# Chapter 7
# Electrochemical Sensor and Biosensors

**Cecilia Cristea, Veronica Hârceagă, and Robert Săndulescu**

## 7.1 General Sensor Concept

From the basic researches of the electrochemistry parents, Volta, Galvani, Sir Humphry Davy, and Faraday, a long period of silence followed in this interdisciplinary field that ends only at the beginning of the twentieth century with the invention of the glass electrode by M. Cremer and the discovery of polarography by J. Heyrovsky. That was the moment when modern electrochemistry was born. The research boom, which leads to the elaboration of the most well-known electrochemical methods and main types of electrodes, continues even today. Electrochemical methods (potentiometry, voltamperometry, and conductimetry) experienced a huge development due to their multiple advantages that they offer, mainly the high sensitivity that allows detection of concentrations in the range of $10^{-8}$–$10^{-10}$ M especially for differential pulse, square wave voltammetry, and stripping techniques.

At the same time the major disadvantage of the electrochemical methods became obvious: lack of selectivity. Practically, all the electroactive species can be reduced or oxidized from a sample or from a matrix and the simultaneous detection in the same sample is possible only in the case when two species possess redox potentials sufficiently separated in the investigated domain of potential. The reduced selectivity was the main issue that pointed the researchers' attention towards the delicate area of the electrode surface, where essential phenomena take place and trigger the race that still continues today having the goal of increasing the selectivity (specificity) for certain analytes. A new domain has been born, the field of modified electrochemical sensors. There are several possibilities today to modify the electrode material or its surface; the general strategies of electrochemical sensor technology will be discussed later.

---

C. Cristea • V. Hârceagă • R. Săndulescu (✉)
Analytical Chemistry Department, Faculty of Pharmacy, "Iuliu Haţieganu" University
of Medicine and Pharmacy, 4 Louis Pasteur St., 400349 Cluj-Napoca, Romania
e-mail: rsandulescu@umfcluj.ro

L.M. Moretto, K. Kalcher (eds.), *Environmental Analysis by Electrochemical Sensors and Biosensors*, DOI 10.1007/978-1-4939-0676-5_7

According to IUPAC[1] a chemical sensor is "a device that transforms chemical information, originating from a chemical reaction of the analyte or from a physical property of the investigated system, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal."

Generally, chemical sensors contain two basic functional units connected in series: a chemical (molecular) recognition system, named *receptor*, which transforms the chemical information into a measurable form of energy, and a physico-chemical *transducer* capable of transforming the energy carrying the chemical information about the sample into a useful analytical signal.[1]

A modern sensor system generally incorporates besides the receptor and the transducer other two key components: a sample delivery unit and a data processor.[2]

The receptor part of the chemical sensors is based on three various basic principles of stimulus: *physical*, where no chemical reaction takes place (e.g., measurement of absorbance, refractive index, conductivity, temperature or mass change); *chemical*, in which a chemical reaction with participation of the analyte gives rise to the analytical signal; and *biochemical*, in which a biochemical process is the source of the analytical signal (e.g., enzyme amperometric sensors, microbial potentiometric sensors, or immunosensors).[1] The last category consists of the well-known biosensors and they can be differentiated according to the biological elements used as receptor, namely enzymes, nucleic acids, aptamers, antibodies, organelles, membranes, cells, tissues, or even whole organisms.

The main function of the receptor is to provide the sensor with a high degree of selectivity for the analyte to be measured. While most chemical sensors are more or less selective (specific) for a particular analyte, some are, by design and construction, only class specific, e.g., sensors or biosensors for phenolic compounds, or whole-cell biosensors used to measure the biological oxygen demand.

The second part of a sensor, the transducer, serves to transfer the signal from the output domain of the recognition system into an output signal (usually electric) which is then amplified by the electronics and converted into useful data.[3]

According to the operating principle of the transducer, chemical sensors may be classified in optical, electrochemical, electrical, mass-sensitive, magnetic, and thermal sensors or sensors based on other physical properties such as radioactivity. The transducer part is responsible for the sensitivity of the device.

