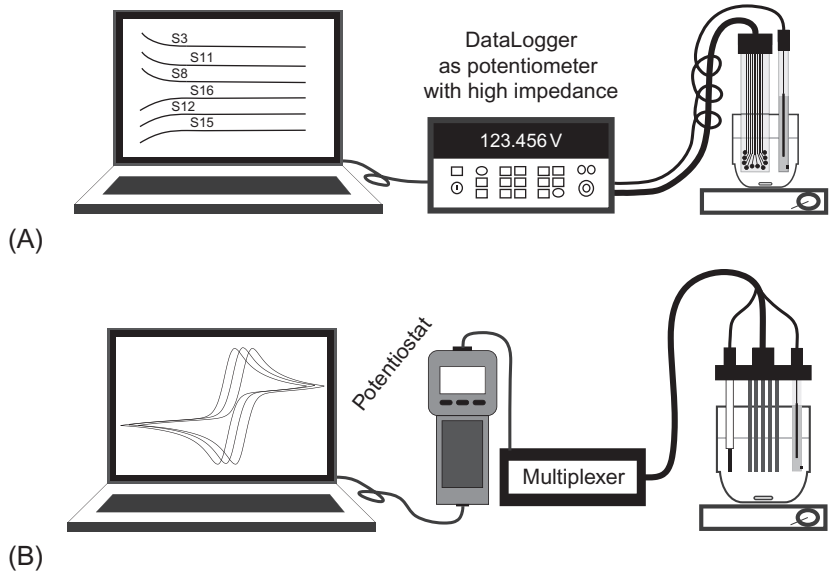

Fig. 13.7 Basic principles of potentiometric (A) and voltammetric (B) sensor arrays analytical systems.

In potentiometric electrochemical devices (Fig. 13.7A), the sensors may be ion selective membranes or polymeric membranes (eg, lipid membranes or others), comprising a mixture of sensor additive chemical compounds, plasticizers and a suitable polymeric matrix, applied using appropriate techniques (eg, drop-by-drop) to a solid support (eg, acrylic body; screen-printed) (Fig. 13.8). The type of sensors and relative

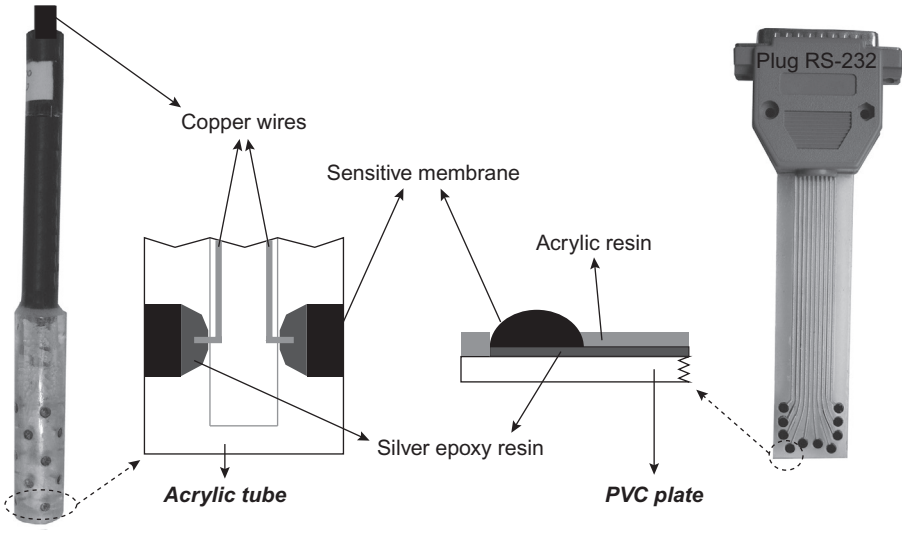

Fig. 13.8 Examples of potentiometric E-tongue devices with different geometries and built with different types of solid supports.

composition included in the E-tongue are established according to their capability to distinguish the different basic tastes and their levels (Baldwin et al., 2011). Also, a RE must be included in the setup (eg, Ag/AgCl). For signal acquisition, a conducting resin (eg, silver epoxy resin) connected to electric wires (eg, copper, silver) may be used (Dias et al., 2008, 2011, 2014).

Voltammetric or even hybrid based E-tongues, which combine sensors from different types to improve the system recognition performance, have also been reported, although with the disadvantage of requiring data processing tools with a high level of complexity (del Valle, 2011). Voltammetric E-tongues (Fig. 13.7B) show high selectivity, sensitivity and low signal-to-noise ratio compared to potentiometric systems (Bagnasco et al., 2014). Usually they are an array of noble metal WEs (eg, gold, palladium, platinum, and silver) and/or electrodes with several coating membranes (eg, polymers and epoxy-graphite). The CE and RE are coupled to the system array and are usually included in screen-printed electrodes (SPE) (Wang et al., 2015), as the signal profile recorded using different voltammetric techniques (eg, CV, SWV). In general, the experimental setup includes one or several WEs, a CE and an RE. In some situations, the potential advantages of merging molecularly imprinted polymers (MIP) with traditional voltammetric E-tongues, leading to novel synergetic hybrid MIP-based E-tongue devices, have also been evaluated (Huynh and Kutner, 2015).

E-tongues have been largely applied in several research fields, as their main applications are still focused on foodstuffs analysis and in particular in beverages evaluation (eg, Apetrei and Apetrei, 2013; de Sá et al., 2016; Phat et al., 2016; Veloso et al., 2016). Nevertheless electrochemical sensor arrays have also been studied for other purposes, such as environmental monitoring (eg, Mimendia et al., 2010; Zadorozhnaya et al., 2015), biomedical research (eg Bandodkar et al., 2013, 2014; Brett et al., 1999; Mc Caffrey et al., 2015; Guinovart et al., 2013; Tahirbegi et al., 2013, 2014) and pharmaceutical applications (eg, Guhmann et al., 2015; Pein et al., 2015; Sidel'nikov et al., 2015; Yoshida et al., 2015). Within the healthcare field, several potentiometric, voltammetric, and/or impedimetric sensor-arrays have been reported, thus highlighting their versatility for medical applications.

