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ENZYMATIC ELECTROCHEMICAL BIOSENSORS AND IMMUNOSENSORS

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ABSTRACT

Electrochemical biosensors have stood out as analytical devices for applications in health, environment, food industry and public safety, due to their sensitivity, selectivity and speed in the detection of specific analytes. These devices represent a viable and efficient alternative to traditional laboratory analysis methods, being able to provide real-time answers, at a lower cost and with greater portability. In this scenario, investing in research and development of biosensors is essential to meet the growing technological demands for early diagnosis, environmental monitoring, and industrial quality control. In addition, the integration of these devices with digital platforms and miniaturized systems expands their potential for application in remote environments. The objective of the chapter is to present the fundamentals, characteristics and applications of electrochemical biosensors, with emphasis on two main classes: enzymatic biosensors and immunosensors. Enzymatic biosensors are based on the immobilization of enzymes on electrodes, allowing the conversion of biological signals into measurable electrical signals. The specificity of enzymes due to their substrates ensures high selectivity in detection, being widely used for the quantification of glucose, lactose, urea and other relevant compounds in health and food. Enzyme immobilization can occur by different methods, such as adsorption, encapsulation, covalent bonding, and trapping in polymeric matrices. Advances in materials engineering have made it possible to improve the stability and efficiency of these devices, making them increasingly effective. Immunosensors, on the other hand, use the highly specific interaction between antigens and antibodies to recognize and quantify substances of interest, being especially useful in the detection of pathogens, tumor biomarkers, and pesticide residues. They can operate in different formats, such as direct or competitive immunoassay, with electrochemical detection based on the variation in current, potential, or impedance generated by the antigen-antibody binding. The appropriate choice of recognition elements and transducers directly influences the sensitivity, detection limit, and reproducibility of immunosensors. With the advancement of nanotechnology, these devices have become increasingly accurate, being able to operate at extremely low analyte concentrations. Thus, electrochemical biosensors represent strategic and innovative detection devices for rapid clinical diagnostics, precise environmental surveillance, and quality assurance in industrial processes and food products. Its continuous development is key to driving accessible and sustainable technological solutions, aligned with the emerging needs of contemporary society.

Keywords: Biosensor. Electrochemical. Enzymatic. Immunosensor.

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1 INTRODUCTION

According to the *International Union of Pure and Applied Chemistry* (IUPAC), a biosensor is a device composed of a biological recognition element associated with a physicochemical transducer capable of providing quantitative analytical information of an analyte1. The transducer is the element that converts a biochemical response into a measurable electrical signal, which can be: amperometric, potentiometric, impedimetric or conductivity. Sensitivity, selectivity, detection limit, reproducibility, response time, and lifetime are the relevant parameters for biosensor applications.

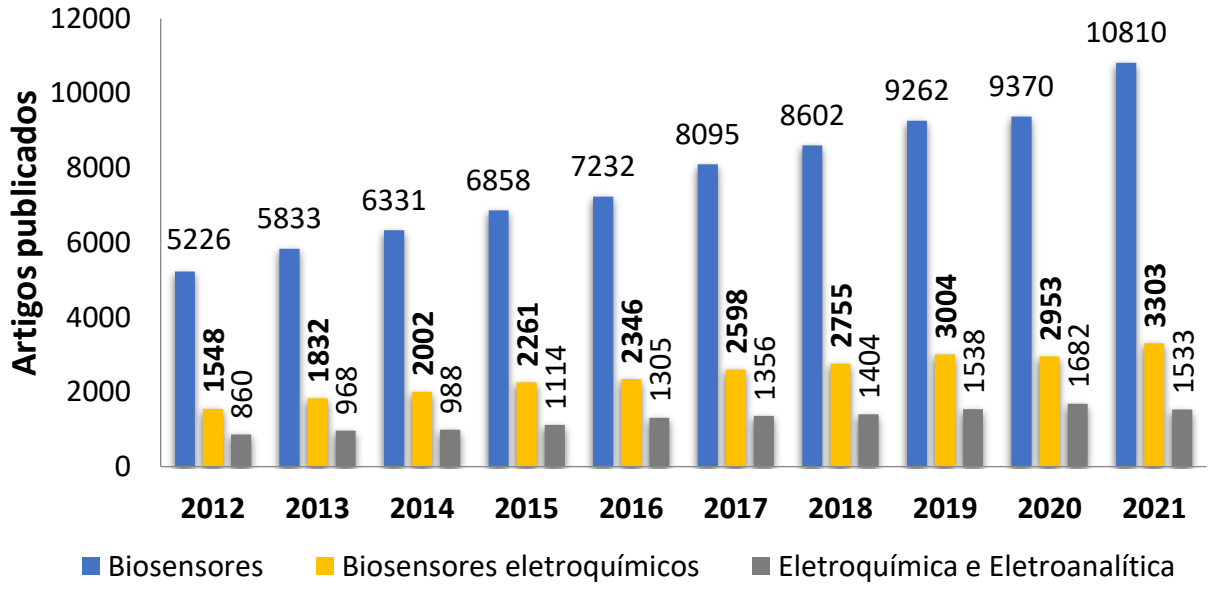
An electrochemical biosensor is a class of biosensors in which the transducer is chemically modified with a biochemical film, and can be composed of an electronic, semiconductor, or ionic conductive electrode. The best known electrochemical biosensor is the glucose meter, which from the initial concept proposed by Clark and Lions2 in 1962, received numerous contributions for more than five decades, until it reached commercial models3. However, the applications of electrochemical biosensors go far beyond glucometers, being used in the detection of viruses4, in the identification of cancer biomarkers5, in the evaluation of pollutants6 and in the analysis of pesticides7, enabling a wide field of activities for fundamental research and technological innovations.

