SENSORS

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Contents

Summary

The exciting role of electrochemical sensors and biosensors as efficient analytical tools in many fields of interest is addressed in this chapter. Starting from some fundamental principles, some examples showing latest trends in this area are presented and commented. Both potentiometric and amperometric and voltammetric sensors are considered, and the efforts to improve analytical characteristics such as selectivity, sensitivity and robustness, especially addressed. Moreover, electrochemical sensors using biological recognition systems, i.e, electrochemical biosensors, are also revised. Enzyme electrodes, immunosensors and DNA biosensors are considered, and some recent relevant examples are given to illustrate the potentialities of these electrochemical devices. Considerations on future developments and requirements of electrochemical sensors and biosensors are also commented.

1. Introduction

Chemical sensors can be defined as small devices that can be used for the direct measurement of an analyte in the sample matrix. Ideally, these devices should respond

to the analyte in a continuous and reversible manner without sample disturbance, thus avoiding the need for sample treatment or even sample uptake. Unfortunately, this ideal situation is very rarely achieved, and sample treatment is often necessary when complex real samples are analyzed.

Among chemical sensors, those based on electrochemical transducers are used in a large extent because they offer some important advantages such as:

1) The electrochemical measurements can be performed in small volumes, even of some few nanoliters, with relative easiness, which is due to the interfacial nature of such measurements. This makes such devices appropriate for "in vivo" monitoring.
2) The obtained signal is obviously electrical and, therefore, the direct transduction of the reaction rate in the readout signal is feasible.
3) The detection limits able to be obtained are usually sufficient and adequate for the detection of numerous analytes of interest.
4) The relative simplicity and low cost of the electrochemical instrumentation allows an easy availability of these devices.

Furthermore, the scarce selectivity associated to electrochemical techniques is strongly minimized when a recognition system with a high selectivity for certain analytes is used.

Although selectivity can be induced in electrodes in several ways, for example by localized electrode heating or by low-frequency sonication, this chapter will deal mostly with electrochemical sensors and biosensors in which a chemical or biochemical recognition element is immobilized or retained at the surface of an electrochemical transducer. This transducer allows the conversion of the chemical or biochemical reaction rate produced in the recognition event in a measurable response, which is further amplified, processed and converted to the readout signal. Figure 1 shows the generalized scheme of an electrochemical sensor.
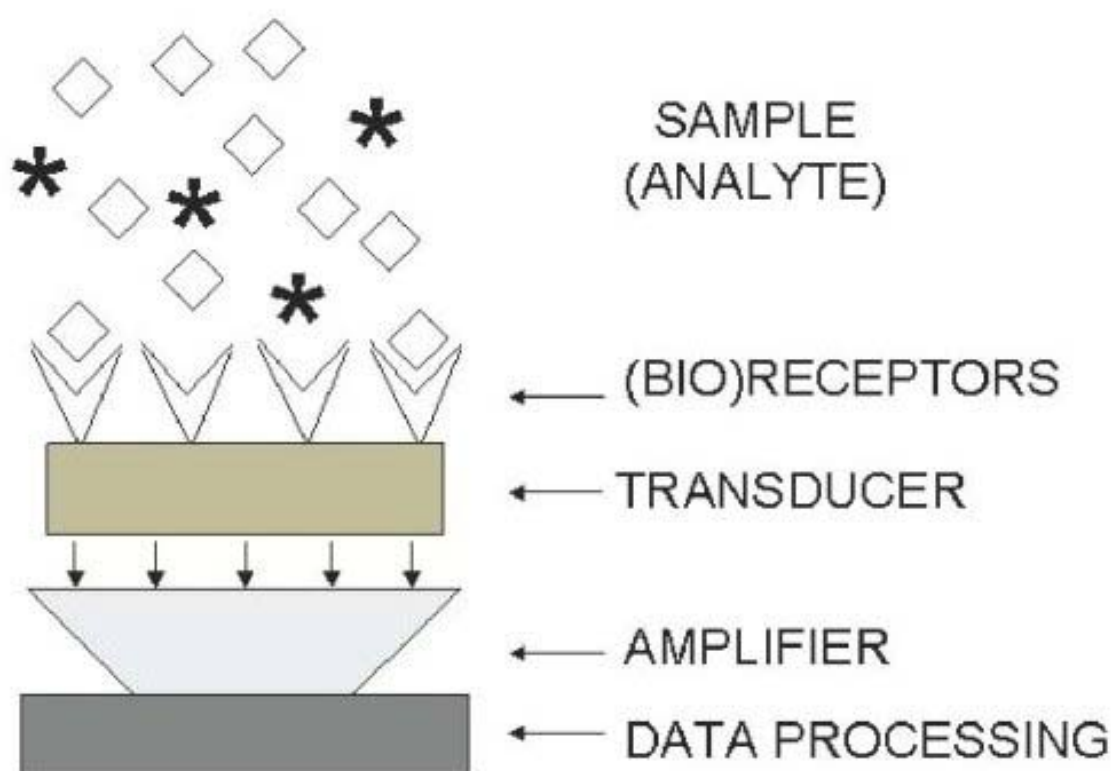
Figure 1. Scheme of an electrochemical sensor