1. Optical devices transform changes of optical phenomena, which are the result of an interaction of the analyte with the receptor part. This group may be further subdivided according to the type of optical properties which have been applied in the chemical sensors: absorbance, reflectance, luminescence, and fluorescence, refractive index including the surface plasmon resonance effect, the optothermal effect, and light scattering.
2. Electrochemical devices transform the effect of the electrochemical interaction which takes place between the analyte (or the generated species from the interaction of the analyte and a biological compound) and the electrode into an exploitable electric signal (current or potential). Such effects may be stimulated

electrically or may result in a spontaneous interaction at zero-current conditions. The following subgroups may be distinguished:

- Voltammetric sensors, including amperometric devices, in which a current is measured in direct or alternating current mode. This subgroup may include sensors based on chemically inert electrodes, chemically active electrodes, and modified electrodes. In this group are included also sensors with and without (galvanic sensors) external current source.
- Potentiometric sensors, in which the potential of the indicator electrode (ion-selective electrode, redox electrode, metal/metal oxide electrode) is measured against a reference electrode.
- Chemically sensitized field effect transistor in which the effect of the interaction between the analyte and the active coating is transformed into a change of the source-drain current. The interactions between the analyte and the coating are, from the chemical point of view, similar to those found in potentiometric ion-selective sensors.
- Potentiometric solid electrolyte gas sensors, differing from classical potentiometric sensors because they work with high-temperature solid electrolytes and are usually applied for gas-sensing measurements.

3. Electrical devices based on measurements, where no electrochemical processes take place, but the signal arises from the change of electrical properties caused by the interaction with the analyte. This group can be subdivided into metal oxide semiconductor sensors, organic semiconductor sensors, electrolytic conductivity sensors, and electric permittivity sensors.
4. Mass-sensitive devices transform the mass change at a specially modified surface into a change of a property of the support material. The mass change is caused by accumulation of the analyte. This group has two subgroups: piezoelectric devices and surface acoustic wave devices.
5. Magnetic devices based on the change of paramagnetic properties of a gas being analyzed. These are represented by certain types of oxygen monitors.
6. Thermometric devices based on the measurement of the heat effects of a specific chemical reaction or adsorption which involve the analyte. Examples of this group are the so-called catalytic sensors. The devices based on measuring optothermal effects can alternatively be included in this group.
7. Other physical properties as for example X-, β-, or $I^-$-radiation may form the basis for a chemical sensor in case they are used for determination of chemical composition.[1]

Among the different classes of transducers employed in sensor and biosensor construction, the electrochemical transducers are the most used ones. They occupy the first position as far as their disponibility on the market is concerned and they have already demonstrated their practical utility. Electrochemistry has superior properties in comparison to other measurement systems because of the rapid, simple, and sensitive characteristics.[4]

The transducer part of an electrochemical sensor is also called a detector, sensor, or electrode, but the term transducer is preferred to avoid confusion.

In case of electrochemical sensors with respect to homogeneous monolithic electrodes, the electrode material fulfilled both roles, as receptor and transducer, but later the run for more selective surfaces has focused the attention of electrochemists to the electrode surface, which is in fact the receptor and is responsible for the sensor's selectivity.

In addition to the classifications of sensors according to the operation principle of the receptor or the transducer part, they can also be classified based on the analyte to be measured: sensors for pH, for metal ions, or for oxygen or other gases, or based on the mode of application: for use in vivo, for process monitoring, etc.

Taking into consideration the abovementioned criterions, attention must be paid to avoid confusion between the terms biosensor and chemical and physical sensors. A biosensor, which by definition incorporates a biological compound, can be used to monitor either biological or non-biological matrices. Chemical sensors, which incorporate a non-biological specificity-conferring part, although used for monitoring biological processes, such as the in vivo pH or oxygen sensors, are strictly speaking not biosensors. Similarly, physical sensors used in a biological environment, even electrically based, such as in vivo pressure or blood flow sensors, are also excluded from the class of biosensors.[5]

## 7.2 Comparison with Biological Sensors

All living organisms contain biological sensors with functions similar to those of the electrochemical devices described above. Generally speaking a sensor, either electrochemical or biological, is a device which receives and responds to a signal when activated.

Most of the biological sensors are specialized cells that are sensitive to a stimulus that can be light, motion, temperature, magnetic field, gravity, humidity, moisture, vibration, pressure, electrical field, sound, and other physical aspects of the external environment, physical aspects of the internal environment (stretch, motion of the organism), environmental molecules (toxins, nutrients, pheromones), internal metabolic milieu (glucose level, oxygen level, osmolality), internal signal molecules (hormones, neurotransmitters, cytokines), and differences between proteins of the organism itself and of the environment or alien creatures.