The global biosensor market was valued in 2021 at USD 24.9 billion8. The demand for low-cost, easy-to-use disposable devices with a short response time has grown significantly in this market segmented into the biomedical, food, agriculture, environment, and biotechnology areas. The biomedical segment dominated the biosensor business in 2021 with 66.5% of commercial applications. In this segment, biosensors are used in the analysis of blood metabolites such as: glucose, lactose, creatinine, urea, cholesterol, and also pregnancy test, drug test, and diagnosis of some infectious diseases. The use of these devices in the environment has grown, being the second largest in volume of applications, especially in the analysis of fungi, pesticides, herbicides, heavy metals and pollutants. The biotechnology segment is the third in market value.

Electrochemical biosensors have advantages over piezoelectric and optical biosensors because they have low detection limits, high reproducibility, and optimal stability. In addition to these advantages, their compatibility with new micro and nanoelectronics technologies makes these devices operationally and economically viable in several segments. The main biosensor industries are: Bio-Rad Laboraories; Abbott Laboratories; Bayer AG; DuPont Biosensor Materials; Johnson and Johnson, Philips; LifeScan, Inc.; Nova Biomedical; Siemens Healthcare and Roche Diagnostics, which produce and market various types of biosensors for biomedical, environmental and biotechnological use.

In the last decade, academic research in the line of biosensors has surpassed, on average, three and a half times the production in the areas of electrochemistry and electroanalytics, in number of publications, according to the evaluation of production indicators in the *Web of Science* database (Thonson Reuters), shown in **Figure 1**. The survey also reveals that the production in the class of electrochemical biosensors is greater than the sum of the productions in electrochemistry and electroanalysis, showing the relevance of this line of research for a large area.

Figure 1 Distribution of publications in the areas of biosensors, electrochemistry/electroanalysis, and electrochemical biosensors. Search conducted in the Web of Science database (Thonson Reuters), keywords: biosensor*; electrochemic* AND electroanal* and electrochemical* AND biosensor*, respectively to each area or line of research.



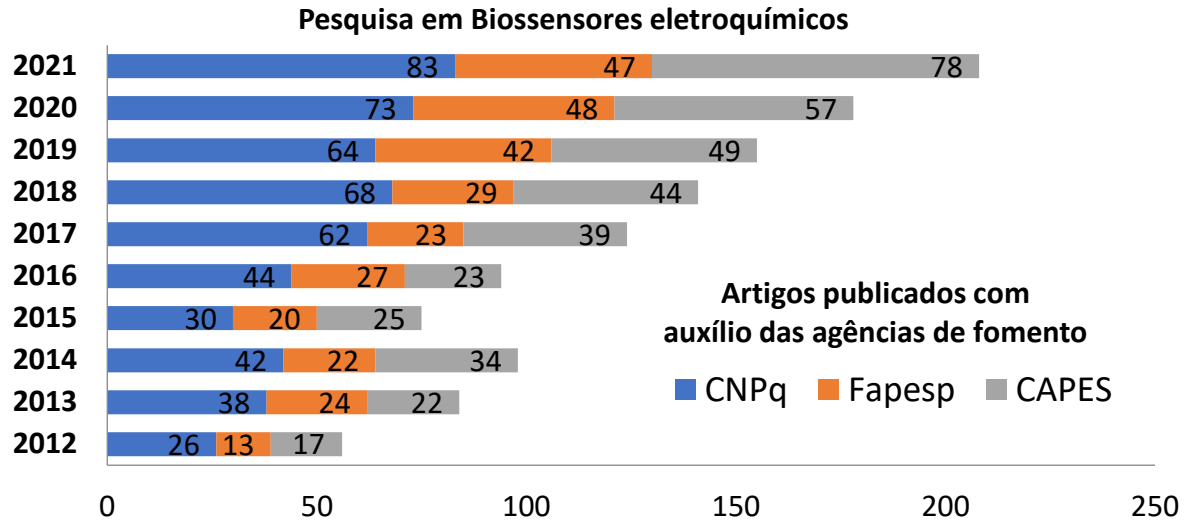
Source: prepared by the author.

The scientific production in electrochemical biosensors in the last decade represents 30% of all research in biosensors of the various classes. In the last ten years, Brazilian participation in research on electrochemical biosensors has occupied 5% of the total, a result obtained by counting the publications that contain in the field "funding agency" the citation of at least one of the three research funding agencies: the National Council for Scientific and Technological Development (CNPq), the São Paulo Research Foundation (FAPESP) and the Coordination for the Improvement of Higher Education Personnel (CAPES).