13.2.2.1 Potentiometric based-sensor arrays for biomedical and pharmaceutical applications

Potentiometric devices have been used to analyze several biological fluids including blood, urine, and sweat. One of the first references regarding the biomedical applications of such devices dates back to 1999 and describes the use of a chemical sensor array for multicomponent analysis of biological fluids (Legin et al., 1999). The authors developed an ISE system for the simultaneous determination of ions (Ca^{2+}, Mg^{2+}, Na^{+}, HCO_3^{-}, Cl^{-}, H^{+}, and HPO_4^{2-}) in solution modeling human blood plasma. The multisensors array, based on nonspecific original PVC materials, had cross-sensitivity towards cations and anions. The results showed that the electrochemical device coupled with pattern recognition tools (eg, partial least squares (PLS) and artificial neural networks (ANNs)) presented a satisfactory quantification precision

(1–4%) within the typical ion ranges found in human blood plasma. The ANNs simulate the biological neural network. The mathematical model uses "… a large number of parallel connected simple arithmetic units (neurons) that can be defined as nonlinear, parameterized, bounded function, so that the variables this function depends on are called the inputs of the neuron and its value is called the output" (Dreyfus, 2005; Marini et al., 2008).

Di Natale et al. (2000) showed that an E-tongue containing metalloporphyrins as sensitive substances and a mixture of PVC and a plasticizer (dibuthylphtalate) could be used for clinical analysis of urine samples. Linear correlations could be established between the sensors signal profiles and several clinical markers determined routinely (eg, pH, specific weight and blood cell concentration). Phillips et al. (2007) used a flow-through multisensor array, assembled in insulated screen-printed chips that comprised cation-selective polymeric membrane electrodes to successfully evaluate Na^+ concentration in undiluted urine after removing interfering compounds (eg, electrically neutral lipids).

Assessing sodium concentration is of clinical relevance as low urine sodium concentration may indicate dehydration, while a relatively high urine sodium concentration suggests acute renal failure. Lvova and coworkers (2009) proposed an E-tongue composed of chemical sensors to detect urinary system dysfunctions and creatinine levels. The electrochemical system was composed of miniaturized metallic sensors and ISEs with PVC solvent polymeric membranes. The device enabled the correct classification of urine samples from healthy volunteers, according to creatinine levels and to predict the creatinine content of urine.

Also, the E-tongue could be used as a noninvasive and low-cost technique for early monitoring of urinary tumors, since it allowed the distinction between urine samples of healthy patients and patients with malignant and nonmalignant tumor diagnosis of bladder. Recently, Yaroshenko et al. (2015) developed a multisensor system with ISE and CSE as an analytical tool to determine the urine ionic composition of healthy people and patients suffering with urolithiasis. The potentiometric data recorded from diluted urine samples enabled the differentiation of samples from healthy and unhealthy people and the quantitative analysis of the sodium, potassium, ammonium, calcium, magnesium, chloride, sulfate, phosphate, urate, and creatinine.

Gonzalo-Ruiz et al. (2009) developed novel and fast disposable screen-printed sensor devices capable of quantifying chloride in sweat, thus being a suitable tool for the early detection of cystic fibrosis (CF). The method relies on the direct measurement of chloride on the skin by a four-electrode configuration (ie, anode and cathode electrodes for sweat generation; WE and RE for potentiometric measurements).

The device performance was assessed using synthetic and real samples of volunteers and deviations lower than 8% were obtained as compared to values determined using conventional clinical techniques. Schazmann et al. (2010) proposed a novel real-time in situ potentiometric quantitative analysis of sodium in human sweat, using wearable ISEs platform, in-house fabricated with PVC tubing used as barrels, which could be used during exercise. The device showed good intra- and interday repeatability and was used to monitor the sweat of CF patients.

Bandodkar et al. (2013, 2014) built a novel tattoo-based solid-contact ISE for non-invasive monitoring of epidermal pH levels. The proposed device exhibited a rapid and sensitive response to a wide range of pH changes. The device was applied to several areas of the human body (eg, lower back, neck, and wrist) and was successfully used for real-time monitoring of pH levels of human perspiration during exercise. Guinovart et al. (2013) showed the potential use of a novel ion-selective all-solid state potentiometric cell containing ammonium-selective polymeric nonactin-based membrane for monitoring ammonium levels in sweat using a temporary-transfer tattoo platform. The results showed that the new wearable tattoo-sensor could be, in the near future, a promising tool for monitoring sports performance or for detecting metabolic disorders.

Tahirbegi and coworkers (2013, 2014) demonstrated the applicability of an all-solid-state differential potentiometric, miniaturized and mass producible pH and potassium ISE sensor integrated in an array, together with an RE, for detecting ischemia at low pH on stomach tissue. The sensor device was designed for enabling its insertion inside the stomach by means of an endoscopic device. The signals' profiles showed good reproducibility for sensors included in the same or in different arrays. The results indicated that ischemic and reperfusion states could be sensed in vivo and that information on tissue damage could also be assessed by the sensor arrays.

Recently, our research group studied the potential use of an E-tongue, operating in a static mode, comprising 40 lipid polymeric membranes with cross-sensitivity, for the analysis of caffeine, analgesic and antipyretic compounds (paracetamol and aspirin, also known as acetylsalicylic acid) (*unpublished data*). The lipid membranes contained PVC (~32%) mixed with different combinations of six plasticizers (~65%; bis(2-ethylhexyl)phthalate, bis(1-butylpentyl) adipate, tris(2-ethylhexyl)phosphate, dibutyl sebacate, 2-nitrophenyl-octylether, or dioctyl phenylphosphonate) and six sensor/additive compounds (~3%; octadecylamine, bis(2-ethylhexyl)phosphate, oleyl alcohol, methyltrioctylammonium chloride, tridodecylmethylammonium chloride, or oleic acid). The final lipid membranes contained lipids with alkyl chains and phosphoric groups negatively charged, and amine lipids with alkyl chains allowing hydrophobic interactions, besides exhibiting a positive charge. The E-tongue system was formed by two cylindrical sensor arrays (Fig. 13.8) built on acrylic bodies, previously described by Dias et al. (2009). Each array had 20 holes filled with conducting silver-epoxy resin connected to copper electric wires. Membranes were formed by deposition of the membrane solution on the silver conducting surface using a drop-by-drop technique. Linear discriminant analysis (LDA), based on the potentiometric profiles of the individual standard solutions of each compound, was used to assess the capability of the device to correctly classify the samples.

The results showed that the E-tongue could correctly classify all the solutions (sensitivity of 100%) for the original grouped data (Fig. 13.9), and for the leave-one-out cross-validation procedure.

