ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Volume 50

ION-SELECTIVE MICROELECTRODES

Edited by Herbert J. Berman and Normand C. Hebert

ION-SELECTIVE MICROELECTRODES

Edited by

Herbert J. Berman
Boston University
Boston, Massachusetts

and

Normand C. Hebert
Microelectrodes, Inc.
Londonderry, New Hampshire

PLENUM PRESS • NEW YORK AND LONDON

Preface

pH and ion-selective microelectrodes are rapidly finding an increasing number of applications in the study and control of living (and nonliving) systems. They are unique in their capacity to measure chemical species without altering natural or controlled environmental conditions. Furthermore, these potentiometric tools measure the activity of the chemical species in contrast to conventional ones that measure total concentration.

The "Workshop on Ion-Selective Microelectrodes" is designed to provide an insight into the principles, theory, fabrication, techniques, present limitations, goals, and applications of some of these tools.

The importance and types of microelectrodes and guidelines for their application in biological systems are discussed by Berman. Their present limitations are reviewed by Durst. He warns that their use in analyzing living matter should be approached with caution because of the ill-defined nature of biologic systems. Techniques are presented next for the fabrication of pH (Hebert), antimony (Green and Giebisch, and Malnic et al.), oxygen (Whalen), then single-barrelled (Wright, Walker and Ladle, Morris and Krnjevic) and double barrelled (Zeuthen et al., and Khuri) potassium and chloride liquid ion-exchanger microelectrodes. Difficulties with and fabrication of reference and glucose electrodes are covered, respectively, by Durst and Wright, and Bessman and Schultz.

Applications of pH and ion-selective microelectrodes are described in microanalysis (Wright), measurement of intracellular ion activity and calculation of equilibrium potentials (Brown and Kunze), and then studies of the kidney (Wright, Malnic et al., and Khuri), brain (Zeuthen et al., Morris and Krnjevic), frog heart (Walker and Ladle), and human skeletal muscle (Filler and Das). In addition, actual and potential clinical applications, respectively, of pH (Filler and Das) and glucose (Bessman and Schultz) electrodes are reviewed.

It is the hope of the organizers, participants and sponsors, The National Heart and Lung Institute, The Microcirculatory Society, and The Host Institute, Boston University, that publication of the Workshop will focus attention on the potential of this new and rapidly developing area, and on the requisite precautions for the proper use of ion-selective microelectrodes in the study of complex systems.

The Editors

Contents

I. Theory, Potential and Existing State of Development

Perspective: Ion-Selective Microelectrodes: Their Potential in the Study of Living Matter *In Vivo*[*]

Herbert J. Berman

Department of Biology
Boston University
Boston, Mass. 02215

Work with ion-selective macro- and microelectrodes is rapidly evolving into a new specialty area in biology. The inherent advantages of the approach are basic: it enables one to measure activity of an ionic species directly, as opposed to concentration, and it does this simply, quickly, and in the presence of numerous other components in a complex system, negating the need of first isolating the molecular or ionic species of interest. Interfering species can be compensated for in most cases. Selective microelectrodes have the additional unique potential of making measurements of ionic species possible for the first time *in vivo* under conditions that approach normalcy, and, in addition, in a continuous manner. Net reactions can therefore be followed *in vivo*. In contrast, most biochemical measurements today are based on *in vitro* procedures that are nonphysiologic. Furthermore, they are frequently limited to only one point in time. Undoubtedly piercing a cell with a microelectrode can alter its functional state. But, as has been debated in neuro- and electrophysiology, if the microelectrodes are sufficiently fine and the area entered small with respect to the total cell, then the injury should be negligible and the measured activity should approximate that present in the normal living system.

---

*The Organizers and Participants of the "Workshop on Ion-Selective Microelectrodes" acknowledge with thanks sponsorship by the Microcirculatory Society and Boston University, and support by the National Heart and Lung Institute, Labtron, Microelectrodes, Inc., and the Transidyne General Corporation.

Our purpose at this meeting was to examine critically, by presentation and discussion of a limited number of selected review and research papers and by practical laboratory demonstrations, where we stand today in the continuum of development and application of these microsensors and to note where we hope to go.

Types of Electrodes.

There are two basic types of ion-selective electrodes; those composed of a solid barrier across which the potential develops, and those with a liquid barrier, such as a hydrophobic solvent between two hydrophilic solutions. They may be categorized further as shown in Table I.

