Biosensors

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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 require 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 a biosensor 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.”[1] The first biosensor, an enzyme-based glucose sensor, was developed by Clark and Lyons. [2] Since then, hundreds of biosensors have been developed in many research laboratories around the world. Over 200 research papers about biosensors have been published each year for the past three years.

The objective of this article is to review the principles of biosensor fabrication and operation, their existing and potential applications in the food and agricultural industries, and to briefly discuss recent research and future trends. For more comprehensive discussion on the topic, the reader is referred to several excellent books and reviews on which most of this article is based.[3–9]

TYPES OF BIOSENSORS

Biosensors can be grouped according to their biological element or their transduction element. Biological elements include 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.[10] 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.

Sensing Elements

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.[11] 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 60°C. Most of the enzymes used in biosensor fabrication are oxidases that consume dissolved oxygen and produce hydrogen peroxide [see Fig. 1(a)]. Enzymes have been immobilized at the surface of the transducer by adsorption, covalent attachment, entrapment in a gel or an electrochemically generated polymer, in bilipid membranes or in solution behind a selective membrane. Several reviews of enzyme immobilization have been published.[12–19] Enzymes are commonly coupled to electrochemical and fiber optic transducers.
Antibodies

Antibodies are 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.\textsuperscript{[20]} Antibodies share 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 immunosensors over traditional immunoassays is that they could allow faster and in-field measurements. Immunosensors usually employ optical or acoustic transducers.

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.\textsuperscript{[21]} Microbial cells have the advantage of being cheaper than enzymes or antibodies,
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,[22] and may require more frequent calibration. Micro-organisms have been immobilized, for example, in nylon nets,[21] cellulose nitrate membranes,[23] or acetyl cellulose.[24]

Other biological elements such as animal of vegetable tissue and membranes as well as organelles and nucleic acids have been researched but are out of the scope of this article. A summary of some biological elements and transducers used in the fabrication of biosensors is presented in Table 1.

**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 electroactive 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

**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 wavelength range that produces the excitation wave. The other, connected to a photodiode, detects the change in optical density at the appropriate wavelength [see Fig. 1(b)]. 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. The basic theory of SPR excitation and some examples of its application to biosensors are presented elsewhere.[25] A few SPR biosensors have been commercialized but no compact inexpensive portable device is available yet.

**Acoustic.** Electroacoustic devices used in biosensors are based on the detection of a change of mass density, elastic, viscoelastic, electric, or dielectric properties of a membrane made of chemically interactive materials in contact with a piezoelectric material. Bulk acoustic wave (BAW) and surface acoustic wave (SAW) propagation transducers are commonly used. In the first, a crystal resonator, usually quartz, is connected to an amplifier to form an oscillator whose resonant frequency is a function of the properties of two membranes attached to it. The latter is based on the propagation of SAWs along a layer of a substrate covered by the membrane whose properties affect the propagation loss and phase velocity of the wave. SAWs are produced and measured by metal interdigital transducers deposited on the piezoelectric substrate as shown in Fig. 1(c).[28]
**Calorimetric.** 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 [29] [see Fig. I(d)].

**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 [30]. 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. Standard BOD5 measurements in which the effluent is pretreated and exposed to bacteria and protozoa require incubation at 20°C for 5 day. In contrast, BOD biosensors have throughputs of 2 to 20 samples per hour and can measure 0 mg L\(^{-1}\) to 500 mg L\(^{-1}\) BOD. When coupled with automatic sampling they can be implemented on-line [30].

**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 processing 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 [31]. 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.

**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.

**REFERENCES**