Biosensor Technology

Fundamentals and Applications

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Preface

The availability of new technology and better communications among engineers, physicists, biologists, and chemists has set the stage for rapid and important advances in the development of biosensors. This new biosensor technology has allowed new applications of classical ideas for biosensors by permitting use of new structures and input functions to probe the transfer functions of systems (contributions by physicists and electrical engineers), development and use of new materials (contributions by chemists), development of specialized and unusual chemistry (contributions by immunologists), and development of new formats for sensors especially for in vivo applications (contributions by physiologists). Those who use the new technologies are stimulating the new research on biosensors.

Although sensors have been used in biological applications for centuries, it was after the discovery of the prototype chemically sensistive semiconductor device that electrical engineers and solid state physicists took up the chemical sensor field and continued the physical sensor developments. Their contributions have been decisive in microminiaturization, especially fabrication of small versions of already practical devices. Similarly, the contributions of physicists in designing quartz crystal piezoelectric devices have expanded the chemical sensor field greatly.

Optical physics has been employed advantageously in sensors. An example is the development of optical absorbance change or luminescence sensors interrogated by probe beams and read by emitted light through optical wave guides such as optical fibers of plastic or glass. Sensors using specially coated optical wave guides, including those that modulate the evanescent wave depending on chemical reactions with the exterior coating, are another development of advanced technology.

Contributions by physiologists and clinical chemists are not so clearly recognized, but they are very important when judged in terms of commercialization by both large and start-up companies. These contributions include the in vivo optical sensors, the micro-encapsulated pH sensing dyes called optodes, and the closely related optrodes. Conversion and specialization of conventional electrochemical sensors into physiologically useful sensors has been a technological triumph. Prior to studies by electrochemists of carbon fiber microelectrodes, microfabricated dots, hemispheres and lines, electrophysiologists had already made 0.1 μm tip capillaries as junction-type reference electrodes and ion exchanger-filled tips for ion assays within single cells.

Symposia and conferences are exceptionally important in a field that employs such a diverse group of scientists. This book contains the proceedings of the International Symposium on Biosensors: Fundamentals and Applications, which was held on the campus of the University of North Carolina at Chapel Hill on September 7-9, 1989. The program was designed to emphasize the physics, engineering, analytical chemistry, and biochemistry (including immunochemistry) as they exist now, and as they may make contributions to sensors technology in the 1990s. Fundamental issues of sensitivity and selectivity were emphasized wherever possible.

The initial session of the symposium was devoted to tutorials on fundamentals of electrochemical, microelectronic, optical and piezoelectric devices. The presentations included:

Richard P. Buck "Electrochemical Sensors and Biosensors; Some Connections with Optical Chemical Sensors"

Jay N. Zemel "Microfabricated Chemical Sensors"

Raymond E. Dessy "Swords into Plowshares - the Way Waveguide, SAW, and Piezoelectric/Pyroelectric Sensors Work"

In addition to the tutorial lectures mentioned above, the program included a number of invited lectures and contributed poster presentations. Manuscripts accompanying many of these constitute these proceedings, although some authors did not elect to submit manuscripts. All of the manuscripts that were received were read by impartial reviewers, and the authors of acceptable manuscripts had the opportunity to make

revisions and corrections in view of the comments by the reviewers. The editors wish to thank the reviewers for their help.

The book consists of four sections. Following an introduction to the field of biosensor technology, there are four chapters devoted to microelectrodes and microelectronic devices. The next section consists of ten chapters on modified electrodes, amperometric, and potentiometric sensors, and the final section of fourteen chapters deals with optical and acoustic wave-based sensors.

The symposium was generously supported by the NC Section ACS, the North Carolina Biotechnology Center,[1] the United State Army Research Office,[2] the North Carolina Microelectronics Center, and Akzo Corporate Research America. The NC Section ACS and the participants in the symposium wish to thank these organizations for their support and for the opportunity to assemble many of the leaders in research on biosensors for presentations of the most recent results on the fundamentals and applications of biosensors.

