ADVANCES IN BIOSENSORS

A Research Annual

Editor: ANTHONY P.F. TURNER
Biotechnology Centre
Cranfield Institute of Technology

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INTRODUCTION

Biosensors have captured the imagination of the world's scientific and commercial communities by combining the multidisciplinary skills of biologists, physicists, chemists and engineers to provide innovative solutions to analytical problems. Biosensors are applicable to clinical diagnostics, food analysis, cell culture monitoring, environmental control and various military situations. Ever increasing demands for rapid and convenient analyses of a wide variety of materials in diverse locations has led to intense interest in this fusion of biology and electronics which mimics our principal concern: the effect of materials and environments on living systems.

A biosensor may be defined as an analytical instrument containing a sensing element of biological origin, which is either integrated within or in intimate contact with a physicochemical transducer. The usual aim is to produce a continuous electronic signal which is directly proportional to the concentration of a chemical or set of chemicals present in a sample. Sensing elements may be biocatalytic (e.g. enzymes, organisms, tissues) or affinity systems (e.g. antibodies, nucleic acids, cell receptors). Optical, electrochemical, calorimetric or piezoelectric transducers are used to convert the biological recognition event into a processable signal. Regardless of the precise definition, however, tangible results of endeavour in this general technological area are become increasingly evident with an exciting range of novel analytical devices emerging onto the marketplace.

The series *Advances in Biosensors* presents a unique compendium of research-level publications, which do not have a place in conventional journals,

but have an increasingly important role to play in completing the primary research literature and offering a more incisive alternative to the full blown, exhaustive review article. In these papers represented by nine chapters in this second volume of the series, eminent authorities in the field of biosensors provide an up-to-date overview of their laboratory's contribution, summarizing the primary research as it has appeared, possibly scattered, in the journal and conference literature and reflecting on their findings. This produces an innovative synthesis of such smaller research efforts into an overall perspective on the topic, which is difficult for the reader to glean from the multifarious original publications. There is lattitude for the inclusion of detail that may have been excised from the original publication and for speculation on future possibilities. The net result is intense, yet highly readable accounts of the state of the art at this leading edge of analytical technology.

Cranfield, Bedford

Anthony P.F. Turner
Professor of Biosensor Technology
Series Editor

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CHEMICALLY CONSTRUCTED AMPEROMETRIC BIOSENSORS

Alexander M. Yacynych

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1. INTRODUCTION

A biosensor is a system of two transducers, biochemical and physical, in intimate contact with each other, which relates the concentration of an analyte to an electrical signal. The biochemical transducer converts the analyte into a chemical and/or physical property, which is sensed and converted into an electrical signal by the physical transducer [1]. Figure 1 shows this schematically.

Biosensors have recently received much attention. There are many reasons, two of which include the development of microelectronics and microcomputers. There has been a general increase in computerization and dedicated microprocessors have been applied to a variety of instrumentation. Prior to this, the ability to process information on a real-time basis was limited and the information provided by the available sensors was generally more than most instrumental systems could handle. Therefore, there was little impetus to develop more sensors. However, the computerization of even simple instrumental systems provided the ability to process large amounts of data quickly. Now the limiting factor is a need for a variety of sensors to meet the opportunities provided by computerization.

Another reason for the renewed interest in biosensors is the rapid growth in biotechnology. Increased research in this area requires new and better analytical techniques, including new and improved biosensors. New products arising from biotechnological research will require careful control and optimization of fermentations and other bioprocesses. It is hoped that biosensors will meet this need.

In addition, new physical transducer technology (e.g. fiber optics, integrated

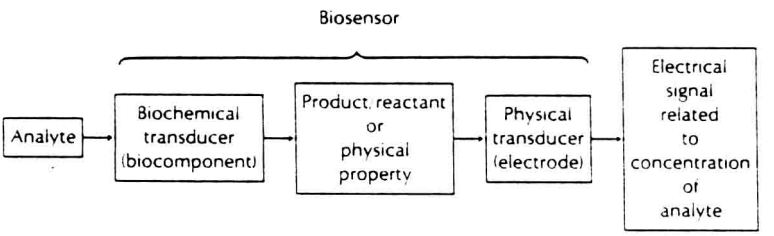

Figure 1. Schematic of a biosensor. (Taken from [137], with permission.)

microelectronic biosensors, piezoelectric crystals and surface acoustic wave methods), new methods of measurement (such as flow injection analysis) and the use of novel biological and biochemical systems (e.g. antibodies, bacteria, organelles, liposomes, receptors and tissues) have greatly expanded research linked to biosensors [2]. These developments have contributed to the increased interest in biosensors.

1.1 Biosensor Components

Table 1 shows various components and properties that are used for biosensors. An item is picked from each column, with the limitation that items from adjacent columns must be compatible. Biocomponents, which function as biochemical transducers, can be as varied as enzymes, tissues, bacteria, yeast, antibodies/antigens, liposomes, organelles and receptors. The biocomponent provides specificity or selectivity for the biosensor. Unfortunately, the excellent specificity of most biocomponents is usually compromised, to some extent, by the physical transducer.