TABLE I

ELECTRODES

YEAR	TYPE	EXAMPLES OF SPECIES SENSED
	SOLID STATE	
	GLASS	H^+, Na^+, K^+
1906	pH	H^+
1957	OTHERS	Na^+, K^+
1966	CRYSTALS (DOPED)	Cl^-, F^-, I^-, CN^-, SCN^-
1950, 1964	HETEROGENEOUS	Ag^+, Pb^{++}, Cl^-, I^-, S^-, SH^-
	PRESSED PELLET	
	IMPREGNATED POLYMER (PVC, PE, SILICONE RUBBER)	
	GRAPHITE	
	LIQUID STATE	
1966	LIQUID ION EXCHANGE	Ca^{++}, Cl^-, NO_3^-, K^+
1967	NEUTRAL CARRIER	K^+ (VALINOMYCIN, GRAMICIDIN)
		Na^+ (MONENSIN)
	VARIANTS	
	GAS	CO_2, SO_2, NH_4
	IMMOBILIZED BIOLOGICAL REACTANTS (ENZYMES)	GLUCOSE OXIDASE
		B-GLUCOSIDASE
		UREASE
		L- AND D-AMINO ACID OXIDASES

The pH electrode, discovered in the first decade of the century (Cremer, 1906; Haber and Klemenslewicz, 1909), typifies the selective barrier that gives rise to a potential. It was not until 1957 (Eisenman et al., 1957) that glass sufficiently selective to Na^+, and still later to K^+ and other univalent ionic species (Eisenman, 1962) was developed, and fabrication at least of Na^+ glass microelectrodes was considered worthwhile (Lavallée et al., 1969). Earlier, Wyllie and Patnode (1950) discovered a generalized method of making solid ion-selective electrodes. They embedded the selective material in an inert solid membrane to form a heterogeneous membrane barrier. However, because of technical difficulties, progress in this area did not commence until about 1966-1967 (Eisenman, 1969; Ross, 1969). Then heterogeneous semi-microelectrodes were made for many ionic species by compression of the inert material containing different active exchange substances into pressed pellets or polycrystals. Different selective substances were also embedded in a number of inert membrane-adaptable materials, such as silicone rubber, polyvinyl chloride and polyethylene, and then in graphite. The types of electrodes were extended to include doped crystals, such as lanthanum fluoride (Frant and Ross, 1966). In 1967 a most significant advance was made: an inert hydrophobic liquid was substituted for the inert solid material composing the barrier and the ion-selective material incorporated into the matrix of the hydrophobic liquid (Ross, 1967). At the same time selectivity by macrotetrolides (macrocyclic antibiotics) for K^+ and Na^+ was pioneered by Mueller and Rudin (1967), Stefanac and Simon (1967), and others. The dates of the references attest to the relative newness of this specialty area, one in which many ingenious innovations may be expected in the future, most potentially adaptable to the microelectrode level. These include electrodes selective for different polutants in air (Kneebone and Freiser, 1973) and others made selective by immobilization of biological reactants, such as enzymes, within membranes or on their surfaces (Baum and Ward, 1971; Buck, 1972; Mohan and Rechnitz, 1972; Gough and Andrade, 1973; Llenado and Rechnitz, 1973).

Techniques and Criteria for Quantification:

The types of measurements that can be made with the ion-selective probes (Table II) include practically all those presently made today by more routine biochemical procedures. Furthermore, the measurements can be made simply, quickly, continuously with time, and in the case of microelectrodes, in an *in vivo* setting under conditions that are beginning to approach normalcy.

Different techniques, such as those noted in Table III, have or are being developed to obtain data. For example, if one can release bound substances that then ionize, or if one can closely

TABLE II

TYPES OF MEASUREMENT

ACTIVITIES
RATES
GRADIENTS
TRANSIENTS
EQUILIBRIA
(CONCENTRATION)

TABLE III

QUANTITATIVE TECHNIQUES FOR MEASUREMENT

DIRECT
KNOWN ADDITION
KNOWN SUBSTRACTION
RELEASE
DILUTION

approximate the distribution of bound to unbound species by addition of a known amount of an ionic species and estimate the amount bound, then one can approximate the total concentration of the ionic species in a continuous manner.