In recent years, it has been reported and verified in many areas of research the drastic reduction of investment by national and state development agencies. Budget cuts in science and technology have caused a decrease in scholarships and resources for inputs

and equipment. However, the line of research in electrochemical biosensors did not suffer a significant impact, signaling its relevance to funding agencies. The scientific production supported by the three main funding agencies that subsidize research in the State of São Paulo, with articles published in indexed journals, has shown growth in the granting of benefits in the last decade, as shown in **Figure 2.**

Figure 2 Promotion of research in the area of electrochemical biosensors, in the last decade, by the three agencies that subsidize research in the state of São Paulo. Search conducted in the Web of Science database (Thonson Reuters), keywords: electrochemical* AND biosensor*; field "funding agency": CNPq, FAPESP or CAPES.

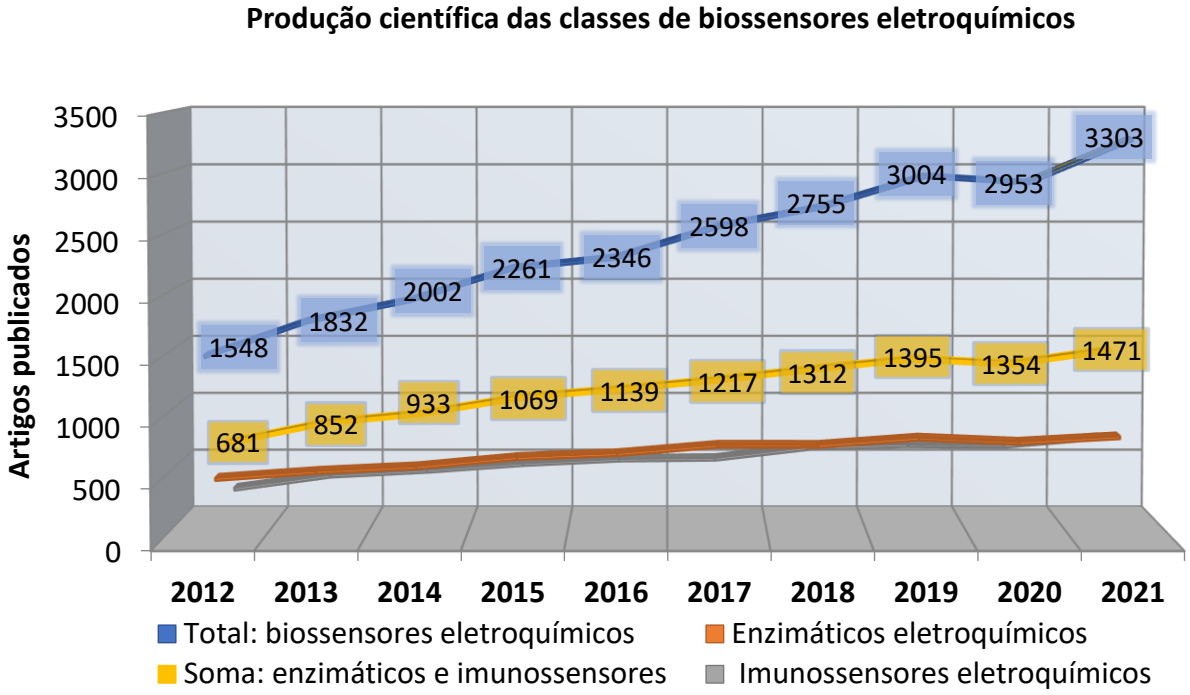


Source: prepared by the author.

Investment in the line of research in electrochemical biosensors, which includes the biomedical, environmental and biotechnological segments, both in the commercial market and in academic research, has grown in the last decade, which shows the wide relevance of this theme within the large area of electrochemistry and electroanalysis.

As for the biological element immobilized in the transducer, electrochemical biosensors can be classified as: enzymatic biosensors, immunosensors, cellular biosensors, genosensors, among others. The classes that provide the highest levels of detectable signal are enzymatic biosensors and immunosensors, used in most applications. **Figure 3** shows that, in 2012, the sum of scientific production in the line of enzymatic biosensors and immunosensors represented 44% of the publications in electrochemical biosensors, maintaining this level at the end of a decade.

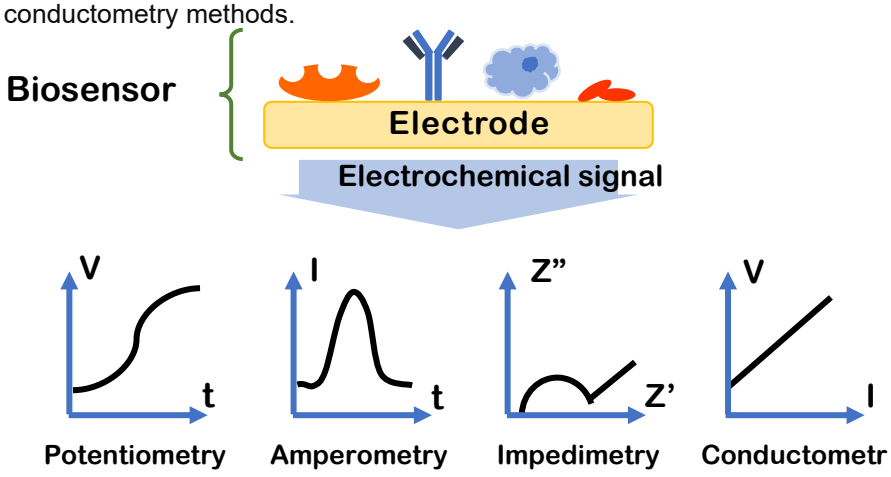
Figure 3 Scientific production on electrochemical, enzymatic and immunosensor biosensors in the total set of publications on electrochemical biosensors. Search conducted in the Web of Science database (Thonson Reuters), keywords: electrochemical* AND biosensor*; enzym*; immun*.