The LDA model had two discriminant functions (explaining 100% of data variability) and included the information of the 12 sensors represented in Fig. 13.9, chosen by a simulated annealing (SA) metaheuristic variable selection algorithm (Bertsimas and Tsitsiklis, 1993; Cadima et al., 2004; Kirkpatrick et al., 1983). In general, the sensitivity

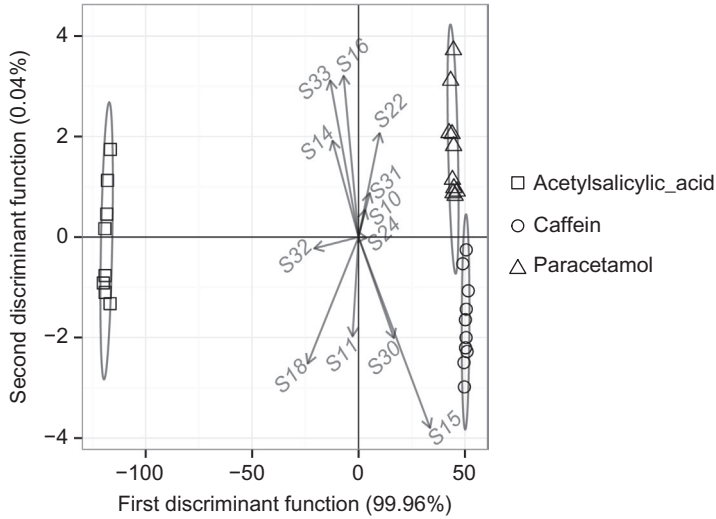

Fig. 13.9 Discrimination of standard solutions containing caffeine, paracetamol, or acetylsalicylic acid using a LDA model based on a subset of E-tongue sensors, selected by the SA variable selection algorithm.

of the lipid polymeric sensors used in the E-tongue decreased in the following order: caffeine > paracetamol > acetylsalicylic acid. The satisfactory qualitative and quantitative results obtained could be tentatively explained considering that

(i) Caffeine can interact with different functional groups present in the plasticizer/sensor additive mixtures that formed the potentiometric multisensor array, namely with the alkane-like regions, amine, and phosphoric groups (Evanics and Prosser, 2005; Shen et al., 2008).

(ii) Acetylsalicylic acid may interact with the quaternary ammonium ions present in the lipid membranes since substituted aromatic carboxylates like salicylates and aspirin are able to interact with lipophilic carriers (He et al., 1992).

Hence, the proposed potentiometric E-tongue seems adequate to quantify caffeine, paracetamol or acetylsalicylic acid in pharmaceutical formulations, although validation of this device using real samples is still required.

Regarding pharmaceutical applications, most reports focus on the use of potentiometric devices as taste-sensing tools for the development of pharmaceutical formulations. E-tongue systems have been used for taste measurement of bitter drug substances towards the development of palatable oral formulations.

Several works published in recent years strengthen the interest in using potentiometric E-tongue in the development of new pharmaceutical formulations. Choi et al. (2014) used an E-tongue to assess the taste masking effect of conventional pharmaceutical sweeteners, including neohesperidin dihydrochalcone, sucrose, sucralose and aspartame. The multichannel taste sensor system, which comprised seven sensors, was used to measure the bitterness degree of typical bitter-tasting model drugs, namely acetaminophen, ibuprofen, tramadol hydrochloride, and sildenafil citrate.

In general, the sensors exhibited a concentration-dependent pattern of the taste-masking-agents showing that the E-tongue could detect small concentration changes of a chemical in solution enabling the evaluation of taste masking using potentiometric data, thus increasing the efficiency of the formulation development process.

Guhmann et al. (2015) described the application of an E-tongue as a taste sensor for the in vitro evaluation of diclofenac taste-masked orodispersible tablet formulations. The potentiometric E-tongue contained an RE and seven lipid membrane sensors for different taste qualities like bitterness (three sensors), sourness, saltiness, umami, and astringency. The results showed that the E-tongue could discriminate the formulations prepared with the same taste-masking agent. Pein et al. (2015) compared different E-tongues for pharmaceutical analysis. Among them, the performances of three laboratory prototypes of potentiometric E-tongues, working in flow or static modes, and comprising different sensors (in number and composition) were evaluated. The potentiometric E-tongues could differentiate samples containing caffeine citrate from API (active pharmaceutical ingredient)-free samples, showing potential for use in the evaluation of taste-masking effects and drug-release kinetics. Yoshida et al. (2015) also studied the use of a potentiometric E-tongue to evaluate palatability of the original and nine generic versions of famotidine orally disintegrating tablets and the bitterness intensities predicted by the E-tongue were in accordance with those assessed by a sensory panel.

13.2.2.2 Voltammetric based-sensor arrays for biomedical and pharmaceutical applications

Voltammetric based-approaches have also been reported for healthcare applications. In 1999, Brett et al. (1999) used poly(methylene blue) (MB) modified electrodes for successfully quantifying hemoglobin in the blood samples of healthy and ill people. The analysis was based on the electro-oxidation of hemoglobin at a fixed potential, which was monitored by CV, using a typical three-electrode cell (a glassy carbon electrode (GCE) as the WE; a Pt gauze as CE and a saturated calomel electrode as the RE). The methodology identified a blood sample as belonging to a patient if the voltammetric signal showed a deviation from the linear correlation established for healthy people (electrochemical method versus conventional clinical spectrophotometric technique).

It was hypothesized that such deviations could be linked to structural changes occurring in hemoglobin variants and so, the E-tongue could be used as a diagnostic tool for anemia, leukemia or excessive loss of blood. Fan et al. (2000) used a modified iodide-silver electrode to determine by CV and DPV the hemoglobin contents in standard solutions and in solutions mimicking real biological fluids. The results showed that a linear relationship could be established between the anodic peak current of the DPV and the protein concentration (detection limit of 3×10^{-7} M).

Mitsubayashi et al. (2003) developed a wearable and flexible oxygen sensor with membrane structure, constructed using microfabrication techniques, and containing a nonpermeable sheet and a gas-permeable membrane with platinum- and Ag/AgCl-electrodes. The sensor device, applied to the skin surface of healthy male volunteers with no history of skin diseases, allowed the safe monitoring, by CV, of the transcutaneous

oxygen pressure without any problems, such as skin inflammation. Iguchi et al. (2005) developed a novel wearable oxygen sensor device with gold and Ag/AgCl electrodes fabricated with gas-permeable membranes using photolithography and sputtering methods and that also contained a membrane filter and a nonpermeable membrane.

The CV and chronoamperometric data recorded showed that the wearable sensor could be used as a new transcutaneous oxygen sensor, enabling evaluating the oxygen level in the conjunctiva of a rabbit without any thermoregulation. An automatic sequential injection voltammetric E-tongue was described by Gutés et al. (2007) to determine oxidizable compounds of clinical and pharmaceutical interest, such as ascorbic acid, uric acid, and paracetamol.