General criteria, such as those listed in Table IV, should be applied to maximize the reliability of the data obtained with microelectrodes. Questions that should be continuously asked and checked include: the stability of the system. Does it drift? If so, how much? How rapidly? Is the drift random or predictable? How stable is the reference electrode? Other questions relate to the limitations of the system. How accurate is the measurement? What is the minimum activity the system can sense? And discriminate? What is the effect of sample volume on the measurement? Is the reactive surface of the microelectrode totally immersed in the substance being studied? What substances are present that may interfere with the true reading? In what way and to what extent do they alter the true reading? Also, can the calibration fluid be made more representative of the experimental fluid and thereby negate the need for or reduce the magnitude of the corrective factor $(k\ A^{n/z}\ Int)$:

$$E_{cell} = E_o + RT/nF \quad \ln(A_{ind.} + k\ A^{n/z}\ Int)$$

TABLE IV

PHYSICAL AND CHEMICAL LIMITATIONS OF SYSTEM OR TECHNIQUE

Proper Functioning of Electrode

Stability of System (Drift, Reference Electrode, Junction Potential, Error)

Selectivity (Interfering Species)

Limits of Sensitivity

Reproducibility (Precision)

Accuracy

Response Time (Rate of Reaction and Degree of Completeness)

Temperature

pH (Buffer) and Total Ionic Strength

where E_o is the constant standard potential for the indicator electrode; R, T, F, and $A_{ind.}$, are the gas constant, absolute temperature, the Faraday constant, and activity of the ionic species being measured; k is the selectivity coefficient; and A_{Int} is the activity of the interfering ions of charge Z. The cell potential thus varies directly with the logarithm of the ionic activity. At 25°C, 2.3 RT/nF has a response slope of 59.2/n mV/pI. That is, at 25°C, the potential of an ion-selective electrode will under ideal conditions change 59.2 mV when the activity of a univalent ion (n) is changed by a factor of ten. (Nernst, 1889; Durst, 1971)]. Assiduous application of these criteria should help to ensure the reliability of the measurement, maximize its accuracy and sensitivity, and hopefully, eventually allow us to achieve the elusive objective of making absolute measurements with high reliability.

Some questions that a physiologist would ask are listed in Table V. He would want to know: Whether or not the system being used can give him the information being sought? How normal the cell or tissue preparation is? And what reliability and confidence he can place on the data being gathered?

Objectives:

Our ultimate objectives are the obvious ones: to determine

TABLE V

PHYSIOLOGIC LIMITATIONS

What are the limitations of the systems and techniques being used? Can they give you the information you seek?

How physiologic is the preparation or cell unit being studied?

Injury - from microelectrode - degree and effect on results?

Exactly where is the microsensing tip located?

What are the physiologic changes with time?
(And those due to the sensor and exposure of the preparation?)

Reproducibility?

Accuracy?

the chemical composition of the material being studied and how it functions without injuring or altering it; also, as an extension of these major objectives, to monitor its functions and learn how to control them so as to optimize the function and the longevity of the system. Furthermore, in applications in pathologic states, we wish to detect the deviation, preferentially in its preclinical state, but, if this is not at first possible, as it occurs, and then to institute corrective measures and monitor their effectiveness, preferably on a continuous and instantaneous basis.

It is unlikely that any one approach will give us all the information needed or sought. Ion selective microelectrodes are a relatively new and rapidly evolving tool applicable to *in vivo* research. It has definite advantages, but like all approaches it has its limitations. For instance, one of the great limitations of several of the present ion-selective electrodes is the concentration range of their response. For example, Ca^{2+} can presently be measured at concentrations as low as $10^{-4.5}$ or 10^{-5}M with reasonable reliability. However, in many cases, we need to sense Ca^{2+} activity rapidly and continuously at concentrations as low as 10^{-8}M. Other new methods applicable to *in vivo* work, some based, e.g., on optics and measurements of transmittance or reflectance at one or more wavelengths, such as in *in vivo* microspectrophotometry (Davila, et al, 1973; Chance, et al, 1971; Liebman, 1969), are also in an early, formative stage of development. It seems most logical that in order to gain greater insight into the complex phenomena challenging us in biology, it would be most advantageous to use all available approaches to elucidate the details of the composition

TABLE VI

OBJECTIVES

THE CHEMICAL AND PHYSIOLOGIC ANALYSIS OF LIVING MATTER AS IT IS.

CONTINUOUS MONITORING OF THE STATE OF BEING AND MECHANISMS OF CONTROL.

MASTERY OF THE CONTROL MECHANISMS.

MAINTENANCE OF THE TOTAL SYSTEM IN ITS OPTIMAL STATE.

and organization of the structures, the details of the reactions within the living system, the precise location at all times of the reactions, the mechanisms of their control, the responses of the reactions to different types and intensities of stresses, and how we may monitor and regulate the reactions in vivo at all levels of integration and complexity.