The editors and symposium organizers also wish to thank Drs. Steve Bobbio, Jack Buchanan, Len Geddes, and Susan Schiffman for their valuable assistance. We also wish to express our especial appreciation and gratitude to Mrs. Linda Caulder for her dedicated efforts which led to a successfully organized symposium and a promptly edited book containing the proceedings of the symposium.

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[1] Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views and policies of the North Carolina Biotechnology Center.

[2] The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

Contents

*Invited speakers are indicated by bold letters.

I
Introduction

1

Sensor Issues for the 1990s: An Introduction to the North Carolina Section American Chemical Society Symposium on Biosensors

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WHY SENSORS NOW?

Analytical chemists have done research on, made and used sensors for years. Ion-sensitive and redox- sensitive electrodes have been known and used for 100 years...since the days of Nernst. Membrane-based glass electrodes were invented in 1906; rudimentary ion selective membrane electrodes appeared soon after synthetic, polymer-based ion exchanging materials were invented in 1935. Continuous electrolytic (coulometric) gas analyzers for SO_2 were made by 1949. Practical amperometric sensors have been around at least since the Keidel Cell Moisture Monitor (continuous electrolytic water monitor of the 1950s). The Clark amperometric ambient and dissolved oxygen partial pressure sensor has been known, commercialized and adapted to various biochemical and clinical analyses for many years.

High temperature, solid state electrochemical cells have been known from the detailed analyses of Wagner in the 1950s, and applications of defect, ion conducting oxide cells to fuel cells and the sensing of ambient oxygen have been known equally long. Optical chemical sensors (color-forming or bleaching reactions, shifts of absorption maxima by dielectric change or stabilizing reactions with ground or excited states) were made as soon as inexpensive spectrophotometers became available in the late 1930s

and especially 1940s. Practical, small physical sensors for temperature, strain, acceleration, velocity and much more, were available during and after World War II.

So...why sensor research now? The answer is compounded from availability of new technology (not specifically chemical) and better communications among engineers, physicists, biologists and chemists than ever seemed possible before.

PRINCIPAL ROLE OF NEW TECHNOLOGY

New technology drives interest in, and development of sensors. Only secondarily have specific needs driven development. New technology has allowed many new embodiments of classical ideas for sensors by permitting use of new structures and new input functions to probe the transfer functions of systems (contributions of physicists and electrical engineers), development and use of new materials (contributions by chemists), development of specialized and unusual chemistry (contribution of immunologists), and development of new formats especially for *in vivo* applications (contributions by physiologists). Practitioners of the new technologies are pushing sensor research. After 1970 and the discovery of the prototype (Bergveld) chemically sensitive semiconductor device, electrical engineers and solid state physicists took up the chemical sensor field and continued the physical sensor developments. Their contributions have been decisive in microminiaturization, especially fabrication of small versions of already practical devices. Applications of the field effect-based sensors *per se* in microfabricated devices seems very much less important than once thought. Similarly the contributions of physicists in designing quartz crystal piezoelectric devices have expanded the chemical sensor field greatly, although the concept of mass sensors for ambient adsorb- and absorb-able gases using the change in resonant frequency of a bulk quartz crystal was exploited some 20 years ago. The forms of devices: thick crystal bulk wave (conventional gas analyzer or film thickness monitor), thick crystal shear wave

(quartz microbalance), SAW delay time (Rayleigh wave along surface of a relatively thick crystal exposed to gases or liquids), SAW resonant frequency excited from the under side of the crystal, and very thin quartz films oscillating in complex ways (Lamb waves) are parts of the emerging technology for applications to gas and to liquid samples. Materials with crystals in the 21 space groups without a center of symmetry show changes of dimension with application of an electric field. Conversely, application of electric fields induces dimensional changes and acoustic waves.

On the same side of the irreversible thermodynamic equation is the pyroelectric effect in which a thermal gradient induces a field and a measureable voltage drop. This latter effect is presently used in the dc mode in contrast with the piezoelectric devices that use ac mode. Many of the same materials show both pyro- and piezoelectric effects. However the rules for selecting materials requires knowledge of crystal symmetry. These new devices are exceedingly sensitive to thermal gradients (heat flux) and seem, at this early date, to be a most profitable direction for chemical sensors using thermal effects: adsorption-desorption, solution-dissolution, simple bond formation (of all types)-dissociation enthalpies. This statement does not intend a criticism of temperature sensors such as thermistors whose physics and chemistry remain an interesting field for research on mechanisms of charge conduction and material properties.

