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Fundamental Aspects of Biosensors

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ABSTRACT
A biosensor is an analytical device which converts a biological response into an electrical signal. The term 'biosensor' is often used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest even where they do not utilize a biological system directly. This very broad definition is used by some scientific journals (e.g. Biosensors, Elsevier Applied Science) but will not be applied to the coverage here. The emphasis of this Chapter concerns enzymes as the biologically responsive material, but it should be recognized that other biological systems may be utilized by biosensors, for example, whole cell metabolism, ligand binding and the antibody-antigen reaction. Biosensors represent a rapidly expanding field, at the present time, with an estimated 60% annual growth rate; the major impetus coming from the health-care industry (e.g. 6% of the western world are diabetic and would benefit from the availability of a rapid, accurate and simple biosensor for glucose) but with some pressure from other areas, such as food quality appraisal and environmental monitoring. The estimated world analytical market is about 12,000,000,000 year^{-1} of which 30% is in the health care area. There is clearly a vast market expansion potential as less than 0.1% of this market is currently using biosensors. Research and development in this field is wide and multidisciplinary, spanning biochemistry, bioreactor science, physical chemistry, electrochemistry, electronics and software engineering. Most of this current endeavour concerns potentiometric and amperometric biosensors and colorimetric paper enzyme strips. However, all the main transducer types are likely to be thoroughly examined, for use in biosensors, over the next few years.

I. INTRODUCTION

Industrial instrumentation for analysis is scarce and often limited to pH and conductivity. There exist on-line optical instruments such as refractometers that may be used to assess composition. However, their applicability to biological material is often limited by the presence of interfering compounds in variable concentration that interfere with the measurement. In most cases, accurate analyses of biological materials are expensive and need to be performed in external laboratories equipped with more sophisticated instrumentation. Most of these analyses require previous purification that requires too much time relative to the processing time, making their on-line implementation impossible for control purposes.

However, in living organisms, biological components like antibodies and enzymes work as natural sensing and controlling "devices." The ability of isolating and purifying these proteins and other biological elements such as cells or organelles has allowed their integration with physicochemical transduction devices to produce biosensors. The most widely accepted definition of biosensors is: "a self-contained analytical device that incorporates a biologically active material in intimate contact with an appropriate transduction element for the purpose of detecting (reversibly and selectively) the concentration or activity of chemical species in any type of sample" (Arnold et.al.,).The first biosensor, an enzyme-based glucose sensor, was developed by Clark and Lyons. Since then, hundreds of biosensors have been developed in many research laboratories around the world.

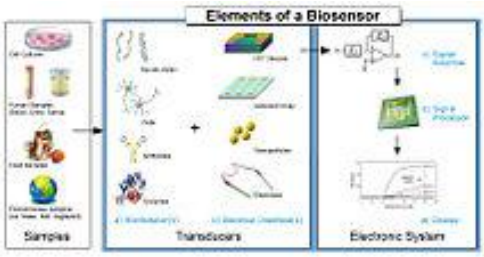
II. DEFINITION

A **biosensor** is an analytical device, used for the detection of an analyte that combines a biological component with a physicochemical detector the *sensitive biological element* (e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc.), a biologically derived material or biomimetic component that interacts (binds or recognizes) the analyte under study. The biologically sensitive elements can also be created by biological engineering

- The ***transducer*** or the ***detector element*** (works in a physicochemical way; optical, piezoelectric, electrochemical, etc.) that transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transduces) that can be more easily measured and quantified;

- Biosensor reader device with the associated electronics or signal processors that are primarily responsible for the display of the results in a user-friendly way.[3] This sometimes accounts for the most expensive part of the sensor device, however it is possible to generate a user friendly display that includes transducer and sensitive element.

III. THE BIOSENSOR SYSTEM



A biosensor typically consists of a bio-recognition component, biotransducer component, and electronic system which include a signal amplifier, processor, and display. Transducers and electronics can be combined, e.g., in CMOS-based microsensor systems.The recognition component, often called a bioreceptor, uses biomolecules from organisms or receptors modeled after biological systems to interact with the analyte of interest. This interaction is measured by the biotranducer which outputs a measurable signal proportional to the presence of the target analyte in the sample. The general aim of the design of a biosensor is to enable quick, convenient testing at the point of concern or care where the sample was procured.