The array comprised three WEs (platinum, gold, and epoxy-graphite discs with 1 mm of diameter) and one integrated RE (Ag/AgCl) for minimizing noise effects. All electrodes were built from metal wires and encased in epoxy resin in PVC tube (6 mm inner diameter) used as the system body. ANN models based on voltammograms data showed a good prediction capability, allowing quantifying ascorbic acid, uric acid, and paracetamol with average errors of 8%, 10%, and 12%, respectively.

Twomey et al. (2011) showed that a four electrodes (Au, Pt, Ir, and Rh) voltammetric E-tongue probe could be used to evaluate different biological fluids, namely gut fluids. CV was used to obtain chemical information (in vivo) from gut model fluids and the different signal profile shapes highlighted the discrimination capability and the potential of such device as a tool for gastrointestinal fluid investigation.

Recently, Mc Caffrey et al. (2015) presented a wireless swallowable multielectrode E-tongue device, incorporating different voltammetric sensing techniques (eg, CV, SWV, and DPV), which enables real-time nonspecific characterization of gut fluids, thus helping in the diagnosis of gastrointestinal diseases such as Crohn's and ulcerative colitis. The satisfactory results obtained, comparable with clinical routine conventional techniques, namely for the electrochemical examination of fecal waters proved the E-tongue's potential as an in vitro or in vivo gastrointestinal tract diagnostic tool.

Tattoo-based electrochemical devices have been recently described for evaluating different physiologically relevant compounds in sweat or skin. Windmiller et al. (2012) developed stamp transfer electrodes for electrochemical sensing on nonplanar and oversized surfaces. The concept was demonstrated towards the voltammetric detection of dopamine, ascorbic acid and acetaminophen, as well as to quantify increasing levels of uric acid in human skin.

Machini and coworkers (2013) developed a voltammetric sensor with hausmannite-type manganese oxide, as a sensing material, for improving sodium ion quantification. The device was satisfactorily used to determine, by CV, sodium ions in diluted urine samples, without prior treatment.

Some works have been reported concerning the use of voltammetric sensor devices for pharmaceutical applications. Sidel'nikov et al. (2015) showed the capability of a voltammetric E-tongue (containing carbon electrodes modified by polyarylene phthalides) to identify antiarrhythmic medicines from several producers. Overall, the results highlighted that the voltammograms of medicines could serve as "virtual prints" and be used for their identification and for quality control.

13.2.2.3 Impedimetric based-sensor arrays for biomedical and pharmaceutical applications

The use of impedimetric-based sensing strategies for biomedical applications is scarce compared to potentiometric or voltammetric bioinspired devices. Even so, some successful applications have been reported. Ivorra et al. (2003) and Genescà et al. (2005) developed miniaturized platinum four-electrode probes for in vivo electrical bio-impedance measurements. The results obtained in vivo using rat kidneys subjected to ischemia showed that this type of probe could be used as a helpful marker of tissue condition. Indeed, since impedance modulus and phase were measured at low frequency it was possible to evaluate extracellular pH and potassium levels. Moreover, the device was also partially successful in identifying kidneys that have suffered previous warm ischemia.

Gonzalez-Guillaumin et al. (2007) proposed a multisensor impedance-pH wireless capsule capable of discriminating between acidic and nonacidic gastro-esophageal reflux. The results suggested that the device could offer a minimally invasive and reliable testing of all aspects of gastro-esophageal reflux disease. Beging et al. (2010) built miniaturized field-effect capacitive Ca^{2+}-sensitive electrolyte-membrane–insulator–semiconductor sensors to determine free Ca^{2+}-ion concentration in native urine of high-risk patients (stone formation) by potentiometry/impedance. The sensor showed almost Nernstian calcium sensitivity and exhibited a detection limit around 0.01 mM, which was below the lowest concentration of Ca^{2+} in native urine.

13.2.3 Aptasensors: Design, development and application

The detection and quantification of protein biomarkers is crucial for clinic diagnostics, as well as for monitoring the treatment of certain diseases. As previously mentioned, electrochemical biosensors exhibit high sensitivity and selectivity for the monitoring of the low levels of protein biomarkers (Saberian et al., 2011; Xu et al., 2009). The use of aptamers to develop biosensors (so-called aptasensors) presents many advantages, including high selectivity and affinity, chemical stability under a wide range of buffer conditions, resistance to harsh treatments without loss of bioactivity, reversible thermal denaturation, adaptability to several targets, ease of storage, and versatility in labeling, immobilization in several surfaces (eg, gold, carbon), signaling and regeneration (Feng et al., 2008; Hianik and Wang, 2009; Li et al., 2010; Radi, 2011; Strehlitz et al., 2008). Additionally, the use of aptamers opens the possibility of performing several analyses with mild alteration of specificity and selectivity of the binding. Aptamers can be isolated by an in vitro selection technique named SELEX (systematic evolution of ligands by exponential enrichment) from random-sequences nucleic acids libraries.

DNA aptamers are suitable for designing reusable aptasensors, whereas RNA aptamers allow single shot measurements since they are more susceptible to nucleases action (Sassolas et al., 2009; Strehlitz et al., 2008). Some chemical modifications can enhance nuclease resistance (ie, biostability in serum) and increase the half-life of RNA aptamers. These chemical modifications can be introduced in the oligonucleotides libraries and either during or after the SELEX cycle (Kuwahara and Sugimoto, 2010; Stoltenburg et al., 2007).

An electrochemical aptasensor is a compact device using aptamers as the bioreceptor element immobilized onto the electrode surface. It comprises the transducer responsible for monitoring the formation of the aptamer-target complex and for the translation of this formation into a measurable signal that can be easily processed, recorded and displayed (Hong et al., 2012; Monošík et al., 2012; Sadik et al., 2009; Thévenot et al., 1999). The electrochemical transduction can be divided into voltammetric, amperometric, potentiometric, and impedimetric (Arshak et al., 2009; Velusamy et al., 2010). In the fabrication of electrochemical aptasensors, different electrode/transducer surface, immobilization methods of aptamers and design strategies can be used depending on the envisaged use (Fig. 13.10).