Starting from this similarity the word receptor or bioreceptor, used for the recognition system of a chemical sensor or biosensor, comes from the sensing systems present in living organisms or systems where the actual recognition is performed by a cell receptor.

Also the term sensitivity can be used for both electrochemical and biological sensors having the same meaning. It indicates how much the sensor's output changes when the measured quantity of the stimulus changes.

Having very similar operating mode and design there are even electrochemical sensors described in the scientific literature called electronic nose[6] or electronic tongue.[7,8]

Nowadays, the term biological sensor is used extensively in the scientific literature. Apart from its meaning as sensing organ in living organisms some authors use it to define sensors that measure a biological component either in vivo or in vitro or for sensors that contain in their structure a biological component as receptor, actually the biosensors.[9]

## 7.3 The Importance of Sensors in Analytical Chemistry

Electrochemical sensors are an important domain of modern analytical chemistry. Understanding sensor devices requires some knowledge of a variety of academic areas. This leads to a very interdisciplinary field populated by physicists, chemists, engineers, biologists and biochemists, materials scientists, electrochemists, and others.

There are many examples of the topics of application of sensors including pharmaceutics, medicine, analytical sciences, synthetic chemistry, biotechnology, materials sciences, and (bio)molecular engineering.

Electrochemical sensors and detectors are very attractive for on-site monitoring of priority pollutants, as well as for addressing other environmental needs. Such devices satisfy many of the requirements for on-site environmental monitoring and have already made a significant impact also on decentralized analysis. Electrochemical sensors can be used for process line monitoring across a range of simultaneous measurement points, bundling different levels of information into the decision-making process.

The possibility of miniaturization and the use of these devices at the so-called point of care or point of action is a strong driver for innovation. This is of great importance especially for biological samples because they generally are available in small amounts and tissue damage must be minimized in case of in vivo monitoring.

A special advantage of sensors for analytical purposes is the possibility of automatization and mass production of miniaturized devices.

Electrochemical sensors have major advantages over traditional analytical methods, which will certainly lead to their even more pronounced use in the near future. They are attractive analytical devices due to their inherent sensitivity and selectivity towards electroactive species, sometimes even due to specificity, accurate and short response times, adaptability, and simplicity of preparation. They are compact, portable, and easy to use; they have a high benefit/cost ratio and present quickness in data collection.

However, many sensors described in the recent literature still display a few drawbacks when compared to other analytical methods. The most difficult problems to overcome are electrochemical interferences in complex sample matrices. Anyway this can be still optimized through different design techniques.

The choice of a suitable working principle and design as well as the layout and constituent materials of a sensor package depends very much on the requirements of sensitivity, selectivity, portability, multivariant or single-use detection, and the specific field of application.[10]

## 7.4 General Strategies of Electrochemical Sensor Technology

In order to construct a successful sensor or biosensor a number of conditions must be met:

1. The receptor must be highly specific for the purpose of the analysis, be stable under normal storage conditions, and show a low variation between assays.
2. The reaction should be as independent as manageable of physical parameters such as stirring, pH, and temperature. This will allow analysis of samples with minimal pretreatment. If the reaction involves cofactors they should, preferably, also be co-immobilized with the receptor.
3. The response should be rapid, accurate, precise, reproducible, and linear over the concentration range of interest, without dilution or preconcentration. It should also be free from electrical or other transducer-induced noise.
4. The method should be sensitive and have limits of detection and quantification adequate to the concentration of the analyte, which is in many cases very low.
5. The complete sensor should be inexpensive, small, portable, automated, and capable of being used by semiskilled operators.
6. For rapid measurements of analytes it is desirable that the sensor can provide in situ real-time results.
7. In case of biosensors for invasive monitoring in clinical situations, the device must be tiny and biocompatible, having no toxic or antigenic effects. Furthermore, the sensor should not be prone to inactivation or proteolysis.[11]

The systematic strategy for designing sensors should consider five features: (1) the detected or measured parameter, (2) the working principle of the transducer, (3) the physical and chemical/biochemical model, (4) the practical application, and (5) the available technology and materials for sensor fabrication.