Bioreceptor

In a biosensor, the bioreceptor is designed to interact with the specific analyte of interest to produce an effect measurable by the transducer. High selectivity for the analyte among a matrix of other chemical or biological components is a key requirement of the bioreceptor. While the type of biomolecule used can vary widely, biosensors can be classified according to common type's bioreceptor interactions involving: anitbody/antigen, enzymes, nucleic acids/DNA, cellular structures/cells, or biomimetic materials:

IV. TYPES OF BIOSENSORS

Biosensors can be grouped according to their biological element or their transduction e includes enzymes, antibodies, micro-organisms, biological tissue, and organelles. Antibody-based biosensors are also called immunosensors. When the binding of the sensing element and the analyte is the detected event, the instrument is described as an affinity sensor.

When the interaction between the biological element and the analyte is accompanied or followed by a chemical change in which the concentration of one of the substrates or products is measured the instrument is described as a metabolism sensor. Finally, when the signal is produced after binding the analyte without chemically changing it but by converting an auxiliary substrate, the biosensor is called a catalytic sensor. (Clark et.al.,)

The method of transduction depends on the type of physicochemical change resulting from the sensing event. Often, an important ancillary part of a biosensor is a membrane that covers the biological sensing element and has the main functions of selective permeation and diffusion control of analyte, protection against mechanical stresses, and support for the biological element. The most commonly used sensing elements and transducers are described below.

Enzymes

Enzymes are proteins with high catalytic activity and selectivity towards substrates (see the article Enzyme Kinetics). They have been used for decades to assay the concentration of diverse analytes. Their commercial availability at high purity levels makes them very attractive for mass production of enzyme sensors. Their main limitations are that pH, ionic strength, chemical inhibitors, and temperature affect their activity. Most enzymes lose their activity when exposed to temperatures above 608C. Most of the enzymes used in biosensor fabrication are oxidases that consume dissolved oxygen and produce hydrogen peroxide. Enzymes have been immobilized at the surface of the transducer by adsorption, covalent attachment, and entrapment in a gel or an electrochemically generated polymer, in filliped membranes or in solution behind a selective membrane.

Enzymes are commonly coupled to electrochemical and fiber optic transducer Antibodies is proteins that show outstanding selectivity. They are produced by b-lymphocytes in response to antigenic structures, that is, substances foreign to the organism. Molecules larger than about 10 kDa can stimulate an immune response. Smaller molecules like vitamins or steroids can be antigenic (also called haptens) but they do not cause an immune response unless they are conjugated to larger ones like bovine serum albumin.

Many antibodies are commercially available and commonly used in immunoassays. Antibodies are usually immobilized on the surface of the transducer by covalent attachment by conjugation of amino, carboxyl, aldehyde, or

sulfhydryl groups. The surface of the transducer must be previously functionalized with an amino, carboxyl, hydroxyl, or other group. A review of conjugation techniques can be found elsewhere. Similar limitations with enzymes. Furthermore, binding may not be reversible and regeneration of the surface may require drastic changes in conditions like low pH, high ionic strength, detergents, etc. Therefore, efforts are being made to produce low cost, single use sensors. Probably the main potential advantage of traditional immunoassays is that they could allow faster and in-field measurements.

Microbes

The use micro-organisms as biological elements in biosensors is based on the measurement of their metabolism, in many cases accompanied by the consumption of oxygen or carbon dioxide, and is, in most cases, measured electrochemically. Microbial cells have the advantage of being can be more stable, and can carry out several complex reactions involving enzymes and cofactors. Conversely, they are less selective than enzymes; they have longer response and recovery times and may require more frequent calibration. Micro-organisms have been immobilized, for example, in nylon nets, cellulose nitrate membranes, or acetyl cellulose.

Transducer elements

Electrochemical, Amperometric and potentiometric transducers are the most commonly used electrochemical transducers. In Amperometric transducers, the potential between the two electrodes is set and the current produced by the oxidation or reduction of electro active species is measured and correlated to the concentration of the analyte of interest. Most electrodes are made of metals like platinum, gold, silver, and stainless steel, or carbon-based materials that are inert at the potentials at which the electrochemical reaction takes place.

However, because some species react at potentials where other species are present, either a selective membrane is used or an electron mediator that reacts at lower potential is incorporated into the immobilization matrix or to the sample containing the analyte.