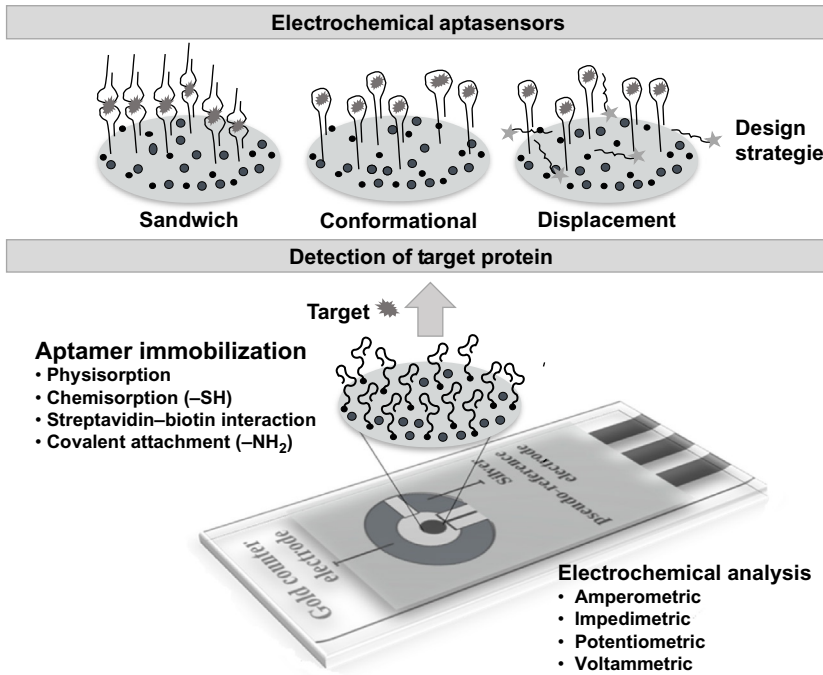

Fig. 13.10 Basic principles of electrochemical aptasensors.

The design of these aptasensors can be divided into three or four types based on the differences in the design of the DNA/RNA-modified electrodes. Cheng et al. (2009) classified electrochemical aptasensors into three categories in which the analyte/target molecule detection relies: configuration, conformation or conductivity changes. For the first, the target binding induces either an assembly or dissociation of the aptamer. In the second configuration type, the target binding induces a modification in the conformation of the aptamer immobilized onto the surface. Finally, for the latter case, the target binding "switches on" the conductivity of the surface-bound aptamer-DNA.

On the other hand, Han et al. (2010) suggested four possible design strategies, viz. target-induced structure switching mode; sandwich or sandwich-like mode;

target-induced dissociation or displacement mode; and competitive replacement mode. These strategies can be used in the development of label and label-free, as well as in "signal-on" and "signal-off" electrochemical aptasensors.

13.2.3.1 Electrochemical aptasensors based on sandwich design

The sandwich design presents an increased sensitivity and selectivity concerning the detection of the target molecule. However, two conditions are required for this sensor arrangement, namely (1) the target must have two independent binding sites (epitopes) to which two different ligands (aptamers) may bind the target molecule without affecting the binding of other targets; and (2) two or more aptamers are selected against the target (Han et al., 2010; Palchetti and Mascini, 2012).

Very few target molecules (platelet-derived growth factor (PDGF) and thrombin (THR)) have two binding sites and two or more isolated aptamers, which limits the use of this type of sensor format. This limitation can be circumvented through the use of the aptamer–protein–aptamer (using the same aptamer) and aptamer–protein–antibody (Han et al., 2010; Palchetti and Mascini, 2012) designs.

Some works have reported the use of aptasensors based on sandwich design for the detection of different proteins, namely THR, C-reactive protein (CRP), vascular endothelial growth factor (VEGF) (Ravalli et al., 2015), mucin 1 (MUC1) (Chen et al., 2015), and PDGF (Wang et al., 2009) (Table 13.1).

Centi et al. (2007) developed a labeled "signal-on" electrochemical aptasensor to detect THR in human plasma samples using the sandwich design with magnetic particles as solid supports. A biotinylated aptamer was captured onto streptavidin-coated magnetic beads through streptavidin–biotin interaction in a carbon electrode surface. The resulting aptamer-modified magnetic beads were incubated with THR. Upon binding, a biotinylated secondary aptamer was added, ready to bind in a further step to a streptavidin–alkaline phosphatase (AP) conjugate. The changes in the electrode surface were detected by DPV and a low detection limit (0.50 nM) was achieved in serum and plasma.

These authors used the same strategy for detecting the CRP. However, in this example a biotinylated RNA aptamer with affinity for CRP was immobilized on the magnetic beads (capture probe) and a biotinylated monoclonal antibody antiCRP conjugated with alkaline phosphatase (AP) (detection probe) was used. The electrochemical detection through DPV allowed a detection limit of 2 nM in serum samples (Centi et al., 2009).

In recent years nanomaterials have been explored for the signal amplification thereby promoting the sensitivity of electrochemical aptasensors. Wang et al. (2011) reported an electrochemical aptasensor for the detection of THR in which nanoparticles were used to increase the surface area (capture probe) and for signal amplification (detection probe). The authors described a sandwich design using a GCE modified with chitosan-hollow CoPt alloy nanoparticle (Cs–HcoPt) film activated with glutaraldehyde (GA) for the immobilization of aptamer-1 modified with amino group. For the signal amplification, aptamer-2 was conjugated with high-quality hollow CoPt bimetal alloy nanoparticles (HcoPt) onto reduced graphene oxide sheet (rGS) with a redox probe

Table 13.1 Examples of electrochemical aptasensors based on sandwich design

Target/aptamer	Aptasensor	Detection limit	Samples	References
THR (DNA)	Label (AP), "signal-on", voltammetric	0.50 nM	Serum and plasma samples	Centi et al. (2007)
THR (DNA)	Label (CdS-QDs), "signal-on", voltammetric	0.55 fM	Standard solutions	Ding et al. (2010)
THR (DNA)	Label (AuNPs–HRP), "signal-on", voltammetric	30 fM	Standard solutions	Zhaoet al. (2011a)
THR (DNA)	Label (NPs–rGS–Thi–HRP), "signal-on", voltammetric	0.34 pM	Standard solutions	Wang et al. (2011)
CRP (RNA)	Label (AP), "signal-on", voltammetric	2 nM	Serum samples	Centi et al. (2009)
VEGF (DNA)	Label (AuNPs–AP), "signal-on", voltammetric	30 nM	Standard solutions	Ravalli et al. (2015)
MUC1 (DNA)	Label (PoPD–AuNPs), "signal-on", voltammetric	1 pM	Serum samples	Chen et al. (2015)
PDGF (DNA)	Label (AuNPs–$[Ru(NH_3)_5Cl]^{2+}$), "signal-on", voltammetric	1 pM	Blood serum	Wang et al. (2009)
THR (DNA)	Label (Cds-QDs), "signal-on", potentiometric	0.14 nM	Standard solutions	Numnuam et al. (2008)
THR (DNA)	Label (AuNPs), impedimetric	0.3 pM	Standard solutions	Ocaña and del Valle (2014)

AP, alkaline phosphatase; *AuNPs*, gold nanoparticles; *CdS-QDs*, cadmium sulfide quantum dot; *CRP*, C-reactive protein; *HRP*, horseradish peroxidase; *MUC1*, mucin 1; *NPs*, nanoparticles; *PDGF*, platelet-derived growth factor; *PoPD*, poly(*o*-phenylenediamine); *rGS*, reduced graphene oxide sheet; *THR*, thrombin; *VEGF*, vascular endothelial growth factor.

thionine (Thi) and enzyme horseradish peroxidase (HRP). This aptasensor presented a low detection limit (0.34 pM) and a good specificity for THR in Tris–HCl buffer.

