Biosensors & Chemical Sensors

Optimizing Performance Through Polymeric Materials

EDITED BY

Peter G. Edelman and Joseph Wang

ACS Symposium Series 487

ACS SYMPOSIUM SERIES 487

Biosensors and Chemical Sensors

Optimizing Performance Through Polymeric Materials

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Developed from symposia sponsored
by the Divisions of Polymeric Materials: Science and Engineering
and of Analytical Chemistry
at the 201st National Meeting
of the American Chemical Society,
Atlanta, Georgia,
April 14–19, 1991

American Chemical Society, Washington, DC 1992

Library of Congress Cataloging-in-Publication Data

Biosensors and chemical sensors: optimizing performance through polymeric materials / Peter G. Edelman, Joseph Wang, eds.

p. cm.—(ACS symposium series, 0097–6156; 487)

"Developed from symposia sponsored by the Divisions of Polymeric Materials: Science and Engineering and of Analytical Chemistry at the 201st National Meeting of the American Chemical Society, Atlanta, Georgia, April 14–19, 1991."

Includes bibliographical references and indexes.

ISBN 0–8412–2218–5

1. Biosensors—Congresses. 2. Membranes (Technology)—Congresses. 3. Polymers—Congresses.

I. Edelman, Peter G., 1956– . II. Wang, Joseph, 1948– . III. American Chemical Society. Division of Polymeric Materials: Science and Engineering. IV. American Chemical Society. Division of Analytical Chemistry. V. American Chemical Society. Meeting (201st: 1991: Atlanta, Ga.) VI. Series.

R857.B54B552 1992
681'.2—dc20 92–6330
CIP

The paper used in this publication meets the minimum requirements of American National Standard for Information Sciences—Permanence of Paper for Printed Library Materials, ANSI Z39.48–1984. ♾

PRINTED IN THE UNITED STATES OF AMERICA

ACS Symposium Series

M. Joan Comstock, *Series Editor*

1992 ACS Books Advisory Board

Foreword

THE ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that, in order to save time, the papers are not typeset, but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the editors with the assistance of the Advisory Board and are selected to maintain the integrity of the symposia. Both reviews and reports of research are acceptable, because symposia may embrace both types of presentation. However, verbatim reproductions of previously published papers are not accepted.

Preface

BIOSENSORS CONTINUE TO BE a field of exciting research. This fact was exemplified at the symposium on which this book is based, where 80 biosensor presentations were spread through seven symposia. Many challenges have been conquered in this field of research, and yet these successes seem overshadowed by the challenges that remain. The widespread optimism of 10 or 15 years ago has been replaced by savvy realism. Many significant transducer developments have been realized, but transducer-enhancing membranes remain the Achilles heel limiting practical applications. Developments in miniaturization of transducers may have progressed, but technology to apply polymers to modify these tiny interfaces has lagged behind.

Research in this field has escalated recently because of a growing awareness of the inherent complexities and limitations of polymers at interfaces. The polymer must fulfill stringent design rules:

- The polymer must possess a desired permselectivity or other attributes.
- The permselectivity or other attributes must not change with time.
- The polymer must be applied in reproducible fashion to the transducer.
- This membrane must remain adhered to the surface to extend the useful life of the sensor.
- The polymer should be compatible with the incorporated biological entity.
- For sensors in contact with blood, not only must the membrane not be fouled in the complex matrix of proteins and lipids, but also the membrane must protect the transducer from fouling.

There are further membrane requirements for a nonthrombogenic in vivo device.

Whereas the focus of this book is polymers for sensor applications, this book is also about interfaces:

- the interface between two multidisciplinary fields (analytical chemistry and polymer chemistry),
- the interface between sample and membrane, and
- the interface between membrane and transducer.

To impart added functionality and reactivity to a sensitive transducer, the interface between transducer and sample can be modified with a polymer. The success of all these interfaces is critical to sensor development and performance.

Research in this discipline is important because of the potential to accelerate the materials development for sensors by applying what has already been learned in other fields, such as electronics, aerospace, and biomaterials. By combining an understanding of sensor issues with a broad understanding of how polymer problems have been solved in other fields, polymer development for specific sensor applications will advance more rapidly. The logical progression from materials development by trial and error is to tailor new materials systematically for each specific application, based on understanding of material science.

