Biosensors

TRAN MINH CANH

Head of Research
Ecole Nationale Supérieure des Mines
Saint-Etienne, France

Translated by
Sarah A. Jackson, *Ph.D.*

CHAPMAN & HALL

London · Glasgow · New York · Tokyo · Melbourne · Madras

Biosensors

TRAN MINH CANH

Head of Research
Ecole Nationale Supérieure des Mines
Saint-Etienne, France

Translated by
Sarah A. Jackson, *Ph.D.*

CHAPMAN & HALL

London · Glasgow · New York · Tokyo · Melbourne · Madras

Published by Chapman & Hall, 2-6 Boundary Row, London SE1 8HN

Chapman & Hall, 2-6 Boundary Row, London SE1 8HN, UK

Blackie Academic & Professional, Wester Cleddens Road, Bishopbriggs, Glasgow G64 2NZ, UI

Chapman & Hall Inc., 29 West 35th Street, New York NY10001, USA

Chapman & Hall Japan, Thomson Publishing Japan, Hirakawacho Nemoto Building, 6F, 1-7-11 Hirakawa-cho, Chiyoda-ku, Tokyo 102, Japan

Chapman & Hall Australia, Thomas Nelson Australia, 102 Dodds Street, South Melbourne, Victoria 3205, Australia

Chapman & Hall India, R. Seshadri, 32 Second Main Road, CIT East, Madras 600 035, India

English language edition 1993

Original French language edition - *Les Biocapteurs* - © 1991, Masson, Paris

Printed in France by Normandie Impression

ISBN 0 412 48190 1

A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication data available

Biosensors

Sensor Physics and Technology Series

Series editors:
Professor K T V Grattan
Centre for Measurement, Instrumentation and Applied Physics
City University
London UK

Dr A Augousti
School of Applied Physics
Kingston University
Kingston-upon-Thames, UK

The *Sensor Physics and Technology Series* aims to bring together in a single series the most important developments in the rapidly-changing area of sensor technology and applications. It will present a snapshot of the range of effort which is being invested internationally in the development of novel types of sensors. New workers in the area of sensor technology will also be catered for with an introduction to the subject through the provision of tutorial guides. Volumes may be sensor technology or applications oriented, and will present recent results from the cutting edge of research in a compact monograph format.

Topics covered will include:

- optical sensors: free-space sensors
- optical sensors: guided wave sensors
- solid state sensors
- biosensors
- microwave sensors
- ultrasonic sensors
- process tomography
- control of networked sensors (system control and data acquisition)
- medical instrumentation
- infrared sensors
- smart sensors
- chemical and biochemical sensing
- environmental sensing
- industrial applications

Titles available

1. Biosensors
   Tran Minh Canh

Preface

Of all the recent discoveries in biotechnology, that of biosensor is one of those which has seen an exponential expansion over the last few years. This evolution corresponds with the increasing need for measuring devices that can follow continuously changing biological processes. Biosensors can meet this need provided that their signals include all the information necessary for an understanding of the process, especially concerning the nature and concentration of the species present in the sample medium.

It is well known that sensors form the basis of all instrumental analysis systems, but they also represent the limiting factors of such systems. In this book, we restrict ourselves to the description and study of sensors, leaving aside the different aspects of signal and data treatment. We believe, however, that it is important to stress the multifaceted character of biosensors, and the applications and economic factors which follow.

Biosensor construction is essentially based on the immobilization of a bioreceptor on the corresponding transducer. The reader will find that there are a large variety of techniques for immobilizing enzymes, cofactors and mediators, and even microorganisms, immunoagents, tissues, and organelles. A large part of this book is devoted to enzyme sensors, which is hardly surprising considering that they have been extensively studied and are now commercially available. Other types of biosensors are discussed, with regard to both the principles of their operation, and their construction.

Biosensors form part of the science of engineering in both concept and application. The contents of this book form part of the Biotechnology option at the *Ecole des Mines* in Saint-Etienne, and at

the *Institut National Polytechnique* in Grenoble. Although this book is aimed at undergraduate students, the in-depth theoretical and technical discussion may also be useful to graduate students in the fields of biotechnology, biomedicine, analytical chemistry, or instrumentation. The bibliography will also give a research scientist, or a student preparing a thesis, a sound basis and an overview of the field.