The selection of materials and fabrication techniques is crucial for an adequate sensor function and the performance of a sensor often ultimately depends upon these factors. Consequently, future developments in sensor design will inevitably focus upon the technology of new materials. Materials used in electrochemical sensors are classified as (1) materials for the electrode and supporting substrate (metals: platinum, gold, silver, and stainless steel; carbon-based materials: graphite, carbon black, and carbon fibre; new mixed materials; or organic electroconductive polymers or salts); (2) materials for improved sensitivity and selectivity (especially nano-sized materials: nanoparticles and carbon nanotubes); (3) materials for the immobilization of biological recognition elements (multifunctional agents: glutaraldehyde and hexamethyl diisocyanate or alternatively non-conductive polymers: polyacrylamide and polyphenol); and (4) biological elements (enzymes, antibodies, antigens, mediators, and cofactors); the last two are applicable for electrochemical biosensors.[12]

Nanomaterials are acquiring a big impact on the development of electrochemical sensors as they bring new possibilities for developing novel electrochemical assays. Nanomaterials can be divided into two categories concerning their utility: nanoscale materials for electrode construction or modification and nanomaterials as tracers for biomolecules. Modified electrodes with nano-sized materials offer the advantages of better sensitivity and selectivity and shorter response time. Nanomaterials have been widely used as biomolecule tracers for electrochemical bio-sensing. Nanoparticles are very stable (compared to enzyme labels); they offer high sensitivity (thousands of atoms can be released from one nanoparticle) and a wide variety of them are available on the market. Nanoparticles are used nowadays as electrochemical labels or as vehicles containing several hundreds or thousands of electroactive labels, pushing the detection limits down to several hundreds of biomolecules.[13]

One of the most important steps in case of constructing electrochemical biosensors is the optimal immobilization of the biocomponent at the surface of the electrode. This optimum immobilization should assure a maximum quantity of bioreceptor immobilized or, more appropriately, a maximum number of functional reactive active sites immobilized in a unity of immobilization substrate as well as its stability and its efficacy.

General strategies of electrochemical sensor technology will cover the development and discovery of new biological molecules and systems, aspects of immobilization and stabilization, micro- and nano-fabrication technologies, challenges associated with measuring signals generated by bio-sensing systems, issues associated with integrating technologies to produce a functioning bio-sensing system, technical interfacing challenges such as sample introduction and handling through to aspects of commercialization, and adoption of bio-sensing technology into chosen markets.

## 7.5 Current Trends and Future Prospects

The requirements and regulations in the fields of environmental protection, control of biotechnological processes, and certification of food and water quality are becoming increasingly urgent. At the same time stricter requirements regarding human and animal health have led to a rising number of clinical and veterinary tests. Therefore, there is a need in developing highly sensitive, fast, and economic methods for detection, quantification, and monitoring analysis. The elaboration of electrochemical sensors is probably one of the most promising ways to solve some problems concerning sensitive, fast, simple, cost-effective, and repetitive measurements with miniaturized and portable devices.

The developed sensors are mainly used in the following three ways:

1. As “off-line” detectors, when samples are taken from the investigated medium and injected in the measuring chamber of the sensor which reads the concentration of the analyte of interest.
2. As “in vivo” detectors, when the sensor is implanted in a living organism and continuously reads the concentration of the analyte of interest.
3. As “online” detectors, when sensors that measure the concentration of the analyte of interest are integrated into a flow line system.[14]

The area of electrochemical sensor research is very active and fruitful. It must be emphasized that most of the challenges in this field remain in the area of selectivity. This may be overcome by their integration into more complex analytical systems that combine online sampling and separation steps or the use of modified electrodes with highly selective chemical or biological recognition layers. However, in cases where direct detection in unmodified samples is possible, the high analysis speed and the capability of detecting extremely small volumes and low concentrations without significantly perturbing the sample remain highly attractive characteristics of electrochemical sensors.[15]

On the other hand, nowadays, the general demands required for analytical systems are extended to multianalyte sensing. Thus, tremendous efforts are focusing on the development of multianalyte assays with the advantages of short analysis time, simplified analytical procedure, decreased sampling volume, improved test efficiency, and reduced cost as compared to parallel single-analyte assays. In this context modified sensors could be promising analytical tools.[16]