In putting the symposium together for the Division of Polymeric Materials: Science and Engineering (PMSE), it was anticipated that the authors would discuss in detail correlations of polymer properties with sensor performance. However, there is still incomplete understanding of material properties, making correlations to sensor performance difficult. This fact makes it even more difficult to go one step further and tailor new materials to meet the design requirements better. This lack of understanding is undesirable, but it creates opportunities for exciting possibilities for biosensor-related materials research. This book captures a moment in time and delineates the current state of the art. It will be interesting to look back at this book in 10–15 years to measure our progress.

This book is intended for two audiences. The first includes those who are involved in sensor research who have polymer-related problems and need to find some potentially elegant remedies. The second audience includes polymer scientists who are looking for a challenging discipline in which to practice their arts. Many of the solutions already worked out for polymers used in electronics or biomedical applications may be adapted to polymers for sensors. As these scientists become more directly involved with sensor-related materials development, better solutions to problems may be realized.

Many other volumes on sensors have been compiled that deal with novel aspects of sensor-related research. This collection is unique in that it is the first sensor book that focuses strictly on polymer and membrane-related research for sensor fabrication.

There are four main goals in putting this book together:

1. to bring together in one volume the current state of materials development for biosensors,
2. to heighten the awareness in the polymer science community of the challenges and opportunities inherent in biosensor development,
3. to emphasize the importance of materials development for biosensor advancement, and
4. to emphasize the necessity for interdisciplinary efforts.

Most of the chapters in this book were presented in symposia organized by two divisions. The majority are from the PMSE division, and most of the remainder are from the Division of Analytical Chemistry. In addition, there is one chapter each from the Divisions of Agriculture and Food Chemistry and of Biological Chemistry. We are fortunate that many excellent authors have agreed to include their works. Three chapters not presented at this symposium were added to round out the volume.

We gratefully acknowledge financial support from Ciba Corning Diagnostics Corporation and the PMSE division. We also thank all those without whose efforts this book would not be possible, especially the authors, reviewers, and the very supportive staff of ACS Books.

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January 21, 1992

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POLYMER MEMBRANES ON PLANAR SUBSTRATES

HYDRATION-DEPENDENT POLYMER APPLICATIONS

Chapter 1

Overview of Biosensors

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Biosensor models have been investigated utilising enzymes, antibodies/antigens, and other cellular components, or even whole cells, to provide a recognition surface for an analyte of diagnostic interest. The realisation of the model brings together many technologies, the components of which must often be studied individually before their union can be achieved. This overview considers the transduction of the analytical parameter and some factors which influence the materials and reagents of the transduction.

Biosensors . . . what does this imply? Traditional chemical and biological analytical methods involve a reaction which takes place in solution following the addition of reagents and sample. A biosensor is frequently described as a 'reagentless' system, but strictly speaking, it is more correct to say that the reagents are already part of the reaction chamber and do not therefore have to be added by the user. It follows from the implications of this statement that Biosensors will most likely be concerned with immobilised reagents - that is to say with reactions at surfaces and their interrogation. To adopt a well utilised definition however, biosensors comprise an analyte selective interface in close proximity or integrated with a transducer (figure 1), whose function it is to relay the interaction between the surface and analyte either directly or through a chemical mediator.

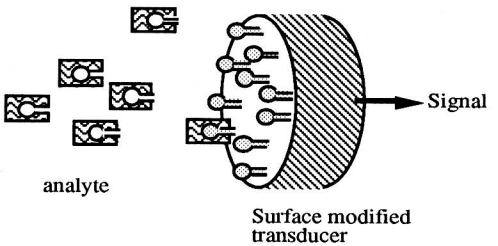

Figure 1. The Biosensor concept

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The nature of the transducer and the transduced parameter will depend on the type of bioanalytical event concerned with detection of the analyte - that is to say, that a system designed for immunoassay where antibody-antigen binding must be detected is unlikely to be appropriate to an enzyme linked redox reaction! However, since several parameters can often alter during an analytical reaction pathway, the choice of device is not necessarily restricted to a single transducer. This book is concerned primarily with the developments of materials appropriate for the realisation of biosensing transduction devices in major areas of this ever broadening field. The nature of the parameters concerned are summarised in figure 2.

