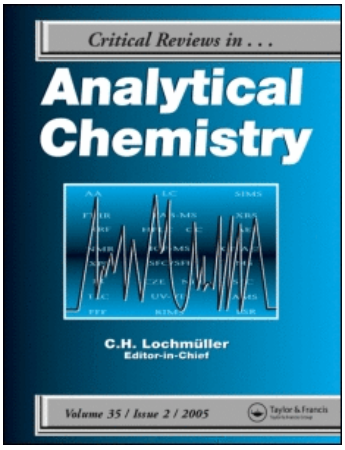


Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Ion-Selective Electrodes in Biomedical Analysis

Robert L. Solsky; G. A. Rechnitz

To cite this Article Solsky, Robert L. and Rechnitz, G. A.(1982) 'Ion-Selective Electrodes in Biomedical Analysis', Critical Reviews in Analytical Chemistry, 14: 1, 1 — 52
To link to this Article: DOI: 10.1080/10408348208542756
URL: http://dx.doi.org/10.1080/10408348208542756

ION-SELECTIVE ELECTRODES IN BIOMEDICAL ANALYSIS

Author: **Robert L. Solsky**
Photo Products Department
Experimental Station
E.I. Du Pont de Nemours & Co.
Wilmington, Delaware

Referee: G. A. Rechnitz
Department of Chemistry
University of Delaware
Newark, Delaware

TABLE OF CONTENTS

I. INTRODUCTION AND SCOPE

The ion-selective electrode is an electrochemical sensor characterized by a selective response to a particular ionic species. The wording "selective" rather than "specific" is considered since it is very rare that an electrode responds to a single ion exclusive of all others. The ion-selective electrode is further described as a membrane sensor which responds in a potentiometric manner. That is, the potential which develops across an ion-selective membrane separating two solutions is measured at virtually zero current. This is a rather unique and intriguing event which has founded an expanding analytical field which has come of age within the last 15 years.

Potentiometry has successfully evolved to cross interdisciplinary lines and is finding applications in virtually every scientific field. One of the more useful and appropriate penetrations has been into the realm of biomedical analyses. The use of ion-selective electrodes in clinical assays is an obvious application when considering their inherent characteristics. An electrode responds to the activity of an ion in solution rather than to concentration. This is highly significant since activity is the important quantity when dealing with biological systems. Ion-selective electrodes effectively function in turbid samples such as whole blood where other means of detection fail. The presence of particles does not affect the measurement, but does arouse disagreement over the choice of proper standards for calibration.

The acceptance by clinicians of electrode-based analyzers has not been wholehearted as one would expect.[1] Various reasons have been given but the overriding cause seems to be the poor quality of early sensors. Many of these deficiencies have been corrected through the development of improved devices and better instrument designs are making the equipment more friendly. It is clear that electrode-based analyzers are becoming more common as evidenced by the increasing numbers of instrument evaluations in the literature. Also, the general literature concerning ion-selective electrodes is expanding at a tremendous rate when the number of text books[2-15] and review articles are considered.[16-29]

The aim of this review is to provide the reader with an up-to-date synopsis of the application of ion-selective electrodes to biomedical analyses. Since there is such a vast quantity of literature, a comprehensive approach will not be taken but representative examples will be developed where appropriate. The initial sections will illustrate the