Many classifications of electrochemical sensors are possible. In order to achieve simplicity, we have decided to distinguish between potentiometric or amperometric and voltammetric sensors, on the one hand, and between sensors that avoid the use of biological recognition elements, and those based on biological mechanisms or principles for the recognition of particular species (biosensors), on the other hand. Some basic fundamentals and selected examples will be given for each topic. Moreover, some comments and examples on impedimetric sensors will be also made.

2. Potentiometric Sensors

These sensors are based on the measurement of a potential difference between a working electrode and a reference electrode. This measurement can be related with the analyte concentration from the Nernst equation. Ion-selective electrodes (ISEs) is likely the most widely studied group of potentiometric sensors, which, in many cases, are fabricated with a membrane and provide potential readouts associated with the ion of interest. Potentiometric sensors are a popular class of analytical sensors that generally possess long lifetimes and acceptable mechanical stability. For example, they are used to perform billions of clinical measurements each year in practically every hospital throughout the world. The main appeal lies in the simple instrumentation, low cost, and suitability for continuous monitoring. Furthermore, they allow discrimination of free (ionized) and bonded (complexed) ions in a sample, as well as between the activities of the different oxidation states of a specific ion. The development of potentiometric sensors is nowadays a mature discipline which is mainly focused on reaching detection

limits orders of magnitude lower than previously thought possible, and making these devices more robust.

Potentiometric sensors can be classified according to the membrane composition. Ion-selective membranes are or contain species capable to interact selectively with analyte ions. The potential measured with these electrodes is a sort of junction potential formed in the membrane that separates both the analyte and the reference solutions.

2.1. Glass Electrodes

Glass electrodes respond selectively to univalent cations. The most widely used potentiometric sensor is undoubtedly the glass membrane pH electrode. (Figure 2). This is routinely employed in the analytical laboratories since various decades. When the electrode is immersed in the solution, an equilibrium is established across the membrane with respect to the hydrogen ions in the internal and external solutions. Glass hygroscopicity determines the response capacity of membrane in the solution, because is in the hydrated glass where the exchange between the alkaline ions in the membrane and protons in the solution is established. So, the conditioning of the glass membrane in the aqueous electrolyte solution plays an important role on the formation of a hydrated gel layer and subsequently on the electrochemical properties of the pH sensor. The activities of hydrogen ion in the inner and outlet solutions from the membrane determine a potential, whose magnitude is a function of the ratio between the activities of both solutions.