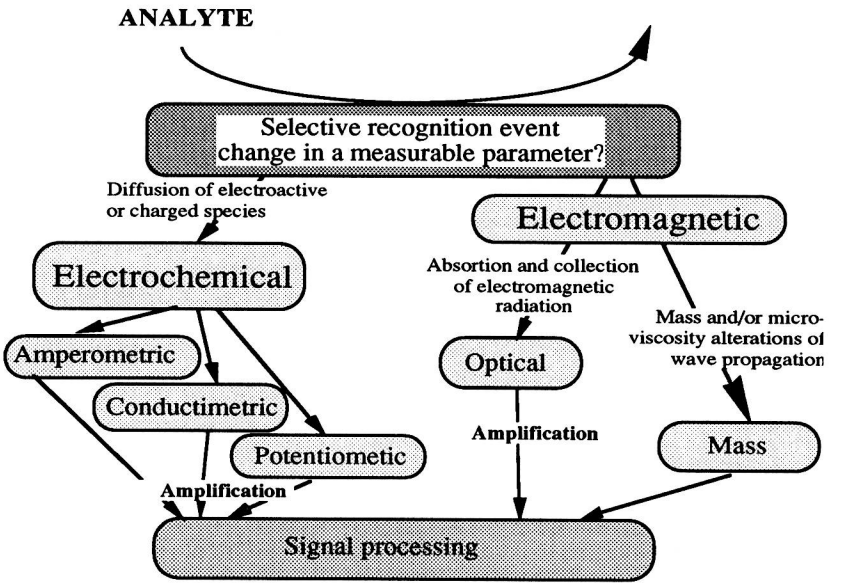


Figure 2. Transduction parameter and device type

Choice of Transduction Parameter

Amperometric. Discounting the centuries old use of live birds or fish to detect the sudden presence of toxins in air or water respectively (the species dies, thus 'transducing' an alarm signal!) the first report of a biosensor can probably be attributed to Clark & Lyons (1) who described an 'enzyme electrode' using glucose oxidase as a selective biorecognition molecule for glucose. The enzyme was held next to a platinum electrode in a membrane sandwich. The Pt electrode, polarised at +0.6V vs SCE, responded to hydrogen peroxide, produced by the enzyme reaction (Scheme 1):

$$\text{Glucose} + O_2 \xrightarrow{\textit{glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

Scheme 1

This lead to the development of the first glucose analyser for the measurement of glucose in whole blood (Yellow Springs Instrument 23 YSI, 1974).

In the early days of this first device, the major effort in biosensor research was probably concerned with amperometric systems utilising redox enzymes. This is perhaps hardly surprising, since nowhere better in available biosensor formats, is the reaction parameter, namely the electron transfer concerned with the redox enzyme, better matched with the transduction parameter - i.e. electron transfer at the electrode. Nevertheless, as can be deduced from the scheme above this can hardly be a straightforward electrode transfer between enzyme and electrode, since the signal that was monitored is that due to the oxidation of a product of the transformation - hydrogen peroxide. Looking at the scheme more closely, the electrode fails to compete with the natural oxidant (molecular oxygen) and the transduced electrode signal is therefore an indirect one, rather than one due to the direct oxidation of enzyme.

In practical terms, as far as a biosensor is concerned, this may be of no consequence. However, utilising H_2O_2 as a measurand requires a constant supply of oxygen (hardly ideal for low or fluctuating O_2 environments) and a measuring potential (+0.6V *vs* SCE) where many interferents (eg Vitamin C) can add to the signal.