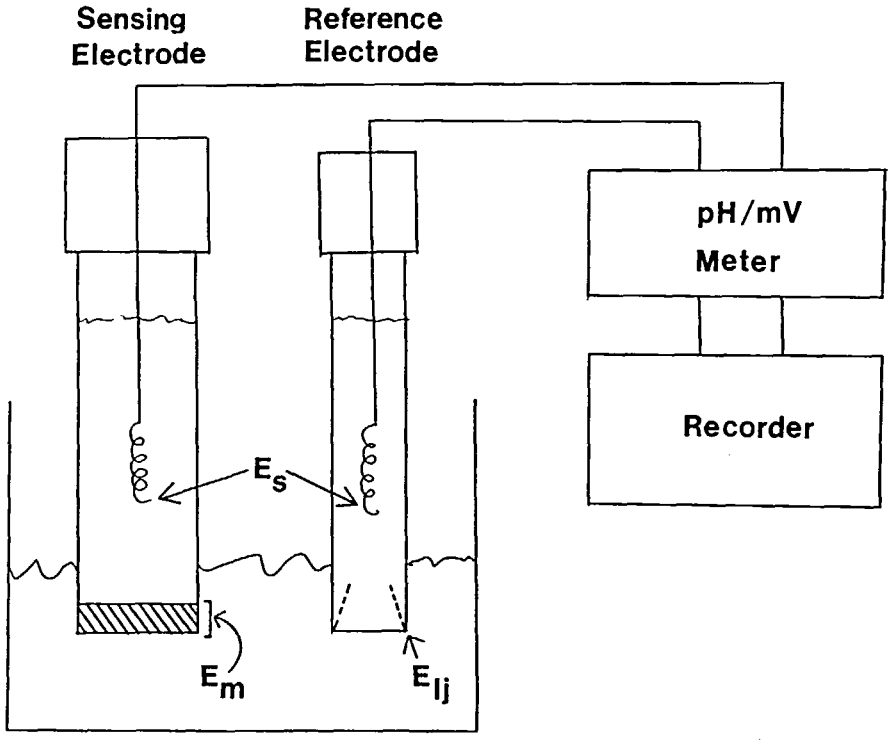


FIGURE 1. Schematic diagram of the typical electrode set-up.

fundamental approaches and techniques which are used to couple biochemical events with selective electrode sensors in order to develop assay systems for a variety of analytes. The basic electrodes themselves will be described and limitations will be noted when applicable.

II. THEORETICAL CONSIDERATIONS

A. Response Characteristics

Ion-selective electrodes respond to the activities of ions which are present in solution. The indicating electrode is always used in conjunction with a counter electrode to complete the electrochemical measuring circuit. This counter electrode is most often a reference electrode which can maintain a relatively stable potential regardless of solution conditions.

The usual experimental arrangement is illustrated in Figure 1 which also indicates the potentials that are involved in the measurement. The standard electrode potentials, E_s, are constant since the internal electrolyte remains constant in both. The liquid junction potential, E_{lj}, can be considered constant for all practical purposes because the internal electrolyte solution is chosen judiciously. However, it should be noted that this is highly oversimplified for this discussion. The membrane potential, Em, is actually composed of two interfacial potentials and a membrane diffusion potential component. The internal interfacial potential remains constant while the external interfacial potential is perturbed by changes in the activities of selected ions in solution. The diffusion potential takes different roles depending on the type of membrane which is operating.

The potential generating processes which occur at membrane interfaces have been

thoroughly described in great detail elsewhere[20,26,27,30,31] and it is not the purpose of this review to reiterate these concepts.

The casual user of ion-selective electrodes need only be aware that all electrodes respond to ion activities as described by the Nernst equation which relates the potential of an ion-selective electrode to the activity of the ion being measured.

$$E = E^\circ + \frac{RT}{nF} \ln a \qquad (1)$$

E is the measured potential, E° is a constant and RT/F is known as the Nernst factor where R is the gas constant (8.314 j K^{-1}), T is the absolute temperature, and F is Faraday's constant (96486.7 C mol^{-1}). The sign and magnitude of charge of the ion is n and the activity of the ion in solution is given by a. The ionic activity is related to the bulk ionic concentration by:

$$a = fC \qquad (2)$$

where f is the activity coefficient and C is the bulk concentration. The activity coefficient depends on the types of ions present and on the total ionic strength of the solution.

The response of an ion-selective electrode may be evaluated by examining the performance of the electrode. Namely, the slope of the calibration curve and the limit of detection are easily defined while other factors such as response time and operating characteristics complete the description.

A typical calibration curve for both mono- and divalent cations is illustrated in Figure 2 to show how the potential-concentration plot appears. The limit of detection is shown while the theoretical slopes differ (59.1 vs. 29.6 at 25° C) for the respective ions. While many electrodes display theoretical slopes, many of the sensors that will be described in later sections respond with sub-Nerstian slopes are indicative of nonideal operation.

B. Selectivity

It was stated earlier that the ion-selective electrode was not entirely specific in its response except for a few isolated examples (i.e., the F^- electrode). More often than not, electrodes are selective in their response to a variety of ions. This means that the observed potential results from the sum of individual potentials for several ions in solution. The relationship which accounts for the total potential is given by the Nicolsky equation.

$$E = E^\circ + \frac{RT}{nF} \ln (a_i + K_{ij}a_j + \cdots K_{ix}a_x) \qquad (3)$$

The selectivity factors (K_{ij}) give a quantitative way to describe an electrode's preference for one ion over the others. The "i" and "j" subscripts refer to the primary and interfering ions, respectively, while all the other terms have the same meaning as in the Nernst equation (Equation 1). If an electrode responded equally well to two different ions, the selectivity factor would be 1.00. The preferred situation is where the K-terms are much smaller than one so that the product is insignificant to the primary ion activity.