The pH-sensitive membrane consists of a three-dimensional silicate network with i.e. a composition as 72% SiO_2-22% Na_2O-6% CaO (Corning Glass 015). These electrodes provide a specific response to hydrogen ion up to pH 9, approximately.
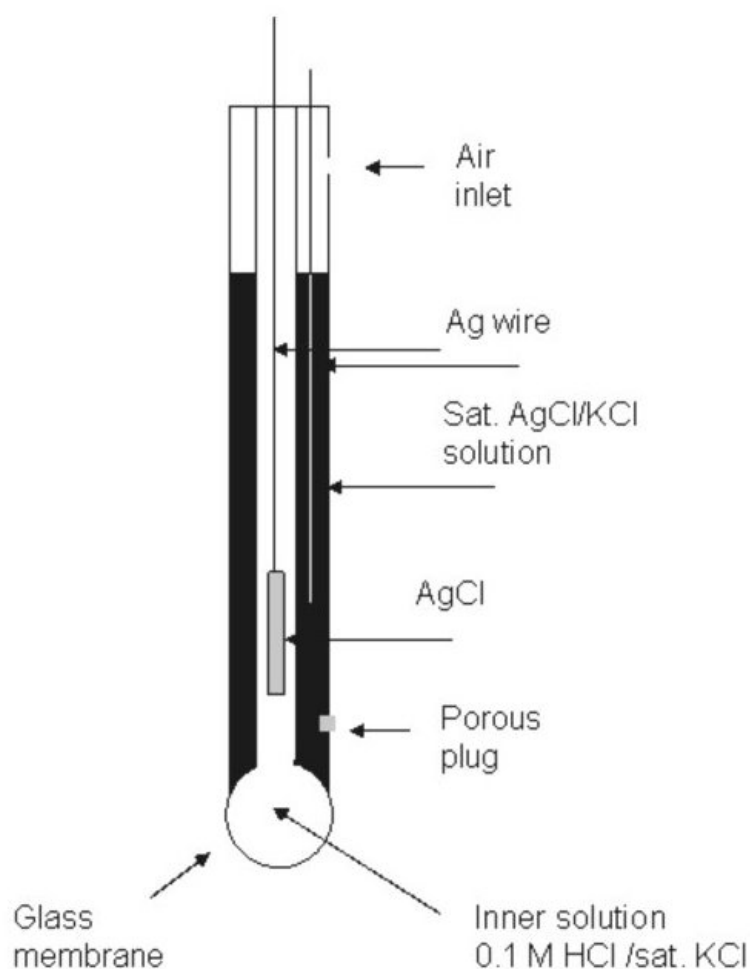
Figure 2. Glass membrane pH electrode

At higher pHs, the glass begins to give a certain response to Na^+ and other monovalent ions. The limitations of this sensor, mainly due to the alkaline and acid errors, have been tried to be avoided through the substitution of the glass by a variety of organic compounds which are carriers of hydrogen ion (non-glass pH sensors). The resulting electrodes exhibit excellent selectivity, reproducibility and accuracy, but their dynamic range is lower than that of glass electrodes. A variety of compounds have been tested as carriers, such as amines and ethers derived from nicotinic acid. For example, several amine derivatives capable of being protonated and also of giving a pH-sensitive response have been investigated. Among them, methyldioctylamine (MDODA) was the first compound studied for that purpose. Later, in order to obtain a carrier that gave a nernstian response over a wide pH range, other compounds with functional groups appropriated to provide responses in both acid and alkaline regions, were synthesized. For example, 4,4´-bis(N,N-dodecylamine methyl azobenzene (DAMAB) made it possible to obtain a wide linear response (pH 1.7 - 13.2), with a slope value of 57.4 mV pH^{-1}. Electrodes chemically modified with amine derivatives have been also prepared with the aim of being used as pH-sensors, and allowing an easy miniaturization.

Ion-selective electrodes for other monovalent ions than protons have been prepared by changing the membrane composition and replacement of the inner solution. For example, sodium and ammonium selective glass-membranes have been constructed from aluminosilicate glasses.

