

BIOSENSORS

E. Galindo

Institute of Biotechnology, National University of Mexico, Cuernavaca, Mexico

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Summary

A biosensor is the combination of a biological element (which is able to produce a perturbation) with a conventional transducer (which is able to generate a measurable

signal). Enzymes, antibodies, tissues and microorganisms have principally been used as biological elements, although DNA and cell receptors have found applications as well. Biosensors will revolutionize the way in which analytical information is retrieved. This will allow the detailed study and automatic control of certain processes, mainly in the fields of clinical analysis, fermentation, food technology and wastewater treatment.

The sensors employ electrochemical, optical, piezoelectric or thermal principles of measurement. The construction of a biosensor is relatively straightforward. However, if the commercialization of prototypes is desired, multidisciplinary teams which include specialists in electronics, mechanical design and biotechnology are required.

Each particular type of biosensor has to be evaluated in terms of its detection range, selectivity, reliability, shelf life, versatility, size and, for some applications, biocompatibility, sterility and suitable packing are crucial. In the specialized literature, hundreds of biosensors have been described for the determination of a very large number of analytes and properties.

1. Introduction

The term “biosensor” is very broad and is used with various different meanings. In the most general of terms, a biosensor is any biological entity capable of being used to identify (either qualitatively or quantitatively) a response to some environmental change. The canary in the coal mine is perhaps the oldest of the biosensors.

Many bioassays for determining cell toxicity can be considered biosensors. However, in more specific terms (and those relevant to the present chapter), a biosensor is a device which can quantitatively detect an analyte and generate a measurable signal.

In the fields of analytical chemistry and automatic control, it is crucial to have methodologies and sensors capable of easy operation and fast measurement. The recording and control of certain biological processes, such as industrial fermentations and wastewater treatment, will be made possible when suitable detectors are available, *i.e.* transducers able to change a given chemical or biochemical parameter into an electrical or optical signal.

Nowadays only a very limited number of commercial sensors are available, which are able to detect physicochemical parameters such as temperature, pH, redox potential, dissolved oxygen, CO₂ and certain ions.

Nevertheless, for the control of these types of processes, it is necessary to measure more than just the mere physical state of a certain culture. It is hence necessary to have transducers which are able to quantify substrates, cell material contents or specific metabolites. The development of the so called “biosensors” has made it possible to achieve this goal.

From a general point of view, a biosensor is a device which is able to detect certain compounds through an electrical or optical signal. These devices consist of basically two components: a transducer, and a biological element able to produce a specific

reaction with the substance that it is required to be measured, which in turn should be able to generate a signal that can be detected by the transducer. Figure 1 shows schematically the general layout of a biosensor.

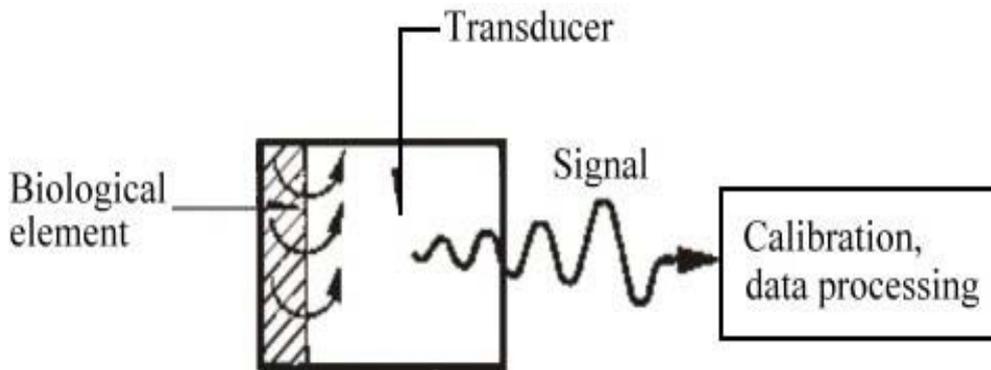


Figure 1: General concept of a biosensor

The detection of compounds by a biosensor has many advantages, including:

- High specificity, a consequence of the high specificity of the biological elements used in the system. Such is the case for a number of enzymes which are able to catalyze only the reaction of a specific isomer or the extremely specific binding of antibody with antigen.
- Fast measurement, which is conducted in a direct way and based on an electrical or optical signal. This also has the additional advantage that a large number of samples can be analysed in a relatively short period of time and, therefore, acts to reduce costs.
- Simplicity, as sample preparation and manipulation are reduced to a minimum.
- Very low reagent usage, as these are only necessary for calibration, maintenance of optimum conditions and, when necessary, sample dilution.
- The biological element can be re-used, as this is usually immobilized.
- On line measurements are possible, an indispensable requirement when continuous recording or automation of a process is the goal. Many processes could be rationally optimized if sensors measuring certain crucial parameters could be configured such that they permitted automatic process control.

