Biosensors

Theory and Applications

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TECHNOMIC
PUBLISHING CO., INC.
LANCASTER • BASEL

Biosensors

a **TECHNOMIC** publication

Published in the Western Hemisphere by
Technomic Publishing Company, Inc.
851 New Holland Avenue
Box 3535
Lancaster, Pennsylvania 17604 U.S.A.

Distributed in the Rest of the World by
Technomic Publishing AG

Printed in the United States of America
10 9 8 7 6 5 4 3 2

Main entry under title:
Biosensors: Theory and Applications

A Technomic Publishing Company book
Bibliography: p.
Includes index p. 213

Library of Congress Card No. 92-61490
ISBN No. 0-87762-975-7

BIOSENSORS

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To my loving wife, Steffie,
whose moral support and spiritual understanding kept me going,
and to my sons, Jesse and Daniel,
whose love and encouragement made the effort worthwhile.

CHAPTER 1

Introduction

A biosensor may be broadly defined as any measuring device that contains a biological element. A schematic drawing for a generalized biosensor is shown in Figure 1.1. A target analyte (illustrated by solid circles) in the external medium must be able to enter the biosensor. The external membrane of the biosensor must be permeable to the analyte, and if possible, exclude other chemical species that the biosensor might also be sensitive to. The biological element inside the biosensor then interacts with the analyte and responds in some manner that can be detected by a transducer. The biological element may convert the analyte to another chemical species (represented by open circles) through a biochemical reaction; produce or release another chemical product in response to the analyte stimulus; change its optical, electrical or mechanical properties; or make some other response that can be reliably quantified. There may be another internal membrane near the transducer which might have different permeability properties than the external membrane. The output signal from a biosensor depends on the type of transducer it uses. The transducer may be a conventional electrochemical sensor, or may be based on another technology.

This book is an overview of the basic theories of operation for a number of specific types of biosensor transducers that have been investigated, with a general survey of some of the many applications using various biological elements that have been tested to date. A major portion of this book has been devoted to electrochemical transducers, since they have been most widely used. In Chapter 2, the theories for operating electrochemical transducers, including amperometric (current), potentiometric (voltage), and coulometric (charge) techniques will be described. Basic theories for conventional electrodes that measure pH, O_2, and other chemical analytes will be discussed further in Chapter 3. Modifications of these conventional

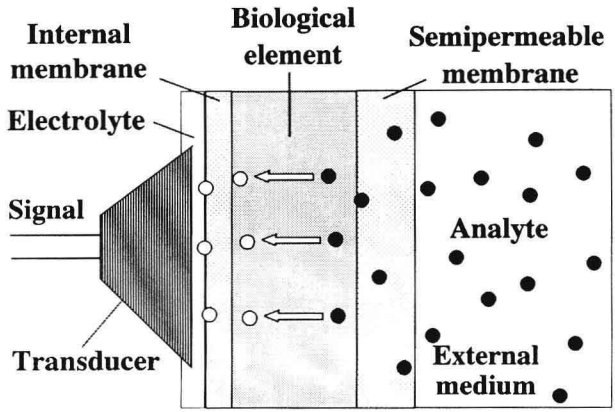

FIGURE 1.1. General principles for a biosensor. The specific chemical target (analyte) is recognized by the biological element, creating a stimulus to the detecting transducer from which a reproducible signal is measured.

electrodes by enzymes will be described in Chapter 4. Miniaturization techniques for electrochemical devices are discussed in Chapter 5. Optical technology will be discussed in Chapter 6, and other technologies in Chapter 7. The rapidly developing field of immunosensors, which allows detection of analytes by very highly specific molecular recognition, is surveyed in Chapter 8. Biosensors using a wide panorama of living biological elements are surveyed in Chapter 9. Finally, Chapter 10 discusses some areas where there are likely to be important applications for commercial biosensors in the near future.

1.1 BRIEF HISTORICAL BACKGROUND

One of the many landmark achievements in our modern era of scientific discovery was the understanding of acid/base chemistry. The Danish scientist Sørenson (1909) described his experimental procedures for determining hydrogen ion (H^+) concentration, defined the concept of pH, and standardized the pH scale. Furthermore, he investigated the effect of pH on rates of enzymatic hydrolysis. He correctly concluded that pH had very important effects on chemical and biochemical processes. By the early 1900s, the concept of electrochemical potential differences due to oxidation and reduction reactions was beginning to be recognized and the theory further refined. The hydrogen gas (H_2) electrode system, consisting of a platinum (Pt) electrode with an electrodeposit of Pt black to increase its surface area, became

the standard for electrochemical measurements. The reduction/oxidation (redox) potential for H^+/H_2 was defined as zero.

The new technology was quickly adopted for biological measurements. Michaelis and Davidoff (1912) used a Pt/H_2 pH electrode to measure the pH of lysed red blood cells. Using the same system, Michaelis and Kramsztyk (1914) measured pH in various mammalian tissues immediately after dissection, then later after boiling the tissues. Buytendijk (1927) was the first to use a solid metal antimony pH electrode for medical purposes. Today, the measurement of blood pH is a vital and routine clinical measurement, and we understand how important a role the H^+ ion plays in respiration and metabolism. Even so, there are still many unanswered questions with regard to the regulation of acid-base balance in the whole organism, in individual organs, and at the single-cell level. Also, there are many new types of pH sensors still under development using a variety of different technologies.