Potentiometric transducers measure the cheaper than enzymes or antibodies, potential of electrochemical cells with very low current. Field effect transistors (FET) are potentiometric devices based on the measurement of potential at an insulator– electrolyte interface. The metal gate of a FET can be substituted by an ion selective membrane to make a Ph transducer (pH ISFET). Enzymes have been immobilized on the surface of such pH ISFET to produce enzyme sensitized field effect transistors (ENFET).

Optical Fiber, optic probes on the tip of which enzymes and dyes (often fluorescent) have been co-immobilized are used. These probes consist of at least two fibers. One is connected to a light source of a given wave length range that produces the excitation wave. The other, connected to a photodiode, detects the change in optical density at the appropriate wavelength Surface Plasmon resonance transducers, which measure minute changes in refractive index at and near the surface of the sensing element, have been proposed. Surface plasmon resonance (SPR) transducers have been proposed. SPR measurement is based on the detection of the attenuated total reflection of light in a prism with one side coated with a metal. When a p-polarized incident light passes through the prism and strikes the metal at an adequate angle, it induces a resonant charge wave at the metal/dielectric interface that propagates a few microns.

The total reflection is measured with a photodetector, as a function of the incident angle. For example, when an antigen binds to an antibody that is immobilized on the exposed surface of the metal the measured reflectivity increases. This increase in reflectivity can then be correlated to the concentration of antigen. (Kress-Rogers, E et.al.,)

Calorimetric transducers measure the heat of a biochemical reaction at the sensing element. These devices can be classified according to the way heat is transferred. Isothermal calorimeters maintain the reaction cell at constant temperature using Joule heating or Peltier cooling and the amount of energy required is measured. Heat conduction calorimeters measure the temperature difference between the reaction vessel and an isothermal heat sink surrounding it. Using highly conducting materials ensure quick heat transferred between the reaction cell and the heat sink. Finally, the most commonly used is the isoperibol calorimeter that also measures the temperature difference between the reaction cell and an isothermal jacket surrounding it. However, in this case the reaction cell is thermally insulated (adiabatic). This calorimeter has the advantage of being easily coupled to flow injection analysis systems. (Turner et al.,)

V. APPLICATIONS

One of the major driving forces for the development of biosensors is biomedical diagnosis. The most popular example is glucose oxidase-based sensor used by individuals suffering from diabetes to monitor glucose levels in blood. Biosensors have found also potential applications in the agricultural and food industries. However, very few biosensors have been commercialized.

Agricultural Industry

Enzyme biosensors based on the inhibition of cholinesterases have been used to detect traces of organophosphates and carbamates from pesticides. Selective and sensitive microbial sensors for measurement of ammonia and methane have been studied. However, the only commercially available biosensors for wastewater quality control are biological oxygen demand (BOD) analyzers based on micro-organisms like the bacteria Rhodococcus erythropolis immobilized in collagen or polyacrylamide.

Food Industry

Biosensors for the measurement of carbohydrates, alcohols, and acids are commercially available. These instruments are mostly used in quality assurance laboratories or at best, on-line coupled to the produced and measured b line through a flow injection analysis system. Their implementation in-line is limited by the need of sterility, frequent calibration, analyte dilution, etc. Potential applications of enzyme based biosensors to food quality control include measurement of amino acids, amines, amides, heterocyclic compounds, carbohydrates, carboxylic acids, gases, cofactors, inorganic ions, alcohols, and phenols. Biosensors can be used in industries such as wine beer, yogurt, soft drinks producers. Immunosensors have important potential in ensuring food safety by detecting pathogenic organisms in fresh meat, poultry, or fish.

VI. CURRENT RESEARCH AND TRENDS

Because in many cases the transduction technology is well established, most of the research is focused on improving immobilization techniques of the biological element to increase sensitivity, selectivity, and stability. While critical, the latter has received relatively little attention probably in part because there is a tendency to design disposable devices that are most useful in quality assurance laboratories but do not allow on-line implementation for process control.

Another dynamic area of research is miniaturization of sensors and flow systems. Development of these technologies is mainly driven by the need for in vivo applications for medical diagnosis and may not find immediate use in the agricultural and food industries. After almost 40 yr of research in biosensors, a wide gap between research and application is evident. The lack of validation, standardization, and certification of biosensors has resulted in a very slow transfer of technology. With faster computers and automated systems this process should accelerate in the future.

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