Source: prepared by the author.

The electrochemically monitored reaction can generate a signal in the electrode system that indicates the emergence of current, accumulation of charge or potential, change in impedance or change in the conductance of the medium. This signal can be interpreted by the electroanalytical methods of amperometry/voltammetry, potentiometry, impedimetry and conductometry, respectively9. The detection methods can be organized according to the category of the signal: current, potential, impedance or conductance and unfold in the electroanalysis techniques, as illustrated in **Figure 4.**

Figure 4 Biosensor and the types of electrochemical signals measured by potentiometry, amperometry, impedimetry and conductometry methods.



Source: prepared by the author.

The ease of operation, portability and simplicity of electrode construction are the qualities that justify the prominence of electrochemical detection in biosensors, in particular, the identification of a measurable signal of the biochemical reaction does not depend heavily on the volume of the sample. Recently, Mello, Bueno and Mulato10 compared the electrochemical response of enzymatic biosensors structured in thin polyaniline films with the optical response. The study shows, for example, for glucose detection, a detection limit of 0.16 µmol L-1 for the impedimetric biosensor and 2.33 µmol L-1 for the optical biosensor.

The electrochemical detection of complexes such as antibody-antigen is little affected by sample components such as chromophores, fluorophores, and particles that interfere with spectrophotometric detection. Therefore, electrochemical assays can be performed on cloudy samples, such as whole blood, without significant interference from fat globules, red blood cells, hemoglobin, and bilirubin11.

The significant number of publications in the line of enzymatic biosensors and immunosensors reflects the great scientific and commercial success of the first representatives of this class, glucose meters. The robustness and accuracy of glucometers continue to inspire research and application in the search for solutions for the detection of numerous emerging analytes in the biomedical, environmental and biotechnological areas. The objective of this chapter is to present a review of the concepts of these two most relevant classes of electrochemical biosensors. The text is structured in four parts: introduction; enzymatic biosensors; immunosensors and conclusion.

2 ENZYMATIC BIOSSENSORS

The enzymatic biosensor is a bioanalytical device in which a catalytic enzyme immobilized in the electrode reacts chemically with the target analyte generating a measurable signal12. Glucometers, or glucose meters, are the most widely used and commercially produced enzyme biosensors. Parameters such as enzyme origin, operational stability, and storage, as well as immobilization procedure are critical when preparing an enzyme biosensor13.

Enzyme biosensors can be classified as electrochemical probes with thin film of enzyme immobilized on the surface of the working electrode. The electroactive product can be directly monitored using amperometry, in which a current is produced in response to a constant potential applied, with enzymatic catalysis being responsible for the significant amplification of the signal in the biosensor14. The use of enzymes in biosensors continues to grow, because due to the complexity of these molecular structures and their unique

specificity with the target molecule, they can detect individual analytes in a complex mixture, such as urine or blood, with great selectivity15.

The development of enzymatic biosensors in biomedical applications in the 1960s began with the monitoring of blood glucose mainly due to intense research in the area. Electrodes functionalized with the enzyme glucose oxidase (GOx) have been widely used in glucose detection since the pioneering work of Clark and Lyons2 between 1950 and 1960. This amperometric biosensor became known as the first generation of enzymatic biosensors. In this **first generation,** the oxidase enzyme is immobilized between a semipermeable membrane and the surface of a platinum electrode. GOx is a stable, inexpensive enzyme easily obtained from *Aspergillus niger*, a common fungus of many fruits and vegetables. GOx is highly specific for β-D-glucose which can be detected by the following reaction expressed in equations (1), (2) and (3):

$$\beta\text{-D-glucose} + \text{GOx-FAD} \rightarrow \text{GOx-FADH}_2 + \delta\text{-D-gluconolactone} \quad (1)$$

$$\text{GOx-FADH}_2 + O2 \rightarrow \text{GOx-FAD} + H2O2 \quad (2)$$

$$H2O2 \rightarrow 2e^- + O2 + 2H+ \quad (3)$$

However, the first generation of biosensors continuously needed oxygen as a substrate to effect the enzymatic reaction. As oxygen is poorly soluble in aqueous solutions, there was a limitation of the current produced in the presence of the analyte, since direct redox reactions between enzymes and electrodes are very rare due to the tendency of denaturation of these proteins when in contact with the electrode surface. Since a limited number of enzymes, such as turnip peroxidase , are able to transfer electrons directly from the active site of the enzyme to the electrode, the initial idea has been modified.