A simple and ultrasensitive aptasensor for the detection of THR was reported by Zhao et al. (2011a). The aptamer-1 (Apt1) was immobilized onto core/shell Fe_3O_4/gold magnetic nanoparticles (AuMNPs) and by chemisorption onto a gold electrode, and the aptamer-2 (Apt2) was coupled with gold nanoparticles (AuNPs) and HRP for signal amplification. This electrochemical aptasensor using DPV and containing two different aptamers (AuMNPs–Apt1/THR/Apt2–AuNPs–HRP) allowed a detection limit of 30 fM for THR in PBS buffer (phosphate buffered saline). In addition, it showed good reproducibility and selectivity for THR.

Recently Ravalli et al. (2015) described a single-use electrochemical aptasensor for the detection of VEGF based on a sandwich design with two different aptamers, AuNPs and an enzyme-amplified detection. The graphite SPEs were modified by electrodeposition of AuNPs for thiolated aptamer-1 immobilization by chemisorption. After Apt1-VEGF recognition, the biotinylated Apt2 was added and the resulting complex was coupled with streptavidin–AP for amplified detection. The electroactive product of the enzymatic reaction was detected by DPV. The "signal-on" aptasensor response was found to be linearly related to the target concentration between 0 and 250 nM in Tris buffer; the detection limit was 30 nM.

Using a similar strategy, Chen et al. (2015) reported a "signal-on" electrochemical aptasensor for the detection of MUC1. The poly(*o*-phenylenediamine)–AuNPs (PoPD–AuNPs) film was used as a capture probe for the immobilization of aptamer-1. On the other hand, the AuNPs functionalized silica/multiwalled carbon nanotubes core-shell nanocomposites (AuNPs/SiO2@MWCNTs) were used to enhance the immobilization of the secondary aptamer, as well as to load large amounts of Thi electrochemical probe. The presence of MUC1 induced the formation of one complex between the PoPD–AuNPs–Apt1 and the Thi–AuNPs/SiO2@MWCNTs–Apt2. The current changes resulting from the formation of such complex were registered by DPV and a detection limit of 1 pM was obtained in serum.

Numnuam et al. (2008) reported the first potentiometric aptasensor using ion-selective microelectrodes for THR detection. In the sandwich design, the THR was captured by a thiolated aptamer attached to the surface of the gold electrode and the measurements were based on cadmium sulfide quantum dot (CdS-QDs) label of the secondary aptamer. The potentiometric aptasensor presented good sensitivity allowing the detection of up to 28 fmol of THR in PBS buffer solutions (detection limit of 0.14 nM).

A THR impedimetric aptasensor using sandwich design was developed by Ocaña and del Valle (2014). The aptasensor used gold–streptavidin nanoparticles (strep–AuNPs), silver enhancement treatment for signal amplification and two different biotinylated THR aptamers (AptTHRBio1 and AptTHRBio2). The fabrication of the aptasensor involved several steps, namely: (1) immobilization of AptTHRBio1 onto an avidin-graphite epoxy composite electrode surface by biotin–avidin interaction; (2) sandwich formation between the AptTHRBio1–THR–AptTHRBio2; (3) binding strep–AuNPs with AptTHRBio2; and (4) silver enhancement treatment. The EIS aptasensor showed high selectivity and sensitivity for THR with a detection limit of 0.3 pM and a good linear range (0.1–100 pM) in PBS buffer.

13.2.3.2 *Electrochemical aptasensors based on target binding-induced aptamer conformational changes*

This strategy has been widely used in the development of electrochemical aptasensors for the detection of different proteins, such as THR (Düzgün et al., 2010; Ocaña et al., 2012; Xiao et al., 2005a), interferon gamma (IFN-γ) (Liu et al., 2010), MUC1 (Hu et al., 2014; Liu et al., 2015; Ma et al., 2013), VEGF (Zhao et al., 2011b), osteopontin (OPN) (Meirinho et al., 2015), and PDGF (Degefa and Kwak, 2008; Lai et al., 2007) (Table 13.2). It exploits the ability of aptamers to form several secondary and/or tertiary structures after binding to their target protein.

Table 13.2 Examples of electrochemical aptasensors based on target binding-induced aptamer conformational change

Target/aptamer	Aptasensor	Detection limit	Samples	References
THR (DNA)	Label (MB), "signal-off", voltammetric	6.4 nM	Blood serum	Xiao et al. (2005a)
THR (DNA)	Label-free, potentiometric	80 nM	Standard solutions	Düzgün et al. (2010)
THR (DNA)	Label-free, impedimetric	4.5 pM	Standard solutions	Ocaña et al. (2012)
IFN-γ (DNA)	Label (MB), "signal-off", voltammetric	0.06 nM	Standard solutions	Liu et al. (2010)
MUC1 (DNA)	Label (MB), "signal-off", voltammetric	50 nM	Standard solutions	Ma et al. (2013)
MUC1 (DNA)	Label (AuNPs–HRP), "signal-on", voltammetric	2.2 nM	Standard solutions	Hu et al. (2014)
MUC1 (DNA)	Label-free, "signal-off", impedimetric	0.1 nM	Standard solutions	Liu et al. (2015)
VEGF (DNA)	Label (MB), "signal-on", voltammetric	5 pM	Human whole blood	Zhao et al. (2011b)
OPN (RNA)	Label-free, "signal-off", voltammetric	3.7 nM	Standard solutions	Meirinho et al. (2015)
PDGF (DNA)	Label (MB), "signal-on", voltammetric	50 pM	Blood serum	Lai et al. (2007)
PDGF (DNA)	Label-free, "signal-off", voltammetric	1–40 nM	Standard solutions	Degefa and Kwak (2008)

HRP, horseradish peroxidase; *IFN-γ*, interferon gamma; *MB*, methylene blue; *MUC1*, mucin 1; *OPN*, osteopontin; *PDGF*, platelet-derived growth factor; *THR*, thrombin; *VEGF*, vascular endothelial growth factor.