This chapter describes the biological elements involved in biosensors, how they can be constructed, their principal response characteristics and working principles. The basic concepts are described and discussed using certain examples.

2. Transducers

Four basic types of transducers are used for the construction of biosensors. Figure 2 shows schematically the basic features of electrochemical, optical, thermal and piezoelectric transducers.

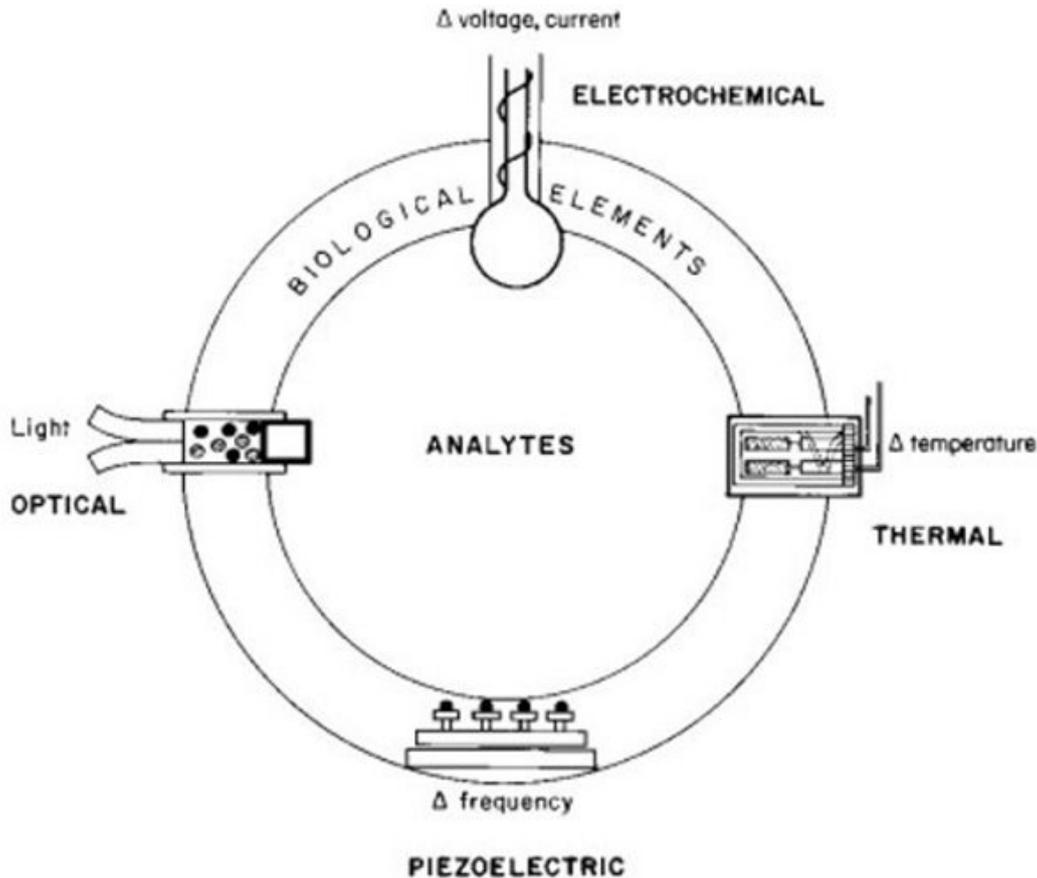


Figure 2: Transducers used in the construction of biosensors

2.1 Electrochemical

According to its type of operation, electrochemical sensors can be divided in two groups: amperometric and potentiometric. In the case of the former, the response (a current) is a linear function of the concentration of the compound of interest. In the latter, the response (a voltage) is a logarithmic function of the concentration. Currently available commercial electrodes basically belong to four types: those detecting cations, anions and gases, and platinum electrodes which measure the current in redox reactions. The ion selective electrodes, as well as those measuring pH, CO_2 and NH_4^+ use the potentiometric principle. Another important class of potentiometric transducers are semiconductor devices, such as field transistors (FETs), of which there are two main types: metal oxide (MOSFETs) and ion-selective (ISFETs). On the other hand, platinum electrodes, and those measuring O_2 and H_2O_2 , use the amperometric principle.

2.2 Optical

Conventional optical transducers were originally used for the measurement of dissolved oxygen, carbon dioxide and pH. Several types of photometric behaviour are useful for the construction of biosensors, namely:

- Visible/Ultraviolet absorption,

- Fluorescence,
- Chemi or bioluminescence,
- Reflection spectroscopy and
- Laser light scattering.