Another landmark achievement in science this century was the development of modern electrochemistry. Important new electrochemical techniques were discovered by Heyrovsky (1922) in the early part of this century in Prague, Czechoslovakia. He developed an instrument that used the surface of a growing drop of mercury to measure oxidation and reduction potentials for a number of different chemical species. This chemical sensor became known as a dropping mercury electrode polarograph. The electrode surface was formed by a continuous, slow stream of mercury through a glass tube, which formed a spherical drop that eventually dropped from the end. The mercury was recycled. A number of practical applications for the dropping mercury electrode polarograph were developed in his laboratory. During a visit to Stanford University in 1933, Heyrovsky presented his polarograph results to scientists in the United States. Subsequently, a large number of investigators began using this new electrochemical technology. Heyrovsky was the recipient of the Nobel Prize for chemistry in 1959 in recognition of his pioneering work.

Some of the early research using this technology was directed to measurements of O_2 concentrations in several different biological media. By monitoring the current from the dropping mercury electrode with a sensitive galvanometer, Müller and Baumberger (1935) measured O_2 in biological fluids. Their instrumentation allowed them to make more accurate measurements than previous scientists had been able to make. They found that the partial pressure (PO_2) in fluids could be measured within $\pm 1\%$ accuracy in the range from 10^{-3} to 1 atmosphere (1 atm = 760 mmHg = 101.3 kPa). Later, Baumberger (1938) used the dropping mercury electrode polarograph to determine the oxyhemoglobin equilibrium curve for O_2. Also, he was the first to measure skin PO_2. In another biological applica-

tion, Petering and Daniels (1938) used the mercury polarograph to measure O_2 consumption rates for algae, yeast, and blood cells. Beecher et al. (1942) attempted to use the polarograph clinically.

One of the major advantages of the dropping mercury electrode was that the surface was continually being renewed with each drop. Thus the detecting element was always fresh and did not depend on the prior history of measurements. However, there were a number of technical difficulties encountered by scientists using the dropping mercury electrode and large measurement artifacts were possible. The polarograph was not always stable for sufficiently long time periods, and required frequent calibrations. Electrode fouling was the most severe problem, and the mercury often needed to be cleaned before it could be reused for another measurement. Also, relatively large samples were required for the analysis, and the sample needed to be well mixed. Vibrating or rotating electrode systems were designed to maximize convective transport to the sensor. A significant drawback of the dropping mercury electrode for biological studies was the inherent toxicity of mercury to living organisms. It soon became clear that better measurement techniques were needed.

1.2 EARLY APPLICATIONS FOR NOBLE METAL ELECTRODES

The first steps towards the development of O_2 sensors began nearly a century ago in Göttingen, Germany. Ludwig Danneel (1897, 1898) scientifically investigated the current generated between two large Pt electrodes held at a 20 mV potential difference. The resulting current was found to be linear with O_2 concentration, but instabilities were observed and difficulties in making biological measurements were reported. Glasstone (1931) investigated the use of Pt electrodes for polarographic measurements in Heyrovsky's laboratory. Although he obtained favorable results, this technique did not receive as much early attention due to the success of Heyrovsky's polarograph.

Blinks and Skow (1938) replaced the dropping mercury electrode with Pt electrodes, and were successful in measuring O_2 evolution by algae during photosynthesis. They found a current-voltage plateau for O_2 in the polarization voltage range from -0.3 to -0.7 V (Pt cathode negative relative to the anode). They were the first to demonstrate that the limiting current at a constant voltage within this plateau range was linear with O_2 concentration from 0 to 99.5% O_2. Their bare cathode electrode had a response time of < 1 second. Further development of the polarographic method using gold (Au) and Pt metal electrodes took place during World War II in the laboratories of the Johnson Foundation at the University of Pennsylvania in Phila-

delphia. The principal scientists involved in this work were Philip Davies, Detlov Bronk, and Frank Brink, Jr. They were particularly interested in using O_2 electrodes to investigate O_2 metabolism of the brain and nervous system. To achieve the spatial resolution needed for meaningful physiological measurements, Davies and Brink (1942) developed the first true O_2 microelectrodes. However, the bare noble metal O_2 electrodes usually suffered from a gradual loss of sensitivity when exposed to blood and tissue, and measurement artifacts were encountered.