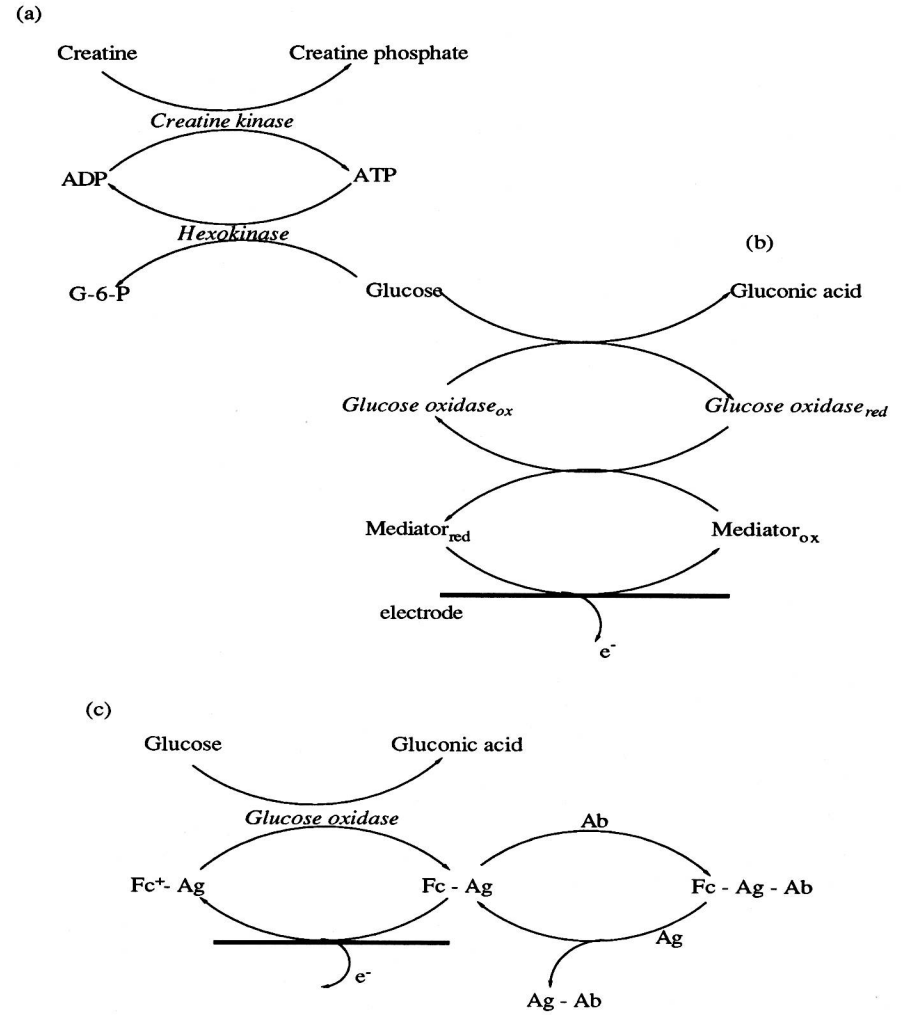
Mediators. Since direct electrode transfer between enzyme and electrode can rarely be performed with repeated efficiency, it was obvious that a synthetic replacement must be found for oxygen, and there thus began the hunt for modified electrodes and enzyme mediators. Many electron acceptor molecules and complexes have been considered for the role of mediator. Amongst the favourites ferrocene (η^5-bis-cyclopentadienyl-iron) and its derivatives still attracts attention - almost as a redox mediator standard (*2,3*).

The cyclic reaction processes which result from the use of such systems (scheme 2b) make them particularly attractive for deployment in a biosensor, since the only molecule which is now consumed by the sensor is the analyte itself and the reagents are regenerated within the assay.

Optimisation of one enzyme linked assay in this manner can also open doors to adjacent schemes and have implications beyond the analyte of original interest. For example schemes 2a/b show how the original system can be applied in a 'competition' for glucose with other enzymes using glucose as a substrate, and thus leading to an assay for say creatine, hexokinase or creatine kinase (*4*). Where the mediator is also an antigen label it can be employed in an enzyme linked immunoassay (scheme 2c). This latter scheme (*5*) depends on electrochemical and mediator inactivity of the ferrocene labelled antigen-antibody complex and the activity of the uncomplexed labelled antigen.

Such mediator-linked assays, even at the simplest original one enzyme level can seem an elegant solution for a biosensor. Nevertheless, optimisation of the reagents alone is not the complete solution. As discussed earlier, the reagents must be immobilised to interact with both analyte and transducer as a self contained system, before the 'biosensor' label is attached. This prerequisite is far from trivial and is a major preoccupation in all branches of biosensor development.