Overall, the principle of measurement is as follows: an immobilized reagent, able to interact with the analyte, forms a complex with distinctive optical properties which can hence be monitored by the sensor. Usually, the biological element is immobilized at one end of an optical fibre, with both the excitation and detection components located at the other end. For absorption-fluorescence-based transducers, the most widely used system has been NAD(P)⁺/NAD(P)H-dependent dehydrogenases because NAD(P)H is known to absorb light strongly at 340 nm and fluoresce at 460 nm. Luminescence and reflection spectroscopy have been particularly useful in immunoassays. The main principle involves the labeling of an antigen with a substance (like luminol or its derivatives) which, when oxidized, produces visible light, and the labeling of the antibody with a fluorescence compound such that emission from the luminol will excite fluorescence. A similar approach can be used with firefly luciferins.

2.3 Thermal

As most biologically-catalyzed reactions generate heat, the accurate measurement of this heat generation, together with the specificity of the biological element, can be used to construct a biosensor. Basically, this device is a small calorimeter, instrumented with highly sensitive thermometers, usually able to detect temperature changes in the range of 0.0001-0.05° C. This technique can detect analyte concentrations as low as 10⁻⁵ M.

2.4 Piezoelectric

Piezoelectric transducers are the smallest of balances. Crystals, such as those of quartz, have no center of symmetry and produce an electrical signal when stressed mechanically (*i.e.* by applying some pressure on them). A crystal oscillates at a certain frequency, which can be modulated by its environment. When the crystal is coated with some material, the actual frequency depends on the mass of the crystal and the coating. The resonant frequency can be measured with great accuracy hence making it possible to calculate the mass of analyte adsorbed onto the crystal surface. This means, that with these devices, detection limits are down to the picogram level. Antibodies, enzymes and antigens have been used as biological elements in these devices.

3. Biological elements

In the case of a biosensor, a biological element is, on the one hand, any biological entity capable of causing a specific reaction or a binding with the compound or parameter that one wishes to analyse, and on the other hand, is able to generate a signal detectable by a conventional sensor. Figure 3 shows the basic types of biological elements used in biosensor construction. The most common biological elements are enzymes, antibodies, tissues and microorganisms, but nucleic acids and receptors have also been used.

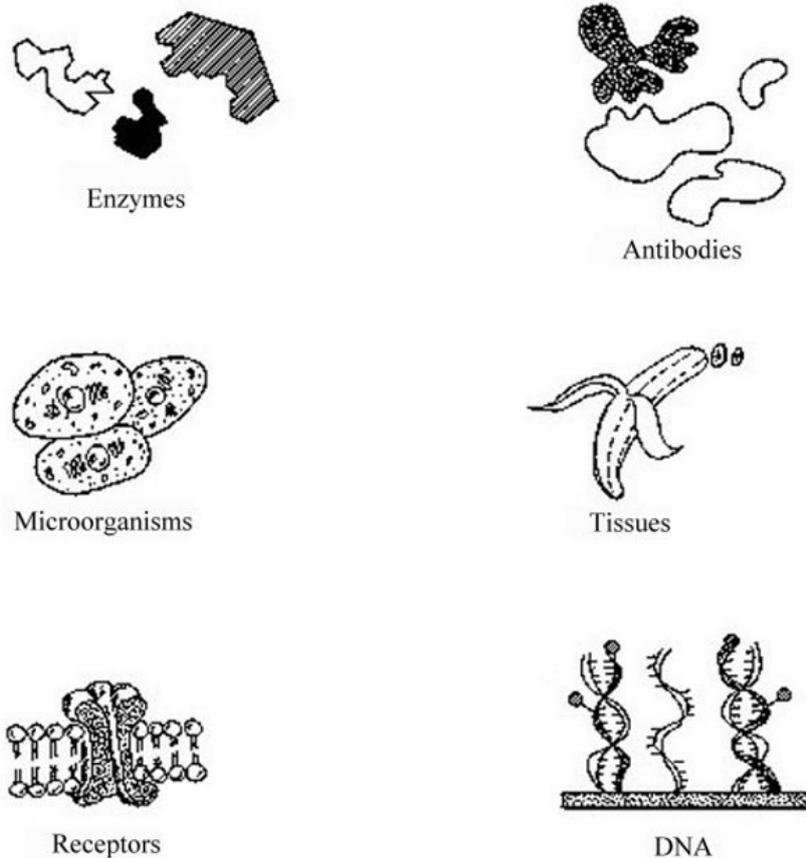


Figure 3: Biological elements used in biosensor construction

The conventional transducer will measure any one of a number of possible variables, including the product of an enzymatic reaction, the consumption of a substrate, the use of a cofactor, the respiration or growth of microorganisms, the production of a certain metabolite, the binding of an antigen, the response of a receptor, etc.