Xiao et al. (2005a) developed a label "signal-off" aptasensor for the detection of THR in blood serum. The aptamer against THR was immobilized onto a gold electrode by chemisorption. Before binding to the protein, the 3′-end MB-label aptamer is close

to the electrode surface, thus allowing the transfer of electrons with the electrode surface due to the flexible structure of the aptamer.

After THR binding, the aptamer forms a stable and rigid structure (G-quadruplex structure) and the MB-label is kept away from the electrode surface, the electron transfer is inhibited, thus resulting in a current decrease. The electrochemical measurements were performed by alternating current voltammetry (ACV) and a detection limit of 6.4 nM was reported. The same strategy was used by Liu et al. (2010) for the detection of IFN-γ. The aptamer against IFN-γ was 5′-end thiolated, conjugated with 3′-MB-label and immobilized onto a gold electrode by chemisorption. The IFN-γ binding induced an aptamer conformation change, increased the distance of MB-label from the electrode surface, led to a decrease of the electron transfer and consequently of the current. SWV was used to quantify the current decrease and a detection limit of 0.06 nM was obtained. This reusable, label "signal-off" electrochemical aptasensor proved to be sensitive and specific to IFN-γ in the presence of overabundant serum protein.

The same strategy was reported by Ma et al. (2013) for the development of a label "signal-off" aptasensor to detect MUC1. For that purpose, a specific DNA aptamer MB-label was immobilized onto a gold electrode surface by chemisorption. In the absence of MUC1, one hairpin structure was formed and the electrode transfer between MB-label and the gold electrode was facilitated. Upon MUC1 binding, a conformational change of the aptamer was induced, the MB-label was removed from the electrode surface and a decrease in the current was observed. The electrochemical measurements were performed by SWV, a detection limit of 50 nM and a large dynamic response range (up to 1.5 μM) were reported. This electrochemical aptasensor showed good specificity in the presence of other proteins and could be seen as a promising tool in clinical applications.

On the other hand, Lai et al. (2007) reported a labeled "signal-on" electrochemical aptasensor for the detection of PDGF in clinical samples. The aptasensor was constructed by immobilizing a MB-label aptamer onto a gold electrode through a thiol group. In the absence of PDG, the MB-label is far away from the electrode surface. Upon PDGF binding, the aptamer forms a stable structure holding the MB-label close to the electrode surface, improving the electron transfer. ACV was used to monitor the changes at the electrode surface and detection limits of 1 nM in blood serum and of 50 pM in serum twofold diluted with buffer were obtained. This aptasensor showed excellent stability in real samples and could be regenerated by disrupting the aptamer–PDGF complex.

Zhao et al. (2011b) reported a labeled "signal-on" aptasensor for the detection VEGF in human whole blood. The aptasensor was fabricated through self-assembly monolayer at the electrode surface, using a polycrystalline gold disk electrode as WE and thiol- and MB-modified DNA aptamer. Upon VEGF binding the partially unfolded aptamer formed a conformational stem-loop structure. This conformational change forces the MB-label to be in close proximity to the electrode, thus resulting in a significant increase in the electron transfer and current signal. A detection limit of 5 pM was achieved in 50% of blood serum.

Using AuNPs–HRP for signal amplification, Hu et al. (2014) developed an ultrasensitive electrochemical aptasensor for the detection of MUC1. AuNPs were used to

manufacture this aptasensor, as well as the enzyme HRP and a GCE modified with multiwalled carbon nanotubes and streptavidin.

The aptamer was modified with thiol at the 5′-end and biotin at the 3′-end, and both aptamer and HRP were immobilized on the AuNPs (GCE–Apt–AuNPs–HRP). After the aptamer–MUC1 interaction, the aptamer was disrupted and the biotin exposed was captured by the streptavidin-modified electrode. This "signal-on" aptasensor exhibited good linear dynamic ranges (8.8–353.3 nM) and a low detection limit (2.2 nM) for MUC1 in PBS buffer.

Applying the target binding induced aptamer conformational change strategy, a label-free approach was reported by Degefa and Kwak (2008) for detecting the PDGF cancer-related protein using $[Fe(CN)_6]^{3-/4-}$ as a redox probe. The DNA aptamer against PDGF self-assembled in a monolayer onto the gold surface by chemisorption, which, in the absence of the PDGF showed a dynamic state and efficient electron transfer between the redox probe and the electrode surface. Following the PDGF binding-induced aptamer conformation change, the electron transfer was significantly affected and a linear decrease of the peak current with the protein concentration (1–40 nM) was observed. Using the same approach and the CV, our research team (Meirinho et al., 2015) developed an aptasensor for OPN detection. An RNA aptamer was immobilized on a streptavidin-modified gold surface through the streptavidin–biotin interaction. After binding the OPN, the current decreased due to the alteration of the aptamer structure. The label-free "signal-off" electrochemical aptasensor showed sensitivity and selectivity towards OPN (detection limit of 3.7 nM) and was able to detect OPN in the presence of other interfering proteins, with the exception of THR.

A potentiometric aptasensor for THR detection using single-walled carbon nanotubes (SWCNT) as a transducing element was reported by Düzgün et al. (2010)). The THR binding aptamer was covalently linked to the carboxylic groups of the SWCNT and the aptamer–THR interaction led to a direct potentiometric signal. The aptasensor showed a good response in the THR concentration range of 10^{-7}–10^{-6} M and a limit of detection of 80 nM. The aptasensor presented also good selectivity against elastase and bovine serum albumin and was easily regenerated.

On the other hand, Ocaña et al. (2012) reported a simple, label-free impedimetric aptasensor for the detection of THR in PBS buffer solutions. In the design of the aptasensor, a graphite epoxy composite was used and the aptamers were immobilized onto the electrode surface by physical adsorption. A detection limit of 4.5 pM was achieved using EIS in the presence of the $[Fe(CN)_6]^{3-/4-}$ redox probe. The aptasensor was regenerated for several cycles. Recently, Liu et al. (2015) developed an impedimetric aptasensor, using AuNPs for signal amplification and the redox probe $[Fe(CN)_6]^{3-/4-}$, for detection and quantification of MUC1 in PBS buffer solutions.