The field of biosensors was born out of the collaboration between scientists from a variety of different disciplines: physicists, chemists, medical and pharmaceutical scientists, biologists, electronic engineers, computer scientists, mathematicians, and engineers. All of these scientists will be able to consult this work without any difficulty and find information to complement their original training. Any criticisms or remarks from these readers will be most welcome.

This book is dedicated to the memory of my parents. I am also grateful to my wife Josiane for typing and preparing the manuscript on the word processor. I particularly appreciated her cheerful acceptance of the extra time I needed for editing this book, especially as it occurred so quickly after the publication of the French monograph *Les Biocapteurs*. I also extend my appreciation to my sons Mân and Duc, and to my daughter-in-law Catherine, for their patience and spirit during the preparation of this book.

Abbreviations

AChE	acetylcholinesterase
ADH	alcohol dehydrogenase
ADP	adenosine diphosphate
AFP	alpha-fetoprotein
AMP	adenosine monophosphate
APTES	aminopropyl triethoxysilane
ATP	adenosine triphosphate
BOD	biological oxygen demand
BPT	benzopyrenetetraol
BSA	bovine serum albumin
Con-A	concanavalin A
D-AAO	D-amino acid oxidase
DB18C6	dibenzo-18-crown-6
DIFP	diisopropylfluorophosphonate
DIMP	diisopropylmethylphosphonate
DNP	dinitrophenol
EDTA	ethylene diamine tetraacetic acid
EIA	enzyme immunoassay
ELISA	enzyme linked immunosorbent assay
ENFET	enzyme field-effect transistor
FAD	flavin adenine dinucleotide (oxidized form)
$FADH_2$	flavin adenine dinucleotide (reduced form)
FAPAPP	ferrocenyl amidopentyl amidopropylpyrrole
FAPP	ferrocenyl amidopropylpyrrole
FET	field-effect transistor
FITC	fluorescein isothiocyanate
FMN	flavin mononucleotide (oxidized form)
$FMNH_2$	flavin mononucleotide (reduced form)
GA	glutaraldehyde
GABA	γ-aminobutyric acid
GASFET	gas-sensitive field-effect transistor
GOD	glucose oxidase

HABA	2[(4-hydroxyphenyl)azo] benzoic acid
HCG	human chorionic gonadotropin
HPLC	high pressure liquid chromatography
HPTS	8-hydroxy-1,3,6-pyrenetrisulfonic acid
HSA	human serum albumin
IgG	immunoglobulin G
IMFET	immuno-field-effect transistor
ISE	ion-selective electrode
ISFET	ion-sensitive field-effect transistor
L-AAO	L-amino acid oxidase
LDH	lactate dehydrogenase
LED	light emitting diode
MOS	metal oxide semiconductor
MOSFET	metal oxide semiconductor field-effect transistor
NAD^+	nicotinamide adenine dinucleotide (oxidized form)
NADH	nicotinamide adenine dinucleotide (reduced form)
$NADP^+$	nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NMA^+	N-methyl acridinium
NMP^+	N-methyl phenazine
P	inorganic orthophosphate
PAM	pyridine aldoxime-methyl
PMS	phenazine methosulfate
PP	inorganic pyrophosphate
ppb	parts per billion ($1/10^9$)
ppm	parts per million ($1/10^6$)
PTFE	polytetrafluoroethylene
PVC	polyvinylchloride
Q^+	quinolinium
REFET	reference field-effect transistor
SAW	surface acoustic wave
SCE	standard calomel electrode
SPR	surface plasmon resonance
$TCNQ^-$	tetracyanoquinodimethane
TELISA	thermometric enzyme linked immunosorbent assay
$TMPA^+$	trimethylphenylammonium
TPA^+	tetrapentylammonium
TTF^+	tetrathiafulvalene
VDRL	venereal disease research laboratory
XO	xanthine oxidase

Contents

1

Introduction

1.1 Biosensors - nature's tools?

Living beings develop and evolve by exchange and communication with their environment. The information gathered enables them to meet their needs, reproduce or survive. Messages to their various senses are received at receptor sites and used to locate food, detect danger, or find a sexual partner. Some bacteria possess specific chemical receptors which, through a positive or negative chemotactism, enable them to locate nutritious substances and distance themselves from poisons.