Even though an electrode is not absolutely specific for a certain ion, it can be successfully used for analysis when the selectivity numbers and the levels of interferents are known. Methods for evaluating selectivity factors are well known, and those employing electrodes should be able to assess whether an electrode is applicable for use. The preferred method is a mixed solution technique where the selectivity is evaluated

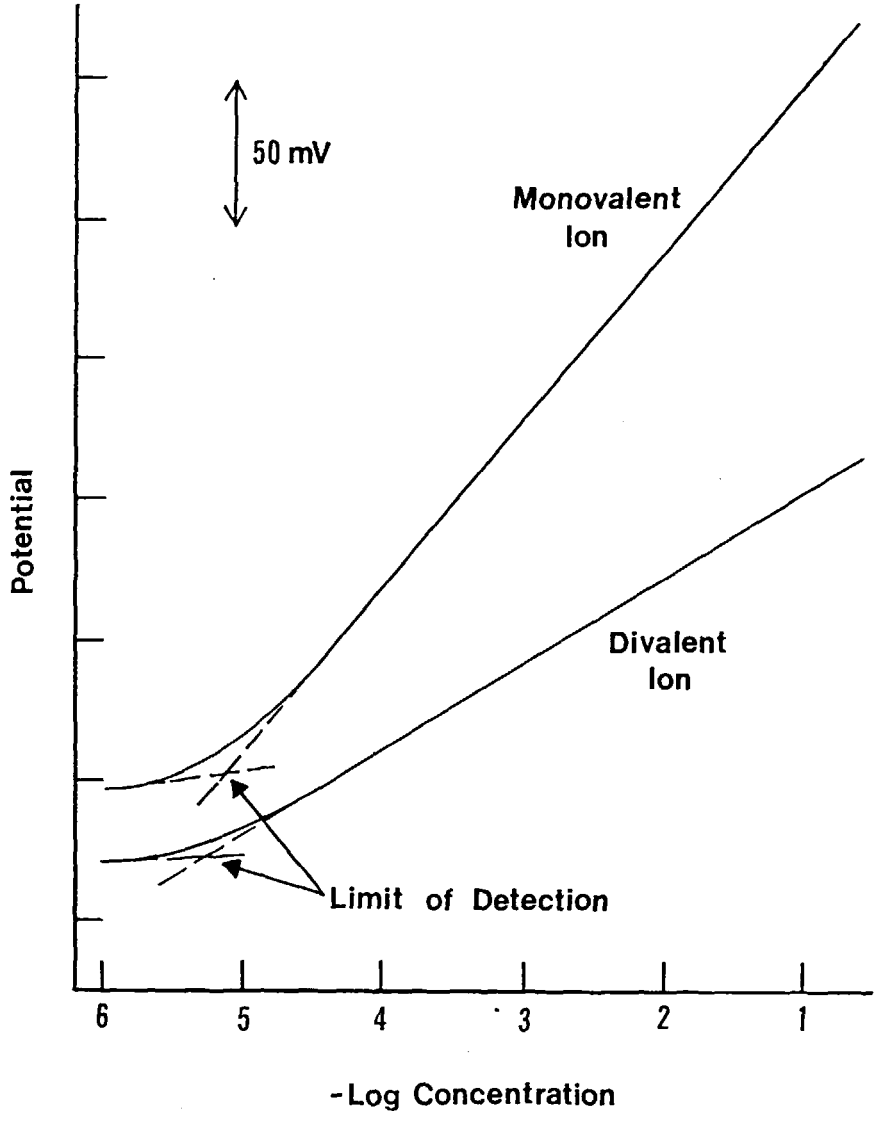


FIGURE 2. Response curves for mono- and divalent cations.

under conditions of actual use.[32] Other approaches using single solutions may be easier and faster to conduct but result in unreliable numbers.

C. Measurement Considerations

Ion-selective electrodes can be used in a number of operational ways. However, it must be remembered that the electrode responds to activities and not concentrations. More often than not we use concentrations for convenience and this is where trouble begins, especially when working in biological samples.

The most widely practiced method of analysis involves a direct measurement of the analyte. In this case, the response slope of the electrode must be known in the region of analyte concentration. The accuracy of this approach is directly related to how well the ionic strength can be controlled from standards to the sample. This is usually

accomplished by adding a total ionic strength adjusting buffer to all samples and standards. However, as biological samples are measured without dilution the nature and composition of the standards are made to resemble the sample so as to avoid the ionic strength effects.