For the fabrication of the aptasensor, a complementary DNA (cDNA) sequence was designed and immobilized on the gold electrode via chemisorption. For signal amplification and to capture the MUC1 protein, the aptamer was functionalized with AuNPs (Apt–AuNPs). After MUC1 binding, a well-defined 3D structure was formed, which led to the Apt–AuNPs/MUC1 complex formation. The proposed label-free aptasensor showed a low detection limit (0.1 nM) exhibiting high sensitivity and good selectivity against carcinoembryonic antigen (CEA) and tumor necrosis factor-α (TNF-α).

13.2.3.3 Electrochemical aptasensors based on target-induced aptamer displacement

This strategy is grounded mainly in the strong affinity of the label-free aptamer to its specific target with a labeled aptamer-complementary sequence (Han et al., 2010). Upon binding of the target-aptamer, the dissociation/displacement of the complementary sequences of the aptamer occurs, resulting in detectable signals.

Xiao et al. (2005b) developed a "signal-on" aptasensor using an aptamer immobilized onto a gold electrode by chemisorption and then hybridized it with a partially cDNA sequence labeled with MB. Upon THR binding, the aptamer adopted a G-quadruplex conformation and labeled aptamer-cDNA sequence pushed MB closer to the gold electrode surface, thus leading to a detectable current that could be measured by ACV. This aptasensor allowed the detection of THR in a linear concentration range up to 80 nM with a detection limit of 3 nM in Tris–HCl buffer.

In turn, Lu et al. (2008) developed a "signal-off" aptasensor for the same protein, using a ferrocene (Fc) labeled short aptamer-cDNA sequence to form a double-strain DNA (dsDNA) with the aptamer immobilized on a gold electrode. The target binding led to the dissociation of the label-sequence that was followed by a decrease in the DPV current signal due to the distance between the Fc-label and the gold electrode. The aptasensor was found to be reusable and highly specific with a detection limit of 2 nM in Tris buffer.

This strategy has been reported by Li et al. (2010) and it used AuNPs to increase the signal of an electrochemical aptasensor designed for lysozyme (LYS) detection. The fabrication of the aptasensor included three steps, namely pretreatment of the gold electrode, self-assembly of AuNPs and immobilization of duplex DNA onto the AuNPs-modified gold electrode (aptamer hybridization with an aptamer cDNA sequence label with Fc). In the absence of LYS, the duplex DNA presumably lies down and places the Fc-label in the close proximity of the electrode to give a significantly strong faradaic current. In the presence of LYS, whenever the aptamer binds the protein, the duplex denatures and releases the aptamer complementary DNA sequence into the solution, thus producing a detectable signal decrease. The "signal-off" aptasensor showed a detection limit of 0.1 pM in PBS buffer solutions and high specificity for LYS.

13.2.3.4 Electrochemical aptasensors for multiple protein detection

For the diagnosis and monitoring of diseases, often the detection of a single target analyte is not sufficient and the simultaneous detection of multiple targets is envisaged. Therefore new designs of electrochemical aptasensors for the simultaneous detection of multiple targets are required (Xiang et al., 2011).

Some strategies with this aim have been reported using, for example, a dual-functional aptamer (Min et al., 2010; Xie and Walton, 2010), an aptamer-cDNA that hybridizes with two aptamers (Zhao et al., 2012), or nanomaterials (Bai et al., 2012; Qian et al., 2010).

Zhao et al. (2012) reported a simple label-free "signal-on" electrochemical aptasensor for simultaneous determination of two tumor markers, MUC1 and VEGF in

standard solutions. In this aptasensor, the Fc-labeled aptamer-cDNA sequence was immobilized onto a gold disk electrode that hybridizes with both MUC1 and VEGF aptamers to form a long double-strand blocking the electrochemical signal. In the presence of the proteins, the hybridization reaction between the cDNA and the aptamers was inhibited and, Fc-cDNA was close to the electrode surface leading to an increase in the current that could be detected by SWV.

Based in a dual signal amplification strategy, Bai et al. (2012) reported a sandwich design for simultaneous detection of PDGF and THR in PBS buffer. The aptasensor was fabricated using AuNPs–SWCNTs as a biosensor platform to capture a large amount of primary aptamers and amplify the detection response. The redox probes toluidine blue (Tb) and Fc were attached onto the rGS coated with platinum nanoparticles (PtNPs–redox probes–rGS nanocomposites). The synthesized PtNPs–Tb/Fc–rGS nanocomposites were then used as carriers for glucose oxidase (GOD) and HRP and the aptamer-2 (PDGF- and THR-binding), respectively. Using DPV it was found that the linear range of PDGF was 0.01–35 nM with a detection limit of 8 pM, while the linear range was 0.02–45 nM with a detection limit of 11 pM for THR.

Recently, Cheng et al. (2015) reported a simple electrochemical aptasensor for multiple detection of THR and LYS proteins in Tris–HCl buffer. In this study, dabycyl-labeled aptamer modified metal nanoparticles (DLAPs) were immobilized on the β-cyclodextrins (β-CDs) modified gold electrode by means of host–guest recognition technique. During the detection, the aptamers' structure changes due to the specific binding with corresponding proteins that force DLAPs far away from the electrode (previously modified with β-CDs).

The DPV responses of the proposed aptasensor after incubation with different concentrations of both proteins showed a detection limit of 64 and 17 pM for THR and LYS, respectively. The reusable aptasensor showed good response, high specificity and practical applicability in complex biological samples.

13.3 Conclusions and future perspectives

This chapter discussed the versatility and broad range of applicability of electrochemical based-sensors, namely sensor arrays/E-tongues and aptasensors, for biomedical and pharmaceutical applications. The potential use of these low-cost, fast, and green bioinspired analytical techniques for medical purposes has been demonstrated in standard solutions, complex biological matrices, and in real samples.

Several design strategies for the development of electrochemical (bio)sensors have been presented, as well as their potential uses. The results reported in the literature offer a first clue about the feasibility of using electrochemical devices as tools for disease diagnosis and monitoring. However, nowadays, sensor arrays/E-tongue and/or aptasensors are not yet fully exploited as routine diagnosis techniques despite their promising suitability for biological applications. Transposing sensor devices from laboratory to real-world applications is one of the major challenges that must be addressed in the near future.

The success of such tasks may reside in the incorporation of new bioinspired sensor materials, which can increase selectivity and lifetime, together with the adaptation and development of new signal processing techniques that could minimize the need of chemical interpretation of sensor signals. For this purpose an interdisciplinary approach must be undertaken, by means of a collaborative effort of biologists, chemists, physicists, material scientists, and computer scientists. Although there is still a long way to go, the overall quality of the available reports and the consolidated current status of electrochemical sensors undercovers a strong driving force that might provide guidelines for the next years of research and for the application of E-tongues and aptasensors to biomedical purposes, as reliable measurement systems.

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