The state of development of our present tools may seem inadequate to achieve our objectives. But it is first by their primitive innovative development, then their use and step-by-step improvement that we have come this far. It is by further refinement of existing approaches, development of new ones, and their ingenious application that one may hope to approach and achieve our ultimate objectives of studying living matter or other materials as they are without production of injury or change, and, in addition, learn how to regulate and optimize their state of being.

REFERENCES

Baum, G. and Ward, F.B., 1971. General enzyme studies with a substrate-selective electrode: characterization of cholinesterase. Anal. Biochem. 42: 487-493.

Buck, R.P., 1972. Ion-Selective Electrodes, Potentiometry, and Potentiometric Titrations. Anal. Chem. Reviews. 44:270R-285R

Chance, B., Lee, C.P. and Blasie, K.J., Eds., Probes of structure and function of macromolecules and membranes. Vol. I. Probes and Membrane Function. (Proc. 5th Colloquium of the Johnson Research Foundation. Apr. 19-21, 1969). Academic Press, N.Y. and London, 1971.

Cremer, M., 1906. Uber dreursache der elecktromotorischen Eigenschafter der Gewebe, Zugleichein Beitrag zur Lehre von den polyphasischen elektrolytketten. Z.F. Biol. 47:562-608.

Davila, H.V., Salzberg, B.M., and Cohen, L.B., 1973. A large change in axon fluorescence that provides a promising method for measuring membrane potential. Nature, N.B. 241:159-160.

Durst, R.A., 1971. Ion-selective electrodes in science, medicine, and technology. American Scientist 59:353-361.

Eisenman, G., Rudin, D.O., and Casby, J.U., 1957. Glass electrode for measuring sodium ion. Science 126:831-834.

Eisenman, G., 1969. "Theory of Membrane Electrode Potentials". Chapter 1 in Ion-Selective Electrodes. R. A. Durst, ed., NBS Spec. Publ. 314, U.S. Gov. Printing Office, Washington, D.C.

Eisenman, G., 1962. Cation selective glass electrodes and their mode of operation. Biophys. J. 2 (Part 2): 259-323.

Frant, M.S. and Ross, J.W., 1966. Electrode for sensing fluoride ion activity in solution. Science 154: 1553.

Frant, M.S. and Ross., 1970. Potassium ion specific electrode with high selectivity for potassium over sodium. Science 167:987-988.

Gough, D.A. and Andrade, J.D., 1973. Enzyme electrodes. Science 180: 380-384.

Haber, F. and Klemenslewicz., 1909. Uber elektrische phasengrenzkrafte. Z.F. physik. Chem. (Leipzig) 67:385-431.

Kneebone, B.N. and Freiser, H., 1973. Determination of nitrogen oxides in ambient air using a coated-wire nitrate selective electrode. Anal. Chem. 45:449-452.

Lavallée, M., Schanne, O.F. and Hebert, N.C. 1969. Glass Microelectrodes. John Widey and Sons, Inc.

Liebman, P.A., 1969. Microspectrophotometry of retinal cells. In Data Extraction and Processing of Optical Images in the Medical and Biological Sciences. Annals, N.Y. Acad. Sci. 157:250-264.

Llenado, R.A. and Rechnitz, G.A., 1973. Ion-electrode based autoanalysis system for enzymes. Anal. Chem. 45:826-833.

Mohan, M.S. and Rechnitz, G.A., 1972. Ion-electrode study of the calcium-adenosine triphosphate system. J. Am. Chem. Soc. 94: 1714-1720.

Mueller, P., and Rudin, D.O., 1967. Development of K^+-Na^+ discrimination in experimental bimolecular lipid membranes by macrocyclic antibiotics. Biophys. Biochem. Res. Comm. 26:398-404.

Nernst, Walther, 1889. Die elektromotorische Wirksamkeit der Ionen. Z.F. physik Chem. (Leipzig) 4: 129-181.

Rechnitz, G.A. and Mohan, M.S., 1970. Potassium-adenosine triphosphate complex: formation constant measured with ion-selective electrodes. Science 168:1460.

Ross, J.W., 1969. "Solid-state and liquid membrane ion-selective electrodes". Chapter 2 in Ion-Selective Electrodes, R.A. Durst, ed., NBS Spec. Publ. 314, U.S. Gov. Printing Office, Washington, D.C.

Ross, J.W., Jr. 1967. Calcium-selective electrode with liquid ion exchanger. Science 156:1378-1379.

Stefanac, Z. and Simon, W., 1967. Ion specific electrochemical behavior of macrotetrolides in membranes. Microchem. J. 12:125-132.

Wyllie, M.R.J. and Patnode, H.W., 1950. Equations for the multi-ionic potential. J. Phys. Chem. 54:204-227.