The trend of using novel materials in electrochemical sensing systems for improved selectivity and sensitivity is constantly increasing, with their success largely due to the continuous design and development that meets the needs of modern electrochemical (bio)sensor technology. Materials ranging from carbon composites, beads or microspheres, molecular imprinted polymers, or quantum dots are playing an important role in these sensing systems. Nanomaterials (e.g., nanoparticles and CNTs) are the core of an emerging technological revolution. The main advantages of these materials are unique thermal, mechanical, electronic, and biological properties not found in conventional materials. Combining these outstanding properties with their remarkable recognition capabilities has resulted in systems with significantly improved performance for analytical applications. Most of the exceptional characteristics of nanomaterials are linked to their surface properties (area, roughness, energetic characteristics, and electron distributions) which enable improved interactions with many chemical and biological entities. These characteristics result in improved stability and selectivity of nanomaterials, and finally of the whole electrochemical sensor device.[17]

The electrochemical sensors are adaptable also to flow injection systems that have the advantages of high sample throughput.[18]

One of the main challenges faced nowadays by the analytical chemist is the development of methods that respond to the growing need to perform rapid "in situ" analyses. The advancement in miniaturization and microfabrication technology has led to the development of sensitive and selective electrochemical devices for field-based and *in situ* environmental monitoring. Electrochemical sensing devices have a major impact upon the monitoring of priority pollutants by allowing the instrument to be taken to the sampling site (rather than the traditional way of bringing the sample to the laboratory). Such devices can perform automated chemical analyses in complex matrices and provide rapid, reliable, and inexpensive measurements of a variety of inorganic and organic pollutants.[19]

Some other expectations of the nowadays practice is the development of analytical devices that are able to monitor in real time in vivo parameters. In this case electrochemical biosensors have played an important significant role in the transition towards point-of-care diagnostic devices. Such electrical devices are extremely useful for delivering the diagnostic information in a fast, simple, and low-cost fashion in connection with compact (handheld) analyzers. Such modern electrochemical bioaffinity, DNA, or immunosensors have been developed with remarkable sensitivity essential for early cancer detection.[20]

One of the most important driving forces for research in many areas of modern analytical chemistry is miniaturization, simplification, automation, and computerized instrumentation of the whole analytical procedure, the so called lab-on-a-chip or micro total analysis (μTAS) technology. The speed for generating the results (high throughput), the amount of information (simultaneous or multiparametric), and the autonomy (portability) are obvious advantages. Other characteristics of miniaturized systems are the simplicity of use and the cost efficiency, combined with small reagent consumption and small waste generation. Recent advances in electrochemical sensor technology include the introduction of microfabrication and ultramicroelectrodes. Although exciting to contemplate, electrochemical or other types of "chips" have not been widely accepted yet in the commercial area. So far these techniques have neither demonstrated the compelling, cost-effective benefits required to displace current technology or workflow nor have they shown the ease of use needed to induce users to change. The major benefit claims of minimum sample requirement and solvent and reagent savings from these approaches seem however encouraging.[21]

Another reason to consider electrochemical sensors as attractive alternative in modern analytical chemistry is the possibility of mass production, the flexibility of the design, and the simplicity of manufacturing especially if we think of screen-printed electrodes.

Originally the biological recognition element in case of electrochemical biosensors was assumed to be isolated from a living system, but now there are prospects of synthesizing this component or employing genetically modified enzymes to increase some specific features of the biorecognition-based assays such as sensitivity, selectivity, and stability. In order to improve these experimental parameters genetic engineering should emphasize two main fields for biosensors—genetically transformed cells and genetically engineered receptor molecules.

Genetic modification already showed the potential for selection of enzyme variants that are specific for a range of individual compounds. Even more novel gene fusions have also resulted in more sensitive biosensors.[22]

Transferability of the analytical data is related to the appropriate analytical validation in order to provide robust and standard operational procedures for practical use in routine analytical work or in control programs. In case of electrochemical sensors the validation step is still a hurdle to be overcome that requires additional further works.[23]

Sensor science generates thousands of new publications each year. Undoubtedly, the development of sensor applications, in the course of these last years, is and has to remain multidisciplinary with research and engineering opportunities straddling across chemical engineering, biology, chemistry, physics, materials, processing science, surface science, information science, and other engineering disciplines, so the innovative ideas described in the scientific literature will reach the marketplace in the future.