The electrons produced in the reaction of a catalytic enzyme cannot always be transferred quickly and easily to the surface of the electrode. The widely accepted Marcos Theory shows that electron transfer drops exponentially with distance17. Therefore, enzymes often require a helper that transfers electrons to the transducer's surface. Artificial redox mediators act as helpers to this transfer and are small soluble molecules capable of providing oxygen for the enzymatic reaction in glucose biosensors. Although many organic compounds are capable of acting as mediators, organic-metallic redox compounds are the most common. This class includes: quinones, organic conductive salts, dyes, Ruthenium complexes,

ferrocene and ferricianide derivatives9. The reaction of the GOx enzyme in the presence of mediators can be expressed by equations (4) and (5):

$$GOx\text{-}FADH2 + 2\ MediatorOx \rightarrow GOx\text{-}FAD + 2\ Mediator_{Red} + 2H+ \quad (4)$$

$$2\ MediatorRed \rightarrow 2\ MediadorOx + 2e- \quad (5)$$

Enzymatic biosensors with mediators, called **second generation,** perform much better than first generation biosensors, mainly due to the elimination of dependence on oxygen in solution. The incorporation of redox mediators also allowed the use of other oxidoreductase enzymes, such as peroxidases and dehydrogenases, which expanded the list of possible target analytes.

The **third generation of enzymatic biosensors** has the biorecognition component coupled through the coimmobilization of the enzyme with the mediator on the surface of the electrode. This can be achieved by direct electrical contact between the enzyme and the electrode, immobilizing the enzyme and mediator in a conductive polymer or a metal electrode. This new generation was initially described by Heller18 and are ideal biosensors for repeated measurements, as neither the mediator nor the enzyme need to be added. Recent studies by Bueno19 show that in systems such as these, electron transfer and quantum conductance are correlated. These studies gained relevance in the fundamental aspects, combining the theory of Rudolf Marcus and Landauer, which expanded the understanding of the processes of storage and transfer of cargo. The evidence of this correlation was confirmed by the computer simulation study by Feliciano and Bueno20. These studies have contributed to the understanding of the fundamental aspects and allowed the improvement of the efficiency of enzymatic biosensors and the development of new applications.

2.1 METHODS OF ENZYME IMMOBILIZATION

The purpose of immobilization is to provide the stable bond between the enzyme and the transducer's sensing surface without blocking the protein's active site or drastically altering its geometry. There are basically four methods of immobilization: physical imprisonment, covalent bonding, adsorption, and electrostatic bonding14. The stability period of immobilized enzymes depends on the temperature, pH, and method used, ranging from hours to months, depending on the preparation, design, and storage conditions of the biosensor.

The simplest approach is to physically trap the enzymes between preformed membranes on the surface of the electrode. In this method of physical immobilization, the native composition of the enzyme is preserved since it does not involve the formation of a

covalent bond26. The most common enzymatic entrapment procedures are: encapsulation, inclusion in a gel, conjugation in electropolymerized film and incorporation in carbon paste.

The covalent binding of the enzyme to the transducer is the most stable immobilization, because it binds directly to the electrode the functional groups of the protein such as NH2, COOH, OH, and SH that do not act directly on catalytic activity9. On the other hand, adsorption is the least stable method of immobilization whose forces that bind the biorecognition element to the transducer are mainly van der Waals forces, with occasional hydrogen bonding. Therefore, the service life of an adsorption-prepared sensor is quite limited. However, the adsorption method is easier to perform, requires minimal cleaning and does not impair the conformation of the immobilized enzyme, being ideal for the initial studies of enzymatic biosensor construction. In the biosensors of this project, the most appropriate immobilization techniques for each stage of development will be used.

2.2 APPLICATIONS OF ENZYMATIC BIOSENSORS

Disposable home glucose monitoring test strips, attached to manual electronic meters sold in pharmacies, are based on the catalytic reaction of the enzyme glucose oxidase (GOx) or glucose dehydrogenase. In these biosensors a single drop of whole blood, without preparation or purification, is placed on a test strip structured on polymeric substrate that contains conductive tracks and membranes on which the dry reagents have been deposited. The two-electrode system is the most commonly used, and in the working electrode the enzyme and the mediator are immobilized and the other acts as a reference electrode.

Currently, commercially sold glucose test strips are second or third generation and ferricyanide is one of the commonly used mediators. Detection in these enzymatic biosensors is amperometric, under a constant potential applied. The reaction catalyzed by the enzyme produces current that is intensified by the mediator and then quantified by an electronic circuit, or micropotentiostat. Commercially sold glucose meters have a sensitivity range between 1.1 and 33.3 mmol $^{L-1}$ glucose, accuracy of 3% to 8%, and test time of about 30 seconds9.

In addition to glucometers, there are commercially enzymatic biosensors used to detect lactate, an ester of lactic acid produced during the process of cellular respiration when the glucose molecule is broken down. Its concentration in the blood increases from the normal value of 0.9 mmol $^{L-1}$ to about 12 mmol $^{L-1}$ with the intense activity of anaerobic metabolism caused by strenuous exercises such as marathon or triathlon27. The first portable electrochemical lactate meters for use in sports medicine were manufactured by Senslab and Arkray35. These biosensors require blood samples with only 0.5 µL and 5 µL, respectively.