Table 1. Biosensor components.

Analyte	Biochemical transducer (biocomponent)	Product/reactant or physical property	Physical transducer
Enzyme substrate	Enzyme	Product/reactant	Electrode/amperometric
Ion	Receptor	Capacitance	Electrode/potentiometric
Organic chemical	Organelle	Heat	Light/absorbance
Toxin	Liposome	Mass	Light/fluorescence
Drug	Tissue		ISFET
Protein	Antibody/ antigen		Thermistor
Antigen	Yeast		Piezoelectric
Antibody	Bacteria		Surface acoustic wave
Virus			
Bacteria			

There are a variety of physical transducers and one must be chosen to match the chemical or physical property produced by the biocomponent. The diversity of physical transducers includes electrochemical, spectroscopic, thermal, piezoelectric and surface acoustic wave technology. Biosensors are predominantly characterized by the physical transducer: for example, fiber-optic biosensors use the absorbance or fluorescence of light by a product formed or reactant depleted by the biocomponent.

This discussion will be limited to amperometric biosensors, which are one of two main categories of electrochemical biosensors, the other being potentiometric biosensors [1–8]. Potentiometric biosensors [9–11] relate the electrical potential to the analyte concentration, whereas with amperometric biosensors [12–14] the current produced during the oxidation or reduction of a product or reactant is proportional to the analyte concentration.

Amperometric biosensors are usually based on oxidoreductase enzymes, which commonly use oxygen, nicotinamide adenine dinucleotide (NAD^+) or nicotinamide adenine dinucleotide phosphate ($NADP^+$) as the electron acceptor which recycles the enzyme after the substrate reaction. Enzymes using oxygen as the electron acceptor are commonly called oxidases, while those using NAD^+ or $NADP^+$ are called dehydrogenases or reductases. Of these two groups, oxidase enzymes are the most commonly used as biocomponents.

A general reaction for many oxidase enzymes is shown below:

$$\text{Substrate} + O_2 \xrightarrow{\text{Enzyme}} \text{Product} + H_2O_2 \qquad (1)$$

The decrease in oxygen or increase in H_2O_2 concentration can be measured, both of which are proportional to the substrate concentration.

1.1.1 Oxygen-Based Biosensors

The first biosensors (also known as enzyme electrodes) used the Clark oxygen electrode as the physical transducer [15]. The Clark oxygen electrode is also known as a polarographic oxygen electrode, which is technically incorrect, because the term polarographic should only refer to electrochemical techniques using the dropping mercury electrode. Presumably this name was adopted because the reduction current of oxygen is limited by diffusion through a gas-only permeable membrane. The principle of diffusion-limited current is similar in polarography.

The oxygen electrode is generally constructed using a two-electrode system, e.g. a platinum cathode and a silver anode. A gas-only permeable membrane (e.g. Teflon) covers the tip of the cathode. A biochemical layer (most often an enzyme) is then placed on the oxygen electrode and secured with a coarse membrane, which allows the passage of analyte, thus forming a biosensor, as shown in Figure 2.

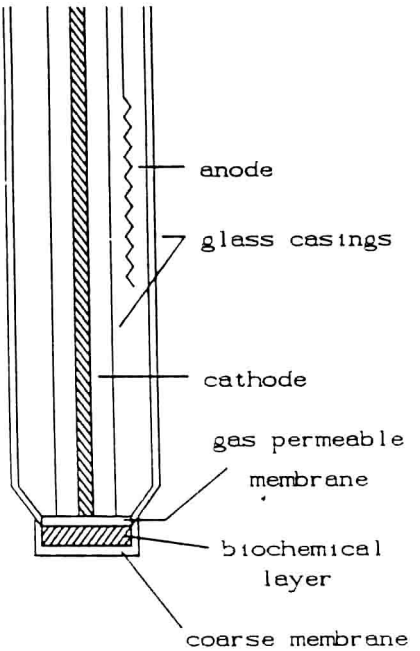

Figure 2. Oxygen-based biosensor. (Taken from [137], with permission).

A negative potential is applied to reduce oxygen at the cathode, as shown in Reaction (2).

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \qquad (2)$$

The reduction current is directly proportional to the oxygen concentration. In the presence of substrate, the enzymatic reaction decreases the oxygen concentration and the decrease in the oxygen reduction current is proportional to substrate concentration.

The principal advantage of an oxygen electrode-based biosensor is its excellent specificity. The specificity of the enzyme is not compromised by the oxygen electrode, because only gases can pass through the membrane and only substances that are reducible at the applied potential can interfere. The disadvantages of this type of biosensor are that it is sensitive to ambient oxygen concentrations and the gas-only permeable membrane can become blocked or clogged; this often requires the use of a second coarse membrane (e.g. dialysis membrane). As the membrane layers become thicker and more complex, the response and recovery time increases, and the biosensor may require more frequent calibration. The maintenance of calibration, for many biosensors, is not only dependent on enzymatic activity, but also on the constancy of the diffusional pathways for reactants and products. The thicker