Cells and Cellular Components. Even in this narrow amperometric biosensor field the reagents may be of widely different natures. In particular, the very nucleus of the analyte selective reaction is not always an isolated enzyme, but may be a whole cell (*6*) or cellular component. Microbial biosensors have been described with a specificity for analytes ranging from CO_2 to Vitamin B. Tissue preparations have also been described utilising sources which appear limited only by the imagination: banana pulp, for example contains a high concentration of polyphenol oxidase which can be used in the estimation of dopamine and catechols (*7*). In the majority of instances these cell based devices have been based on a change in the O_2 tension monitored at an electrode mounted behind the cell layer. Like the H_2O_2 measurement already described, this is an indirect signal. Also like the isolated enzyme counterparts,



Scheme 2. Reaction pathways utilising a redox enzyme linked amperometric measurement via a redox mediator. (b) assay for glucose; (a)+(b) assay for creatine, creatine kinase or hexokinase; (c) competitive immunoassay using Fc-labelled antigen.

artificial redox mediators have been investigated, but in this instance transport is restricted by the cell walls and membranes. A considerable variation in efficiency of mediation has often been noted, even between similar mediators and the same organism, or related organisms and the same mediator; factors such as lipophilicity and surface interactions can also play a more important role than in enzyme preparations.

Cell preparations are also of use in some instances where the specificity of an enzyme linked assay is not required or the nature of the analyte makes it an unsuitable solution.

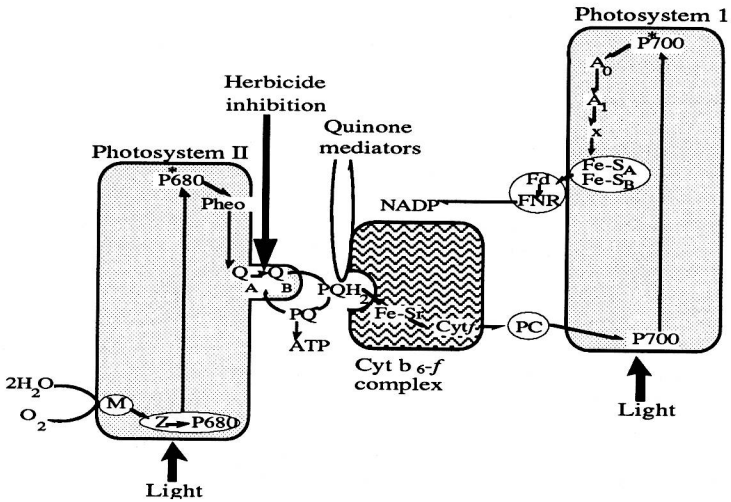


Figure 3. The Photosynthetic electron transport chain (PET)

An elegant example of this is the monitoring of herbicide residues via the photosynthetic electron transport (PET) pathway by utilising cyanobacteria or thylakoid membranes (*8*). For many herbicides the mode of action is as inhibitors of PET, often acting between the 2 photosystems as indicated in figure 3, and the result is a decrease in the photocurrent.

Within the boundaries of a biosensor, immobilisation of both the bacteria and thylakoid membranes together with a redox mediator, capable of charge transfer between PET and electrode, has allowed detection of some herbicides <10ppb (Martens, N. and Hall, E. A. H., Institute of Biotechnology, University of Cambridge, unpublished result). The advantage of this system is that it is not specific to a single herbicide but for any PET active toxin. In an area such as pesticide monitoring where the compounds have not been developed with predetermined properties, but are more the result of random screening, such a solution is more attractive than one which requires the development of many individual, unrelated devices in order to detect several compounds . . . and then probably finally fails to detect the actual herbicide in use!

Potentiometric. Not all analytes can be readily assayed via a redox enzyme and in these instances the assay schemes suggest parameters other than electron transfer which may be probed. Indeed, even where a redox enzyme is available for the analyte in question, it is not always desirable to deploy an amperometric technique.

Potentiometric measurements have been most frequently developed around pH sensitive electrodes and the same analytical reagents have been used in pH-FETs. Any of the enzyme pathways which result in a change in H^+ can be applicable here, but the most studied routes are those involving penicillinase or urease:

$$\text{Penicillin} \xrightarrow{\textit{penicillinase}} \text{Penicilloate} + H^+$$

$$\text{Urea} \xrightarrow{\textit{urease}} NH_4^+ + HCO_3^-$$