It is possible, that for the measurement of a particular compound, there exist a number of different possible combinations of sensors and biological elements. Figure 4 depicts, as an example, the different alternatives available for the measurement of glucose. If an enzyme is chosen, for instance glucose oxidase (which consumes oxygen), the O_2 consumption will be proportional to the amount of substrate and could be measured via the decrease in current using an oxygen electrode. Glucose oxidation produces H_2O_2 and gluconic acid. The oxygen peroxide so produced can be detected by a platinum electrode and gluconic acid by a pH electrode. Because there is a stoichiometrical relationship between these products and glucose, it is possible to establish a correlation between the electrode signals and the substrate concentration. Gluconic acid, in turn, could be titrated with iodine, the consumption of which can be detected by an ion specific electrode and a relationship established (commonly called a standard plot) between glucose concentration and electrode signal. In the case that a microorganism is chosen as the biological element, there are two possible means of measurement. Firstly, the microorganism is alive and one can measure indirectly its growth or respiration. Glucose metabolism [see also - *Cell thermodynamics and energy metabolism*] will

mainly produce more cells and CO₂. The resultant biomass can be detected using a platinum electrode and the CO₂ by means of an ion selective electrode. Because the amount of resultant biomass, as well as the CO₂ produced is proportional to the glucose present, it is hence possible to establish a relationship between these parameters and the glucose concentration. Secondly, it is possible to use microorganisms by employing a given enzymatic activity contained in the cell. In this case, the principles employed are identical to that which would be used when a purified enzyme is used as the biological element.

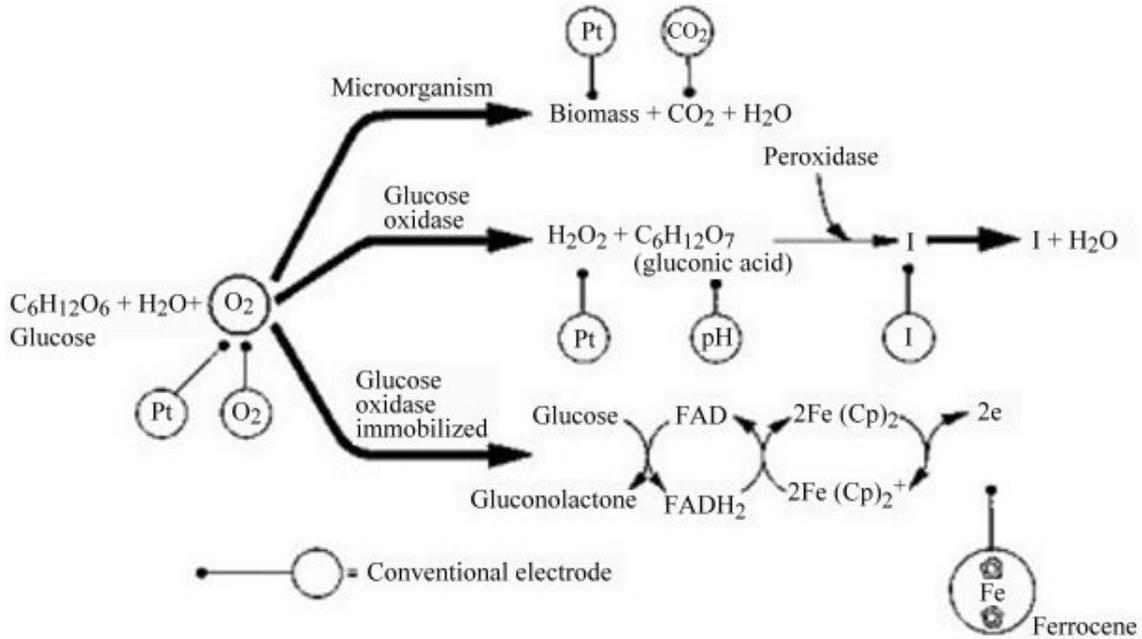


Figure 4: Alternative techniques for measuring glucose concentration using different biological elements and transducers

In section 7, an example is presented of the use of each of the different biological elements.

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Biographical Sketch

Enrique Galindo has a BSc in Chemical Engineering and a MSc and PhD in biotechnology. Presently he is Professor at the Department of Bioengineering of the Institute of Biotechnology of the National University of Mexico. The areas of expertise of Dr. Galindo include the development and optimization of bioprocess, the study of hydrodynamic effects in bioreactors, fermentations scale-down and the development of biosensors. He has a 20 years experience in biotechnological research. His work has resulted in more than 65 scientific publications and 5 patents.