## References

1. Hulanicki A, Glab S, Ingman F (1991) Chemical sensors. Definitions and classification. Pure Appl Chem 63(9):1247–1250
2. Potyrailo RA, Mirsky VM (2009) Introduction to combinatorial methods for chemical and biological sensors. In: Potyrailo RA, Mirsky VM (eds) Combinatorial methods for chemical and biological sensors. Springer, New York, NY
3. Chaubey A, Malhotra BD (2002) Mediated biosensors. Biosens Bioelectron 17:441–456
4. Wang J (1999) Amperometric biosensors for clinical and therapeutic drug monitoring: a review. J Pharm Biomed Anal 19:47–53
5. Thevenot DR, Toth K, Durst RA, Wilson GS (2001) Electrochemical biosensors: recommended definitions and classification. Biosens Bioelectron 16:121–131
6. Stella R, Barisci JN, Serra G, Wallace GG, Rossi D (2000) Characterisation of olive oil by an electronic nose based on conducting polymer sensor. Sensor Actuator B Chem 63(1–2):1–9
7. Peris M, Escuder-Gilabert L (2009) A 21st century technique for food control: electronic nose. Anal Chim Acta 638(1):1–15
8. Woertz K, Tissen C, Kleinebudde P, Breitkreutz J (2011) Taste sensing systems (electronic tongues) for pharmaceutical applications. Int J Pharm 417(1–2):256–271
9. Kang YR, Park EJ, Kim JH, Min NK, Kim SW (2010) Development of bio-nanowire networks using phage-enabled assembly for biological sensor application. Talanta 81(4–5):1425–1430
10. Luttge R (2011) Chapter 6 chemical and biological sensors at component and device level. In: Luttge R (ed) Microfabrication for industrial applications. Elsevier, Amsterdam, pp 179–198
11. Grieshaber D, MacKenzie R, Voros J, Reimhult E (2008) Electrochemical biosensors - sensor principles and architectures. Sensors 8:1400–1458
12. Zhang S, Wright G, Yang Y (2000) Materials and techniques for electrochemical biosensor design and construction. Biosens Bioelectron 15(5–6):273–282
13. Pumera M, Sanchez S, Ichinose I, Tang J (2007) Electrochemical nanobiosensors. Sensor Actuator B Chem 123(2):1195–1205
14. Castillo J, Gáspár S, Leth S, Niculescu M, Mortari A, Bon□idean I, Soukharev V, Dorneanu SA, Ryabov AD, Csöregi E (2004) Biosensors for life quality: design, development and applications. Sensor Actuator B Chem 102:179–194
15. Bakker E (2004) Electrochemical sensors. Anal Chem 76:3285–3298

16. Wu J, Zhang Z, Fu Z, Ju H (2007) A disposable two-throughput electrochemical immunosensor chip for simultaneous multianalyte determination of tumor markers. Biosens Bioelectron 23(1):114–120
17. Scida K, Stege PW, Haby G, Messina GA, Garcia CD (2011) Recent applications of carbon-based nanomaterials in analytical chemistry: critical review. Anal Chim Acta 691:6–17
18. Junqueira JRC, Araujo WR, Salles MO, Paixao TRLC (2013) Flow injection analysis of picric acid explosive using a copper electrode as electrochemical detector. Talanta 104:162–168
19. Hanrahan G, Patil DG, Wang J (2004) Electrochemical sensors for environmental monitoring: design, development and applications. J Environ Monit 6:657–664
20. Wang J (2006) Electrochemical biosensors: towards point-of-care cancer diagnostics. Biosens Bioelectron 21(10):1887–1892
21. Rios A, Zougagh M, Avila M (2012) Miniaturization through lab-on-a-chip: Utopia or reality for routine laboratories? A review. Anal Chim Acta 740:1–11
22. Campas M, Prieto-Simon B, Marty JL (2009) A review of the use of genetically engineered enzymes in electrochemical biosensors. Semin Cell Dev Biol 20:3–9
23. Farre M, Kantiani L, Perez S, Barcelo D (2009) Sensors and biosensors in support of EU directives. Trends Anal Chem 28(2):170–185