Even so, the accuracy of direct measurement is limited by the resolution of the instrument. A 0.1-mV error in readings yields an error of 0.4% for monovalent ions and an error of 0.8% for divalent ions. This reflects the "best case" and is difficult to achieve in routine measurement. A general expression for error has been described by

$$\% \text{ error} \cong 4\ |Z_i|\ \Delta E$$

where Z_i is the valence of the ion and ΔE is the uncertainty of measurement.[33] As can be seen, a 4% error results from an uncertainty of ± 1 mV with a monovalent ion and 8% for a divalent cation. In some cases this is acceptable but for most this represents a level of uncertainty which is too high.

Alternate techniques of measurement include the standard addition methods. When single standard additions are made, the value of the slope is required, necessitating prior calibration. However, when multiple addition techniques are employed, the slope is calculated during the measurements and slope plots are not needed.

In using multiple standard addition, the potential of the sample is measured followed by multiple additions of standard analyte solutions with potential readings taken between additions. The response slope of the electrode is calculated during the analysis by knowing the concentrations and volumes of the standards.

Single point analyte addition is performed by taking a potential reading of a standard solution followed by the addition of the sample. As with the standard addition method, the value of the slope must be known in advance.

Potentiometric titrations are one of the most accurate of the measurement methods. In this type of assay a reagent is added to complex or react with the ion of interest. The complex may precipitate, or the level of ionic activity may be reduced by simple complexation in the solution phase. In either case, the decrease in ionic activity is sensed by the electrode. This method of measurement will be explained in further detail in later sections.

The calculation of error in these and other methods of potentiometric measurement has been reviewed.[34-37] Relevant factors which identify the sources of errors which propagate during measurements are also included. Of particular interest are the limit of detection, the practice of working at or near this limit, and the means of extending the working range of ion-selective electrodes.[38-40] These topics are of utmost importance to those working with biological fluids which tend to be more diverse and challenging in their composition and push the ion-selective electrodes to their limits. However, the simplicity of design and use more than offset the precautions that must be taken to successfully apply these sensors to biological samples.

III. BASIC ELECTRODE CONFIGURATIONS

Ion-selective electrodes are characterized as having some form of permselective membrane separating two electrolyte solutions. The processes which occur are ion exchange events at the membrane-solution interface. There are several types of membranes that are used in electrodes and they have been classified in a number of ways. For this discussion, the basic electrode systems are divided into two groups: those having solid membranes and those having liquid membranes. Others have used the terminology fixed site vs. mobile site.[23] These membrane systems will be described to form a basis of

understanding of the more complicated electrodes that will be discussed in following sections.

A. Solid Membranes

Ion-selective electrodes having solid membranes can be further subdivided into two classes. The membranes may be either homogeneous or heterogeneous in nature.

1. Homogeneous Membranes

The homogeneous membrane electrodes are best recognized by the glass pH electrode. The response of the glass electrode is perhaps the best understood of all the electrode types.[41,42] The experimental evidence indicates that this electrode operates by a cation exchange mechanism involving the selective absorption or binding of cations within the hydrated layer at the surface of the glass membrane. The ion exchange process is dependent on the composition of the glass which determines the nature of the exchange sites. The selectivities can thus be altered by changing the composition of the glass to make electrodes which can respond to different cations. Such electrodes can be used for ammonium and the alkali metal cations.

Another type of homogeneous membrane is the single crystal. The crystal-type membrane electrodes respond to ions by the ability of one of the lattice ions to migrate through the crystal. This conduction proceeds via a lattice defect mechanism whereas a vacancy defect can be filled by a mobile ion moving into it, thus forming a vacancy from where the ion came. Obviously, the crystal must be composed of a salt of the mobile ion and usually the ion of smallest size and charge is the mobile ion in the crystal. The LaF_3 crystal membrane electrode is a good example of this type of structure. In the LaF_3 crystal, the fluoride ion is mobile and since the lattice only allows the fluoride ion to enter, the selectivity of this electrode is exceedingly high.

2. Heterogeneous Membranes

The heterogeneous membrane electrodes are also of the crystal type. However, these membranes are formed of mixed crystals and are thus distinguished from the homogeneous crystal membranes. The mode of operation is similar except that the mobile ion is silver and the membrane is composed of mixed salts of Ag_2S. The crystal membranes operate by virtue of the sparingly soluble salts which form the membrane. There is an equilibrium dissociation of the salt which produces a finite concentration of the lattice ions in solution. The presence of one of these ions in the test solution shifts this equilibrium at the membrane-solution interface thus altering the potential of the electrode. Mixed crystal electrodes can be made which respond to the halides, Cd^{+2}, Cu^{+2}, Pb^{+2}, and CN^- as well as additional analytes.