Blood lactate levels are used as indicators of conditions such as acidosis or bacterial meningitis.

Four enzymes can be used as biorecognition components in lactate biosensors: lactate dehydrogenase, lactate oxidase, lactate monooxidase, and cytochrome b2. Some electrochemical lactate biosensors include mediators such as ferricyanide and nicotinamide adenine dinucleotide (NAD), which is a coenzyme that has two oxidation states: NAD+ (oxidized) and NADH (reduced). NAD is an organic compound found in the cells of living beings and used as an electron transporter in metabolic reactions of oxy-reduction and has a preponderant role in the production of energy for the cell36. These enzymes, conjugated to mediators, when binding with lactate, produce current in the working electrode that is measured amperometrically.

Another possible arrangement in the enzyme biosensor, called the interference biosensor, detects changes in the rate of catalysis of reactions when effectors bind to enzymes, acting as inhibitors or activators. These biosensors are also called enzyme inhibition sensors were developed for the detection of pesticides such as organophosphate and carbamate, respiration toxins such as cyanide (CN−) and azide (N_3^-), and toxic heavy metals such as As, Pb, Cd, Cr, and Hg37. Enzymes used in enzymatic biosensors include tyrosinase, turnip peroxidase, and acetylcholinesterase. Although there are relevant applications commercially available, a large number of analytes still lack devices for their detection, and enzymatic biosensors can be developed for these targets.

3 IMMUNOSENSORS

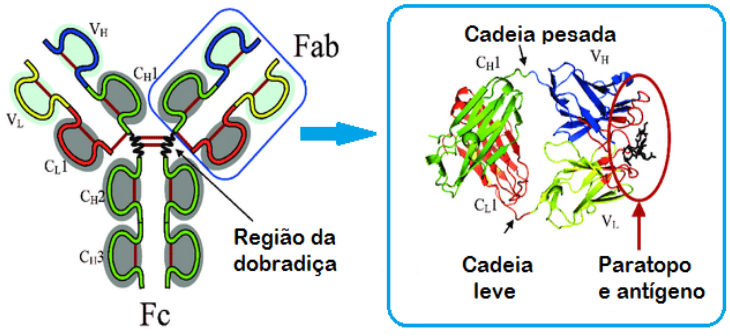
Immunosensors are affinity biosensors based on strong selective binding between antibodies (Ab) and antigens (Ag). The detection of an analyte, in this case an antigen, is performed through its binding to a specific antibody immobilized in the electrode or vice versa21. The Ab-Ag bond generates an electrical signal in the transducer that can be quantified by any of the electroanalytical methods, depending on the immunosensor design. For example, in the immunosensors used in pregnancy and fertility tests, the antibodies immobilized in the electrode have an affinity for binding with the hormone hCG (human chorionic gonadotropin), which is produced when the egg is fertilized. The most common commercial types of these tests are colorimetric and those that detect the bonding reaction electrochemically.

Immunosensors stand out among other types of biosensors, as they detect traces in the order of parts per billion (ppb), have high specificity in antibody-antigen binding (Ab-Ag) and also enable the detection of various analytes such as bacteria, viruses, drugs, toxins,

hormones, environmental pollutants, pesticides, herbicides and other compounds. In addition to these advantages, these biosensors require little sample preparation, reduced use of reagents, and compatibility with automation9, making these devices an attractive alternative to conventional quantitative analytical methods such as chromatography and mass spectrometry. The multiplex design of the immunosensors also allows for simultaneous analysis of multiple analytes, which improves efficiency and makes assays relatively fast and cost-effective. Immunosensors are easier to construct than enzymatic sensors and have four factors involved in their design: electrode configuration, antibody immobilization, minimization of nonspecific interactions, and choice of detection method22.

IgG immunoglobulin, one of the main antibodies (Abs) used in immunosensors, is a "Y"-shaped glycoprotein with a molecular weight of the order of MW~150 kDa produced by a host in response to the presence of a foreign molecule called antigen (Ag)[23]. An antigen is any element that the body recognizes as foreign, such as chemicals, proteins, or particulate matter (dust, pollen, etc.). IgGs have four polypeptide chains, two identical with a molecular weight equal to or greater than 50 kDa and two lighter chains with about 25 kDa, as illustrated in **Figure 5**. The chains of Abs are joined by disulfide bonds and hydrogen bonding interactions,

Figure 5 (a) Structure of an IgG immunoglobulin: Fc – effector fragment, Fab – antigen-binding fragment, V – variable domain, C – constant domain. (b) Binding of an antigen to the Fab fragment.



Source: adapted from reference [23].