2.2. Crystalline-based Electrodes

These solid-state electrodes constitute another important group of potentiometric sensors which undergo ion-exchange reactions at the membrane/solution interface. Classes of membranes are: (a) single crystal, (b) pressed powder and (c) powdered salts held by an inert binder.

Fluoride determination in aqueous solutions making use of a membrane constructed with a lanthanum fluoride (LaF_3) single crystal is a classical example of these potentiometric sensors. This single-crystal device is by far the most successful anion-selective electrode. The crystal is doped with EuF_2 to provide vacancies at anionic sites, and the movement of fluoride by migration through the crystal lattice establishes the potential difference with a Nernstian response $E = K-0.059 \log a_{F^-}$ at concentrations down to micromolar level. The only interfering ion is OH^- because of its similarity in size and charge with fluoride. Thus, the electrode must be used at pH values lower than 8.5.

Solid-state membranes prepared with precipitate-based systems have been used for the potentiometric determination of a number of cations and anions. Silver sulfide mixed with other silver salts, mainly halides, allow the preparation of selective electrodes for the anions forming part of the precipitate. In this case, the mobility of Ag^+ determines the ionic conduction. Furthermore, Ag_2S mixtures with CdS, CuS or PbS provide membranes that are responsive to cadmium, copper or lead ions, respectively. Jalpaite (coprecipitated CuS/Ag_2S) membranes allowed the preparation of a selective electrode for Cu^{2+} adequate for the determination of this ion in sea water with a detection limit at the nanomolar level. This type of solid membranes have been also employed for the determination of Pb^{2+} traces in drinking water, and are useful for speciation of free lead and lead carbonate as well as for biodisponibility studies. A solid-state membrane selective electrode for cadmium has been used for the evaluation of Cd^{2+} ion consumption in cell cultures.

Ion-selective electrodes consisting of chalcogenide glass membranes are used for the determination of metal ions and have been applied for environmental and industrial monitoring. The potential generating process of a chalcogenide glass electrode derives from the formation of a modified surface layer with ability to undergo ion exchange. This layer is probably formed during the conditioning of the chalcogenide membrane in an electrolyte that contains the primary ion. Relevant examples of these electrodes are the development of a portable sensor for real-time detection of iron based on a free Fe^{3+}-selective chalcogenide membrane, $Fe_{2.5}Se_{58.5}\ Ge_{27..3}Sb_{11.7}$, which was applied to the study of the biological cycle of this element in sea water, and a mercury(II) ISE.

Mixed salts can be also suspended on an inert support such as silicone rubber. These configurations are well established for several cations and anions, but little research has

been performed to achieve lower limits of detection, which depend on the solubility product of the materials.

2.3. Liquid Membrane Electrodes

In these configurations, a water-immiscible liquid substance is impregnated in a polymeric membrane, commonly constituted of PVC. This membrane separates the measurement solution from the inner compartment as it is displayed in Figure 3. In this compartment, a silver-silver chloride reference inner electrode is immersed into a stock solution of the ion to be detected, usually in the form of a chloride salt.
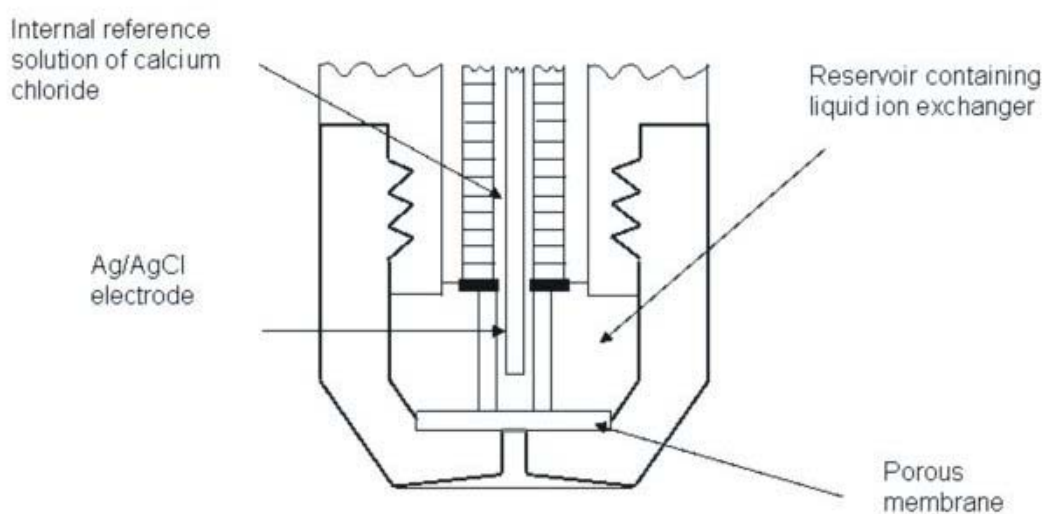