It was not until the modifications by Clark (1956), that the electrochemical detection of O_2 was reliably made. The Clark O_2 electrode has subsequently led to a period of rapid growth for biosensor applications in the medical and biological fields. A schematic drawing of the Clark electrode design will be shown in Chapter 3, along with a discussion of the general theory of O_2 electrodes. The simple modification that made this sensor more reliable than bare cathodes was the physical isolation of the cathode from the measurement medium. This was accomplished by placing a gas-permeable membrane over the cathode. The cathode was either a Pt or Au wire sealed in glass, with an Ag/AgCl wire anode. An electrolyte solution, typically 2 M KCl, was placed inside the body of the electrode housing to complete the electrical circuit between the anode and cathode. Early tests in blood, plasma, urine, and other solutions were successfully made using a polypropylene membrane. The Clark O_2 electrode quickly led to other research efforts that marked the birth of biosensor technology. As will be discussed further in Chapters 4 and 5, the Clark O_2 electrode and similar membrane-covered electrodes are vital components of many biosensors in current use.

1.3 EARLY OPTICAL METHODS

Britton Chance (1991) has recently reviewed the development of modern optical methods, especially with regard to spectrophotometric measurements in tissue. He also presented a historical perspective on the evolution of optical techniques beginning with the early work by Otto Warburg in Germany before World War II. Advances in electronics that had been made during World War II were successfully applied to optical instrumentation by Britton Chance at the University of Pennsylvania, Franz Jöbsis-VanderVliet at Duke University, Dietrich Lübbers in Germany, and many other scientists. The principle of using a differential, dual beam spectrophotometer had been developed by the English physicist John Tyndall in the late 1800s. This concept was applied to biological measurements by several laboratories, and a modified split beam spectrophotometer made commercially by

Beckman Instruments became an important optical instrument in many laboratories. Multiple channel devices for simultaneous scanning of more than one wavelength were designed and improved upon. A wide range of optical methods have become well accepted for biophysical and biochemical measurements. These techniques have also been adopted for use as optical transducers in biosensors.

1.4 IDEAL BIOSENSOR CHARACTERISTICS

The optimum design of electrochemical, optical, and other types of biosensors is dictated by several basic physical properties of the measuring system, as well as those of the media in which the measurement is made. Some of the most pertinent properties and characteristic behaviors of ideal biosensors are listed as follows.

1.4.1 Sensitivity

The sensitivity is usually defined as the final steady state change in the magnitude of the biosensor output signal with respect to the change in concentration of a specific chemical species ($\Delta S/\Delta C$), as illustrated in Figure 1.2. The target analyte is usually not directly detected by the biosensor. More often, changes in concentration of a co-reactant or co-product of a chemical reaction taking place within the biosensor are measured. The sensitivity of the biosensor with respect to the chemical substrate of interest

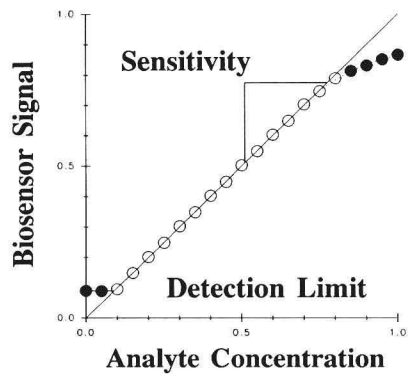

FIGURE 1.2. Calibration curve for a biosensor with a linear range (open circles), showing lower limit of detection and nonlinear behavior at higher concentration (solid circles). Sensitivity is defined as the slope of the linear range ($\Delta S/\Delta C$).

(the analyte) must then be related to the directly detected chemical species through the appropriate stoichiometry of the chemical reaction. In other cases, some physical property has been altered by the biological element, which is then measured by the transducer.

For some biosensor types, measurements are based on the dynamic response of the biosensor. Sensitivity may be defined in this situation as the change in the signal with time for a given change in concentration ($\Delta S/\Delta t/\Delta C$), or some other relationship that depends on time. Time integration, frequency analysis, or other data processing of the time varying signals may also be of value in relating them to the concentration of the analyte.

There are many factors that determine the effective sensitivity of a given biosensor design to a target analyte. These include the physical size of the sensor, the thickness of the membranes and resulting mass transport of chemical species from the sample to the sensing region, and various processes that deactivate the biosensor or otherwise impair its operation over time. Ideally, the sensitivity of a given biosensor should remain constant during its lifetime and should be sufficiently high to allow convenient measurement of the transducer output signal with electronic instrumentation.

1.4.2 Calibration

An ideal biosensor should be easily calibrated simply by exposing it to prepared standard solutions or gases containing different known concentrations of the target analyte. Calibration curves need not require many data points to obtain the sensitivity, especially if the operational behavior of the biosensor is known. Calibration points should bracket the range of values that will be measured, to avoid possibly unreliable extrapolations outside the expected range. Ideally, it should be necessary to perform a calibration procedure only one time to determine the sensitivity of the biosensor for subsequent measurements. In practical terms, however, it is usually necessary to make periodic calibrations at regular intervals to characterize changes in the sensitivity with time.

1.4.3 Linearity

A perfectly linear biosensor will have a constant sensitivity over the concentration range from zero to the maximum substrate concentration that can be physically dissolved in the measurement medium. Practically, the region of linearity may be restricted to a narrower range of substrate concentrations, as represented by the open circles in Figure 1.2. A two point calibration can be made anywhere in the linear range, allowing the measurements in this range to be reliably converted to accurate substrate concentrations.