The role of optical physics cannot be underestimated for the development of optical absorbance change or luminescence sensors interrogated by probe beams and/or read by emitted light through optical wave guides: through transparent optical fibers of plastic or glass. Sensors using specially coated optical wave guides, including optical fibers and films that modulate the evanescent wave depending on chemical reactions with the exterior coating, are another development from advanced technology. The latter technology is not familiar to many conventionally trained chemists because a new parameter: the refractive index of materials. It is

as important in sensor design of as the chemistry of the absorbing or reacting layer.

Contributions by physiologists and clinical chemists are not so clearly recognized, but very important if judged in terms of commercialization by large companies and several start-up companies. In vivo optical sensors, the microencapsulated pH sensing dyes called optodes, were conceived and developed at the Max Planck Institute for Systemic Physiology by Lubbers and coworkers. Later a very closely related concept, optrodes, was developed at Livermore Labs by Hirschfeld and coworkers. Conversion and specialization of conventional electrochemical sensors into physiologically useful sensors has been a technological triumph. Prior to studies by electrochemists of carbon fiber microelectrodes, microfabricated dots, hemispheres and lines, electrophysiologists had already made 0.1 μm tip capillaries as junction-type reference electrodes (for net membrane potential measurements) and ion exchanger-filled tips for ion assays within single cells. One must also mention the role of physiologists and clinical chemists in pointing out the special analytical problems of clinical, intensive care, doctors' offices, and *in vivo* measurements.

A logical combination of 1) the interfacial potential difference (pd) at an electrolyte/membrane interface (whether generated by faradaic or non-faradaic charge separation processes) and 2) the well known solid state physical generation of electron/hole pairs in Si by irradiation by energetic photons, leads to a new kind of device. The notion is 'light addressable potentiometry'. Whatever the interfacial pd is, it can be perturbed and in some sense measured by generation of charge that partially cancels the local field.

THE PRESENT ROLE OF CHEMISTRY

For about 20 years many of the current ideas of sensor chemistry have been known and exploited. These include use of chemically modified redox electrode surfaces as ion selective electrodes

(Michael Sharp radical salt electrodes); use of enzymes in reactive layers to generate from neutral charge substrate, species that can be sensed (potentiometric enzyme electrodes); use of enzymes in amperometric sensors (modifications of Clark oxygen electrodes); use of enzymes and mediators in amperometric sensors (glucose sensors since 1960s); use of mediators in redox reactions, formerly called 'secondary intermediates' in early 1950s (Br_2-Br^- for oxidation of electrochemically inert SO_2, or $Mn^{+2/3}$ for NO_2); use of selective reagents to generate response specificity (crown ethers and natural and synthetic ionophores in monovalent and divalent ion sensors since 1965); and use of selective layers to extract or partition species into surface layers on sensors (King quartz crystal mass sensors in mid 1960s), to name but a few examples.

The new technology, contributed by chemists in recent years, has frequently been a perturbation on existing classical ideas, formats or systems. Electronic-conducting electrodes with surfaces modified with pure ion conductors cannot serve as sensors for non-redox ions since the membrane/electrode interface is blocked (capacitively coupled). However, in the same way that mercury films can accumulate trace metals prior to anodic stripping, ion exchange films can accumulate trace ionic redox species that could be subsequently determined by either anionic or cathodic stripping. Electronic-conducting electrodes, modified with mixed conductors, can be sensors of organic gases that dissolve and increase ion or electron mobilities in a reproducible way.

More importantly, mixed conductor films may selectively catalyze the surface redox reactions of species that are not themselves electroactive in rapid, reversible ways. Redox enzymes involving coenzymes NAD, FAD, and certain quinones need this catalysis by 'promoter' films. Or, enzyme/coenzymes can be incorporated in mixed conductor films with exciting applications to amperometry of electrochemically inert substrates such as glucose and ethanol. At this time, the question of just how