Figure 3. Schematic diagram of a calcium-ion-selective electrode

The active or recognizing membrane element can be an ionic exchanger or a macrocyclic neutral compound. Ions discrimination depends on the nature of the recognition element and the exact composition of the membrane. These ion selective electrodes are particularly important because they allow the direct determination of a fair amount of polyvalent cations as well as various anions. A disadvantage comes from the leaching of the ion present in the inner solution which gives rise to an increase of the activity at the layer adjacent to the membrane, with the consequent increment in the detection limits. The localized accumulation of ions makes impossible the measurement of very diluted samples. In order to avoid this, complexing agents such as EDTA have been added to the inner solution, or a small external current has been applied thus generating a steady flow of cations towards the external compartment of the electrode. One of the most used liquid membrane ion selective electrodes is the calcium electrode (Figure 3). It is based on the ability of phosphate ion to form stable complexes with Ca^{2+}, and uses a liquid cationic exchanger consisting of a phosphoric acid diester, $(RO)_2PO_2^-$, where R are aliphatic groups of 8 to 16 carbon atoms. Calcium ion activities as low as 5×10^{-7} M can be determined with this sensor.

Ion exchangers composed of quaternary ammonium or phosphonium ion salts are employed for the preparation of anion selective sensors. The selectivity of these

electrodes is governed by ion lipophilicity, this giving a selectivity order which is known as the "Hofmeister" series. In this series, large lipophilic anions give maximum response; in decreasing order are, among other anions:

$$ClO_4^- > SCN^- > I^- > NO_3^- > Br^- > ClClO_4^- > SCN^- > I^- > NO_3^- > Br^- > Cl^- > HCO3^- > HCO_3^-$$

So, for example, nitrate selective electrodes suffer interference from perchlorate ions. Recent efforts have been made to find ion carriers that possess specific chemical interaction with the ion of interest, showing an "anti-Hofmeister" response sequence. Neutral carriers such as metallophorphyrins or macrocyclic polyamines offer selective sensing for inorganic and organic anions which differs greatly from the Hofmeister sequence. Sometimes, the interaction with the specific anion involves an exchange of the anion coordinated with the metal center of the organometallic ionophore, with the target anion in the sample solution. In these applications, the role of the central metal is very important. For example, the indium complex of chloro[5,10,15,20-tetra(p-methoxyphenylporphirinate] has a good selectivity for nitrate, allowing a detection limit of 5.1×10^{-6} M. However, the similar complex with aluminum does not respond adequately to this ion. The higher thermal stability and easiness to be synthesized are two important advantages of metal phtalocyanines to be used in this type of applications, and sensors based on these compounds have shown selective response towards some organic anions. For example, a tin(IV)-phtalocyanine complex has been recently used for the potentiometric detection of salicylate.
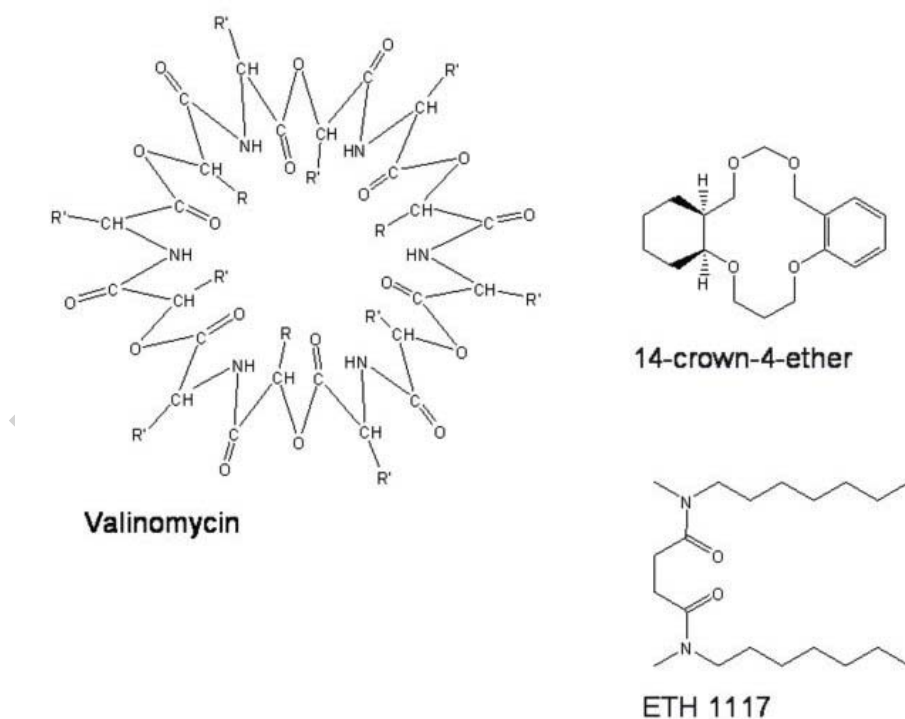

Figure 4. Structures of neutral carriers used in liquid-membrane ion-selective electrodes: valinomycin for K^+; 14-crown-4-ether for Li^+; ETH 1117 for Mg^{2+}

Natural macrocyclic molecules or synthetic cyclic polyethers (crown compounds) have been also used as neutral carriers, these giving an interaction with the analyte ion facilitated by the electron donors present in the polar host cavity (see Figure 4). Most of

them have been used for the recognition of alkaline cations which constitute clinically relevant electrolytes A very popular configuration is the valinomycin ISE, which is usually employed for clinical analysis of blood potassium. This electrode gives a Nernstian response to K^+ activity from 10^{-6} to 0.1 M . Other organic carriers have been also used. For example, the 14-crown-4-ether allows the preparation of highly selective lithium electrodes with no interference from sodium, to measure the levels of this ion in biological fluids from psychiatric patients treated with lithium drugs.

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Biographical Sketches

José M. Pingarrón obtained his Ph.D. (1981) from Complutense University of Madrid. Between 1982 and 1983, he did postdoctoral training at the École Nationale Supérieure de Chimie de Paris. Since 1994, he is a full Professor of Analytical Chemistry at the Complutense University of Madrid. José M. Pingarrón headed the Department of Analytical Chemistry at the Faculty of Chemistry between 1998 and 2006 and he was the President of the Spanish Society of Analytical Chemistry between 1998 and 2001. Professor Pingarrón is the recipient of the Faculty of Chemistry Medal and the Complutense University of Madrid Medal. His research interests focus on analytical electrochemistry, nanostructured electrochemical interfaces and electrochemical and piezoelectric sensors and biosensors. He has over 200 publications including peer-reviewed papers, book chapters and text books. He currently belongs to the government body of the Spanish Royal Society of Chemistry and is its representative in the Division of Analytical Chemistry of the European Association for Chemical and Molecular Sciences. Professor Pingarrón belongs to the Editorial Advisory Boards of the Journal of Electroanalytical Chemistry, Talanta and Electroanalysis. Related links: http://www.ucm.es/info/analitic

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