The antibody-binding region is called the paratope and binds with high affinity to the region of the antigen called the epitope. Ab-Ag interactions can be of the type: hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals forces. When the analyte to be detected is biomedical, antibodies can be found in blood serum, fluids such as saliva and urine, and cell membranes, facilitating the collection and analysis of samples. Electrochemical immunosensors can be classified into three groups regarding electrical transduction24. In the potentiometric approach, the Ab-Ag complex built on the surface of the

electrode changes the potential proportionally to the concentration of the analytes. In the amperometric method, a constant potential is applied to the electrode and the current associated with the reduction or oxidation of electroactive species created by the interaction is measured. However, since most antibodies are not able to induce electrochemical reactions by themselves, mediating agents or enzymes must be functionalized in the biomolecule. In turn, the impedimetric techniques measure the transfer of electrons in response to a small excitation potential, enabling the direct detection of analytes without the use of markers or redox pairs. Impedimetric immunosensors can be configured for impedance or capacitance measurements and show advantages over potentiometric and amperometric sensing.

3.1 PRODUCTION OF ANTIBODIES

There are two types of antibodies: polyclonal (Abs) and monoclonal (MAbs). Abs are heterogeneous in relation to their binding domain and do not always allow the required detection selectivity, whereas MAbs improve detection limits due to greater MAb-Ag binding specificity, and are preferable for the construction of electrochemical immunosensors25. While Abs are a heterogeneous mixture of immunoglobulin molecules secreted against an antigen at the beginning of the body's immune response, MAbs have binding affinity for a single epitope, allowing the detection of small amounts of Ag, which greatly increases the specificity of MAb-Ag bonding.

MAbs can be developed for a wide range of substances, and theoretically, if a MAb can be produced for a specific analyte, an immunosensor can be developed to detect that substance. The cost of reagents for immunology assays continues to decrease due to the development of molecular biology techniques. Currently, there is a significant variety of antibodies being sold by large reagent manufacturers such as Sigma Aldrich. For the detection of emerging analytes, it is possible to order specific antibodies with the appropriate functionalization, from specialized national companies.

3.2 IMMOBILIZATION OF ANTIBODIES AT THE ELECTRODE

Like enzymes and other biorecognition molecules, antibodies are very sensitive to environmental conditions. Normally, MAbs are immobilized on a solid surface for application in biosensors, but depending on the orientation of the biomolecules in this immobilization, there may be a loss in the ability to bind to the antigen21. The antibody arms, the paratope, should be exposed to the sample, and it is necessary to choose immobilization methods that favor the effector fragment to bind to the transducer surface. MAbs can be immobilized on

the electrode surface by methods that include biotin-streptavidin bonds, adsorption to a conductive polymeric matrix such as polypyrrole, and covalent bonds. Interactions of disoriented antibodies on the surface can lead to changes in the binding structure of the paratope21, contributing to the decrease in the immunosensor's detection limit. In addition to the immobilization orientation, the density of the MAbs immobilized at the surface must be adequate to minimize the effects of steric interactions, associated with the overlapping of electron clouds and which can affect binding affinity.

Another determining factor for the construction of an efficient immunosensor is the reduction of nonspecific bonds. Non-specific binding in a biosensor involves adsorption of MAbs with species other than those of the target analyte. There may also be adsorptions of these other species in the empty spaces of the electrode, a phenomenon that increases the background signal, and is the main limitation of detection in electrochemical biosensors7. Therefore, to reduce or eliminate these non-specific bonds, procedures such as the use of nonionic surfactants, Tween 20, bovine serum albumin (BSA), polyethylene glycol, gelatin, or casein should be included. Self-assembled *monolayers* of oligo (ethylene glycol) and dextran layers are also used successfully to prevent non-specific bonds on electrode surfaces. The immobilization techniques described will be used in the development of immunosensors in the proposed low roughness electrodes.

3.3 APPLICATIONS OF IMMUNOSENSORS

There is a vast field for research and application of immunosensors that covers the segments of the biomedical, environmental and biotechnological areas. However, it is the biomedical area that has the largest number of publications and presents the greatest demand for research, with commercial biosensors for some markers. In this segment, currently, the great need is the detection of infectious diseases. In recent years, communicable viral diseases have emerged with great intensity, causing enormous damage in several countries. The main diseases with epidemics in the twenty-first century were: SARS, avian flu, H1N1, Ebola, dengue, chikungunya, zika and the new coronavirus (SARS-COV-2).

The main need in cases of epidemics is the rapid triage of patients, in care centers and hospitals, so that individuals receive appropriate treatment. When it comes to diagnosis, there are two extremes: the rapid colorimetric tests based on the antibody-antigen interaction, and the molecular test known as the reverse chain *reaction* (PCR) method. The first, also called serological test, provides the result in a few minutes, but is effective only when the immune system is already producing antibodies, which happens a few days after infection.

At the other extreme, PCR, the gold standard for diagnosis, analyzes the genome of the virus and is able to provide accurate results even at the beginning of the disease, before the manifestation of symptoms. However, as it is a specialized and more expensive analysis, it is available on a larger scale only in cases of extreme need, as it was in the pandemic of the new coronavirus.

To fill the gap between the two diagnostic extremes, colorimetric and PCR, new proposals have emerged, such as the use of immunosensors for the detection of structural proteins of viruses, such as envelope and capsid proteins. In the literature, this arrangement is called affinity biosensor. This option allows you to extend the diagnostic window to the early periods of the disease using the Ab-Ag28 interaction. A similar proposal can be applied to other viruses, allowing an increase in the detection window, greater sensitivity than colorimetric tests, and lower cost than molecular assays.