Man has long dreamt of employing these wonderful tools, biological sensors, to observe and understand his environment. For the moment, however, he must be content with copying nature, and use the arsenal of cells, tissues, proteins, and enzymes that nature has provided to create measuring devices which convert an observed event into a measurable quantity. As a result of this biomimicry, the sensors he creates are given the name *biosensors*. Biosensors not only help him to examine and understand biological processes, but also have numerous industrial and medical applications. The biological medium is continually evolving and thus requires continuous monitoring. This is impossible via traditional sampling methods, and only biosensors can meet such a requirement. Sensors are well known in medicine for the determination of pH, the partial pressure of oxygen or carbon dioxide. Their eventual employment in intensive care or in surgery could give a constant measure of other important biological parameters such as the concentration of glucose, urea, or other metabolites.

1.2 Historical development

In 1962, Clark and Lyons [1] first mentioned the possibility of using enzyme-containing membranes to transform urea or glucose into a product that was detectable with a pH or oxygen electrode. Later, in 1967, Updike and Hicks [2] prepared an enzyme electrode by polymerizing a gel containing glucose oxidase onto an oxygen electrode. When this electrode is placed in contact with a biological solution containing glucose and oxygen, the two compounds diffuse into the enzymatic membrane. The electrode then oxidizes the glucose into gluconic acid using the oxygen in the solution. The oxygen electrode detects the reduction in oxygen partial pressure, which is proportional to the glucose concentration. This discovery was a decisive step in biological analysis. Electrochemistry only provides sensors that detect anions or cations, but by associating them with a biological system, a multitude of other substances can be detected, which would otherwise only be measurable via long and fastidious procedures. Thus, a new class of sensors was created, biosensors. They were born out of the combination of existing sensors, such as amperometric, potentiometric, thermal, piezoelectric, acoustic and optical sensors, and biological systems, such as enzymes, cells, microorganisms, chemical receptors, and immunological agents.

Naturally, the first enzyme fixation methods [2] involved physical retention, and it was attempted to preserve, as far as possible, the integrity of the biological system. It was later realized that covalent fixation would prevent enzyme loss, and ensure long-lasting immobilization. The immobilization of enzymes is of prime importance. So long as the enzymes remain active, their immobilization enables repetitive and multiple determination. Conventional enzymatic methods discard the enzyme after each separate sampling. Enzymes are expensive because of the various extraction and purification stages they require, and we can immediately understand the economic interest of the new procedure, and its impact on the cost of sample analysis in automated systems.

The immobilization of more than one enzyme on a single support was another important step in the development of enzyme sensors [3]. A single enzyme is not always sufficient to transform the substance under study into a substance that is detectable by a transducer. It is often necessary to resort to several enzymes to perform a series of

transformations; the product of the first reaction serving as the substrate for the second, and so on [4]. In some cases, the enzyme cofactor, for example, NAD, is not associated with the enzyme and will not be attached to the support during the immobilization of the enzyme. A second reaction must therefore be designed to attach the cofactor to the support, and provide it with the mobility required to reach the active site of the enzyme [5].

The immobilization of mediators has recently been achieved. These compounds, such as ferrocene or the organic conducting salts of tetracyanoquinodimethane (TCNQ), can be immobilized on conducting polymers like polypyrroles [6]. Mediators electrochemically reoxidize the cofactor thereby preventing the dependence of enzyme sensors on the oxygen concentration. In the glucose-sensitive electrode without a mediator, for example, oxygen would be essential for the regeneration of the cofactor FAD during the oxidation of glucose. The use of mediators thus represents an important contribution in biomedical analysis, notably for in-vivo measurements where the oxygen concentration is variable. When mediators are present, the biosensor can easily function in the absence of oxygen, which is vital for the study of anaerobic fermentation processes. The regeneration of mediators, however, requires amperometric electrodes to provide the necessary current for the reoxidation of the cofactor.

Enzyme sensors can also be prepared using field-effect transistors. Such biosensors are constructed by attaching an enzymatic membrane to the insulating layer of a semiconductor structure [7]. Transistors are normally sensitive towards protons, but in the presence of enzymes they may also detect organic substances, such as urea or penicillin. They also have the advantage of a low output impedance, a high signal to noise ratio, and a low price because they can be mass produced. This means that biosensors with a one-time use can be envisaged.

A large technological expansion has also occurred in the area of fiber optics. Improvements have been made in the optical quality and mechanical resistance of optical fibers, and they now have many applications in transducers. Optical fibers lend themselves to the construction of biosensors; a suitable biological substance is attached to the tip of the fiber and produces an optical signal when it is in contact with the system under study [8]. Optical biosensors exploit a variety of radiation phenomena, for example, absorption, fluorescence, and