B. Liquid Membranes

This class of membrane electrodes uses a lipophilic organic solvent as a membrane material. This solvent has traditionally been held in place using a porous material such as a Millipore Type VC filter or a porous glass frit. The liquid membrane electrode has a complexing agent dispersed in the solvent which selectively allows the conduction of ions across the membrane.

One of the first liquid membrane systems used diesters of phosphoric acid as a charged exchanger to form a selective Ca^{+2} sensor.[43] The ester functionalities were 8 to 16 carbon atoms long to render the ion exchanger hydrophobic. The liquid ion-exchanger membrane is functionally similar to the solid membrane electrodes except that the sites are mobile instead of fixed. However, in the case of the liquid membrane, the cation-exchanger complex is neutral thus excluding the solution anions from the membrane

phase. In a similar fashion, liquid membranes can also be made to respond to anions by incorporating positively charged ion-exchange materials.

Both types of ion-exchangers are highly dissociated in the membrane which means that the selectivity among the ions is independent of the nature of the exchanger. Instead, the selectivity depends on the relative mobilities of the ions in the organic phase as well as on the partition coefficients of the ions between the aqueous phase and the organic solvent of the membrane phase.

Liquid membrane electrodes can also be prepared using neutral ion carriers instead of charged ion-exchangers. One of the best examples of this type of membrane is the potassium selective electrode based on the macrocyclic antibiotic, valinomycin.[44] Other sensors have been made using similar materials, but the common feature of these neutral carriers is the cyclic arrangement of electron rich atoms which form a cavity capable of coordinating cations. The cyclic crown ethers, i.e., dibenzo-18-crown-6, also have this "puckered crown" of oxygen atoms regularly spaced with interposed methylene groups. This arrangement of atoms is responsible for the selectivity of the neutral carrier electrodes by virtue of the "fit" between the size of the cations and the size of the cavity. This fit can be expressed as the equilibrium association constants for cation complexation by the neutral-carrier. Thus, as a first approximation, the selectivity of an electrode based on one of these carriers for one cation over another can be estimated as the quotient of association constants for the individual cations.[45]

The organic liquid phase can be held in other matrices beside the filter paper supports. Various polymer materials have been used, the most notable being polyvinyl chloride (PVC).[46] The use of polymers for membranes results in improved mechanical stability and increased electrode lifetimes. The pore structure through the membrane is effectively reduced to macromolecular dimensions, thus increasing the effective viscosity of the organic solvent.[47] This reduces convective processes in the membrane to the point where they can be neglected.

Many investigations have focused on the mechanism by which PVC-based ion-selective electrodes operate. Elaborate radio-tracer experiments demonstrated that the neutral carrier is mobile in the membrane and that, as expected, solution anions do not enter the membrane phase.[48-50] Since electroneutrality of the membrane can not be violated, there had to be anionic sites in the membrane. It was demonstrated that PVC uptakes water and that upon exposure to salt solutions there is a net flux of protons from the membrane indicating the existence of immobile, hydrated hydroxyl sites within the polymer.[51] This represents the latest understanding of the neutral carrier ion-selective electrode.

The properties of neutral carrier-based polymer membranes have been reviewed extensively since they are becoming more popular in use.[52,53] Studies on selectivity and lifetime indicate that these sensors should be applicable to commercial instrumentation as some of these membranes have lasted at least 1 year in continuous-use tests.[54]

IV. SENSITIZED ELECTRODES

The concept of sensitized electrodes will be developed be beginning with an operational definition. The recommended IUPAC classification for sensitized electrodes is described by the placement of a membrane or layer of material upon one of the basic electrode types.[25] Thus, the basic electrode is modified by the added layer to impart an enhanced or altered selectivity. One can think of this process as a "building block" approach to form novel electrodes which respond to desired analytes.[55] These modifications have taken several forms. The simplest of these results in what is known as gas-sensing electrodes.