Currently, there are biosensors incorporated in commercial portable instruments such as the *i-STAT,* developed since the 1990s by Abbot and capable of analyzing small volumes (20 µL) of whole blood29. It is a manual instrument, simple to use, with disposable cartridges containing biosensors associated with microfluidics. The instrument simultaneously measures several biochemical parameters such as: hematology, blood gases, coagulation parameters, endocrinology, cardiac markers, however, there are no immunoassays available for this equipment, space to be filled by immunosensors.

The most modern promising portable biosensor instrument is the *Osler Origin*, developed by *Osler Diagnostics*, a *startap* created at the University of Oxford. In the final phase of the regulatory stages, the instrument promises high efficiency, including the most advanced detection techniques, and encompasses all the benefits of biosensors: measurement in whole blood samples, sensitivity to the target at low concentrations, multiplex assay, low cost, easy operation, and delivery of results in a few minutes30. The philosophy of insertable cartridges allows the continuity of research in the development of biosensors for numerous markers not yet supported by *Osler Origin* and *i-STAT*, both in the biomedical area and in the environmental and biotechnological areas. Biosensors developed for *point-of-care* use gain relevance since hospitals, health centers and medical clinics in large urban centers need rapid detection for a large number of analytes.

4 CONCLUSION

Electrochemical biosensors involve a wide field of scientific research worldwide and represent one of the most dynamic and promising areas of technology applied to the detection of chemical and biological substances. Its growing importance is justified by the combination

of high sensitivity, selectivity, speed of response and miniaturization potential, fundamental characteristics for the advancement of detection devices in the areas of health, environment, food safety and industrial control. Throughout this chapter, two fundamental classes of electrochemical biosensors have been addressed, enzymatic biosensors and immunosensors. The emphasis was on its principles of operation, forms of immobilization of biocomponents and applications.

Enzymatic biosensors stand out for the use of enzymes as biological recognition elements, whose specificity in relation to the substrate allows highly selective measurements. This characteristic makes them essential tools, for example, in the determination of glucose in biological fluids, contributing directly to the monitoring of diseases such as diabetes mellitus. Enzymatic immobilization methods, such as physical adsorption, covalent bonding, die entrapment, or encapsulation, exert a direct influence on the stability, reusability, and sensitivity of devices. Recent advances in the development of conductive materials, such as nanomaterials and conductive polymers, have provided significant improvements in the performance of these sensors, making them more robust and reliable. In addition, the use of enzymes genetically modified or stabilized by physicochemical techniques has increased the useful life and efficiency of enzymatic biosensors under different operating conditions.

Immunosensors, in turn, explore the specific interaction between antigens and antibodies, being particularly useful in the detection of pathogens, toxins, tumor biomarkers, hormones, and other compounds with clinical, environmental, or dietary relevance. The sensitivity of these devices can be improved through the judicious choice of antigen-antibody pairs, the optimization of immobilization surfaces, and the application of appropriate electrochemical techniques such as pulse voltammetry, amperometry, or electrochemical impedance. With the advent of nanotechnology, it has become possible to further increase the surface area of electrodes and incorporate functional nanomaterials capable of improving electronic conduction and immobilization efficiency. As a result, modern immunosensors have achieved extremely low detection limits, allowing analyses in complex samples with high reliability.

The comparative analysis between enzymatic biosensors and immunosensors reveals that both have specific advantages and challenges, and the choice of the most appropriate platform depends on the type of analyte, the sample matrix and the requirements of the final application. While enzyme biosensors are widely applicable in continuous and routine analyses, immunosensors are particularly effective for spot diagnostics and detection of substances with biomedical or toxicological relevance. Both types of biosensors, however,

share the common challenge of biocomponent stability and the need for frequent calibration, aspects that continue to be the subject of intense research.

The future of electrochemical biosensors points to integration with digital technologies and wearable devices, such as smartphones, microcontrollers, and Internet of Things (IoT) systems, expanding their applications in remote environments, in real time, and in personalized medicine contexts. In addition, the development of multifunctional and multiplexed platforms, capable of simultaneously detecting several analytes, represents a growing trend in this area of research and development. The interdisciplinarity between chemistry, biotechnology, materials engineering, and data science has the potential to drive significant innovations in this scenario.

In a global context of growing demand for rapid diagnostics, environmental monitoring, and product traceability, electrochemical biosensors play a strategic role as affordable, sustainable, and adaptable devices. The development of innovations based on bioelectrochemical principles can contribute not only to scientific and technological advancement, but also to addressing urgent societal challenges, such as epidemic control, water quality surveillance, and large-scale food security.

Therefore, investing in research, development, and training of human resources in the area of biosensors is essential to consolidate this technology as an integral part of innovative and efficient solutions. The consolidation of partnerships between universities, research centers and the productive sector will be essential for the transfer of knowledge and for the large-scale production of devices for application. In view of this, electrochemical biosensors, especially enzymatic and immunosensors, are consolidated as protagonists in the construction of a future where science meets innovation to promote quality of life, sustainability, and social well-being.

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