Table 1
GAS-SENSING ELECTRODES AVAILABLE

Gas sensed	Internal electrode	Electrolyte equilibrium
NH_3	H^+, NH_4^+	$NH_3 + H^+ \rightleftharpoons NH_4^+$
SO_2	H^+	$SO_2 + H_2O \rightleftharpoons HSO_3^- + H^+$
CO_2	H^+, CO_3^{-2}	$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$
H_2S	H^+, S^{-2}	$H_2S \rightleftharpoons 2H^+ + S^{-2}$
NO_2	H^+	$2NO_2 + H_2O \rightleftharpoons NO_3^- + NO_2^- + 2H^+$
HX	H^+, X^-	$HX \rightleftharpoons H^+ + X^-$

A. Gas Electrodes

Potentiometric gas-sensing electrodes depend on an aqueous equilibrium process which occurs in the space between the surface of a basic electrode type and an overlying gas-permeable membrane. This equilibrium generally involves an acid-base couple but other reactions may be used as well. The primary electrode types and the equilibria involved are listed in Table 1 to illustrate the electrodes that can be constructed to determine gases.

Gas electrodes have been the subject of a number of reviews which serve to describe the study and design of gas-sensing electrodes and illustrate applications for their use.[19,27,56-58]

Another type of gas sensor is the air gap electrode. An air gap, gas-sensing electrode uses an actual air gap across which gas diffusion takes place rather than a hydrophobic, microporous membrane to separate the sample from the aqueous internal solution. These electrodes are normally of the same relative size as the conventional gas electrodes. However, it was recently demonstrated how small these electrodes can be assembled.[59] C. P. Pui et al. fabricated the micro-air gap, gas-sensing electrode shown in Figure 3. The tip diameter of this sensor is 10 μm and the total length of the device is 2.5 cm. The elegant construction of this electrode represents the state-of-the art in micro-gas-sensing electrodes and serves to stimulate numerous imaginative uses for such devices for in vivo applications.

Another concept in gas-sensing probes provides the aqueous internal electrolyte solution as a mobile rather than a static fluid.[60-62] In this case, the internal electrolyte flows past the membrane and the shift in the associated equilibrium is sensed by an electrode located downstream. The arrangement for this type of gas-sensor is shown in Figure 4 and illustrates how the internal solution is continuously refreshed.

This type of flow-through gas sensor can boast higher throughputs of sample because the electrolyte does not have to be reconditioned between measurement. Also, the pH of the donor and recipient streams can be adjusted to enhance the gaseous transport across the membrane, decreasing response time and enhancing sensitivity. The response of this electrode parallels commercial gas electrodes but demonstrates superior response time and detection limit.

Recently, more attention has been focused on the response characteristics of gas sensitive electrodes. Bailey and Riley[63] describe methods for calculating the lower limit of detection of gas electrodes based on the thickness of the internal electrolyte film and equilibration rates of the sensed gas.

Studies conducted using CO_2 and SO_2 sensors describe the measurement range and interferents for these electrodes on both a theoretical and practical basis. The sample pH was found to be of use in limiting the interference for other gases and other conditions are discussed in relation to analysis time and selectivity.[64]

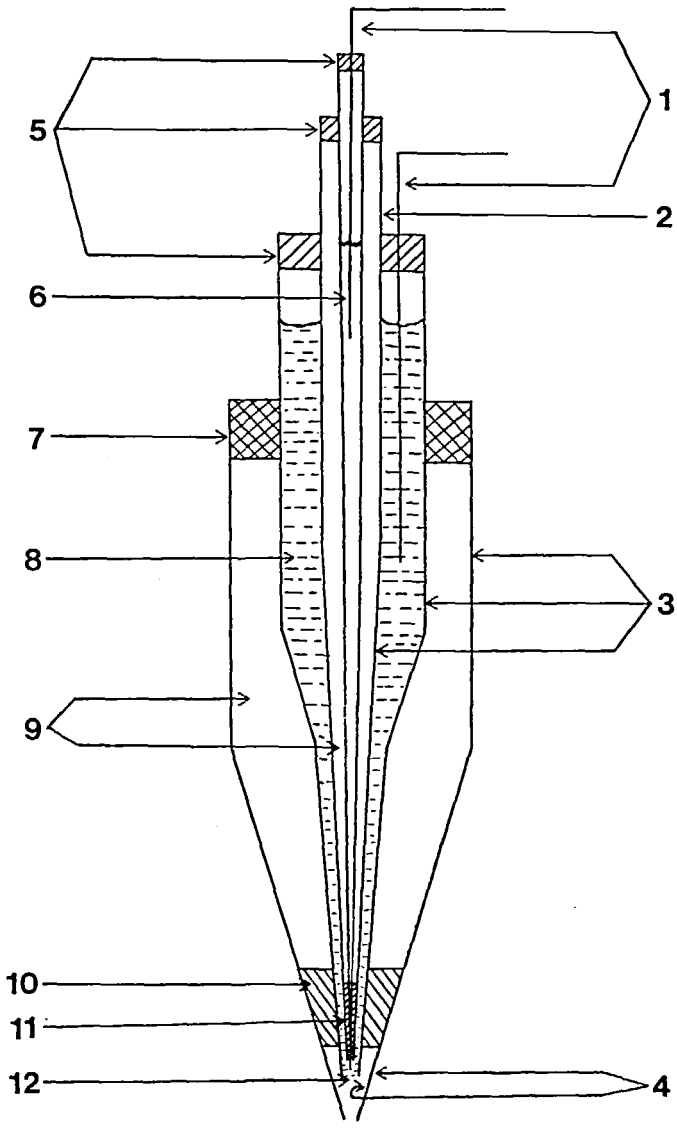


FIGURE 3. Construction of a micro air-gap sensing probe: (1) Ag/AgCl wires, (2) insulated micro pH electrode, (3) ordinary Pyrex glass, (4) hydrophobic surface, (5) glass cover cement, (6) 0.01 *M* HCl, (7) epoxy resin, (8) 0.005 *M* NH_4Cl or $NaHCO_3$ + 0.01 *M* NaCl, (9) air space, (10) high vacuum Apiezon wax, (11) glass cover cement or halocarbon wax, (12) air gap. (From Pui, C. P., Rechnitz, G. A., and Miller, R. F., *Anal. Chem.*, 50, 330 (1978). With permission.)

A theory is described for the estimation of the detection limit for the ammonia air gap or membrane electrode. The linearity of the equilibrium response in the aqueous internal electrolyte and the velocity with which the equilibrium is attained affect the limit of detection. The theory is extended to other gas electrodes as well. Several different conditions are discussed in their relation to the detection limit.[65]

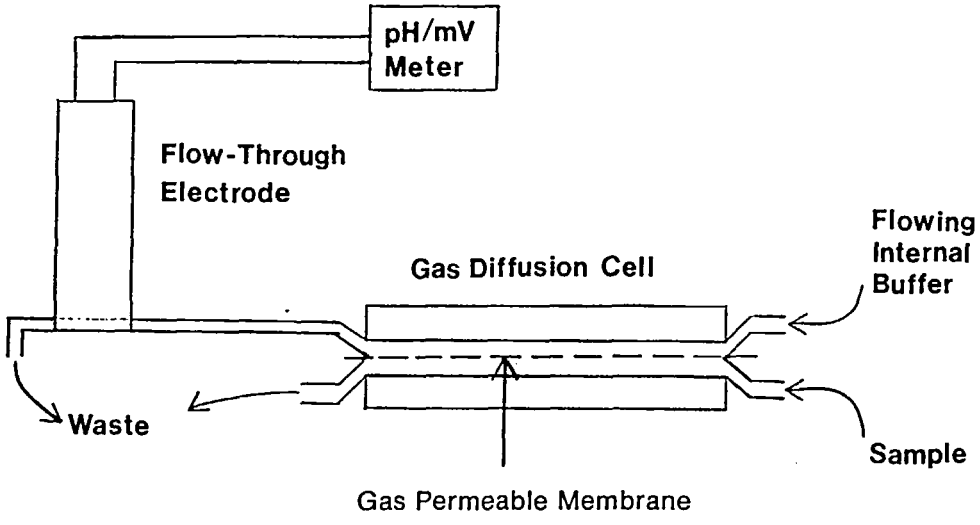


FIGURE 4. Schematic diagram of the flowing internal electrolyte gas-sensing electrode.

B. Enzyme Electrodes

The enzyme electrode is also a sensitized electrode in that a basic electrode is overlayed with an enzyme layer to impart selectivity to a specific substrate. The substrates are various organic compounds which are acted on by the enzyme to either consume or produce an ion or gas which can be sensed by the electrode. In this way a variety of substrates can be sensed as easily as ions with little attention to sample preparation.

Enzyme electrodes have enjoyed a spectacular popularity which was initiated by Guilbault who introduced an electrode for urea based on an ammonium ion-selective electrode.[66,67] Since these first publications, hundreds of papers have appeared for a host of substrates. Numerous review articles tabulate the enzyme electrodes which have been studied and include data on linear range, interferents, and response time. Some of the more notable reviews appear as chapters in books[9,13,68-73] and as review articles in journals.[74-81]

The steady-state response of enzyme electrodes has been evaluated on a theoretical basis.[82] The thickness of the enzyme layer and its activity relative to the substrate concentration contribute to whether the response will be Nernstian.

Probably the most overpublished enzyme electrode is the urea electrode. Another paper is cited here for the way the enzyme is held to the ammonia electrode. An improved Teflon® membrane has urease directly coupled to it.[83] These composite membranes should reduce the response time as the geometry is improved over previous designs.

Enzyme electrodes can be used for a variety of studies beyond substrate measurement. An AMP-selective enzyme electrode has been used to measure the binding interaction between D-fructose-1,6-diphosphatase and AMP.[84] The enzyme electrode was capable of distinguishing free from bound AMP.

Lysozyme enzyme activity was monitored by utilizing live bacterial cells of *Micrococcus lysodekticus* as the substrate.[85] The cells were loaded with a marker ion (trimethyl phenyl ammonium ion) which was released by the action of lysozyme on the cells. This type of coupled "enzyme electrode" was shown to be sensitive and offered numerous advantages over turbidimetric methods of analysis.

The majority of reported enzyme electrodes use an ammonia gas electrode as the internal sensor. Many novel nonammonia electrode systems have been recently reported.

Oxidase enzymes have been used with potentiometric detectors instead of the classical peroxide sensor. In this approach, the peroxide formed reacts with iodide ion to form iodine. The decrease in I^- concentration is proportional to the enzyme substrate. L-Amino acids, alcohols, and glucose have been determined using this method with the respective oxidase enzymes.[86,87]

In another approach, glucose was determined potentiometrically by immobilizing glucose oxidase on a platinum screen. The potential difference between this electrode and a Ag/AgCl reference electrode was related to the glucose concentration in the sample. The hydrogen peroxide formed was thought to be the source of the potential. Early work with glucose only demonstrated an upper limit of 40 mg/dℓ[88] but with further improvements, this range was extended to 150 mg/dℓ.[89] This was accomplished by coimmobilizing catalase with the glucose oxidase and surrounding the electrode with a cellulose acetate membrane to exclude macromolecules.

Proteins have been determined with enzyme electrodes by first cleaving the protein with proteases and releasing ammonia with L-amino acid oxidase.[90,91] An ammonia-sensitive gas-electrode was used to measure the ammonia formed. Proteins were quantitated from 0.1 to 100 μg/mℓ.

Another novel application of enzyme-based electroanalytical sensors is the assay of thyroxine using an iodide electrode. The microsomal fraction of rat liver possesses thyroxine de-iodinating activity which cleaves I^- from T_4. The iodide which is released allows the quantitation of thyroxine at submicromolar levels.[92]

Enzyme electrodes can also be used to monitor compounds other than their respective substrates. Materials which act to slow down or stop an enzyme's activity can also be determined. Various inhibitors to a number of enzymes are known. One of these is fluoride which is known to inhibit urease activity. Thus, F^- can be estimated by exposing the urea electrode to unknown fluoride solutions in fixed amounts of urea.[93]

Urease can also be inhibited by mercury (II) in a similar manner.[94] It was shown that between 0 to 0.7 nmol of Hg_2^{+2} can be quantitated. However, the mercury binds so tightly to the enzyme that thioacetamide and EDTA are required to regenerate the urease activity.

C. Bacterial Electrodes

Highly selective membrane electrodes have been prepared by immobilizing isolated enzymes as biocatalytic layers on ion-selective electrodes. The enzyme is isolated from bacterial or animal tissue sources in a purified form. However, it is known that many isolated enzymes are unstable outside their natural environments. Thus, it should be feasible to utilize the source of the enzyme as a biocatalytic layer to develop membrane sensors while avoiding the troublesome extraction and purification steps.

This concept was put into practice when the bacterium *Streptococcus faecium* was immobilized onto an ammonia gas-sensing electrode.[95] This species is known to possess a high L-arginine diaminase activity which has been utilized for microbiological assays of this amino acid. A commercial ammonia gas-sensing electrode was combined with a layer of bacteria held to the tip of the electrode with a dialysis membrane. This structure is depicted in Figure 5 and represents the typical construction for these types of sensors. The response times for bacterial electrodes are longer than for enzyme electrodes as the substrate must first diffuse into the bacterium then undergo enzymatic conversion to products which must finally diffuse from the cell. The electrode required approximately 20 min to reach a steady state value in the L-arginine concentration range of 5×10^{-5} to 1×10^{-3} molar. The best response of the electrode was observed in pH 7.4 phosphate buffer. The response curve for L-arginine is shown in Figure 6 which also indicates the electrode's selectivity over other amino acids.

It can be seen from this figure that L-asparagine and L-glutamine interfere

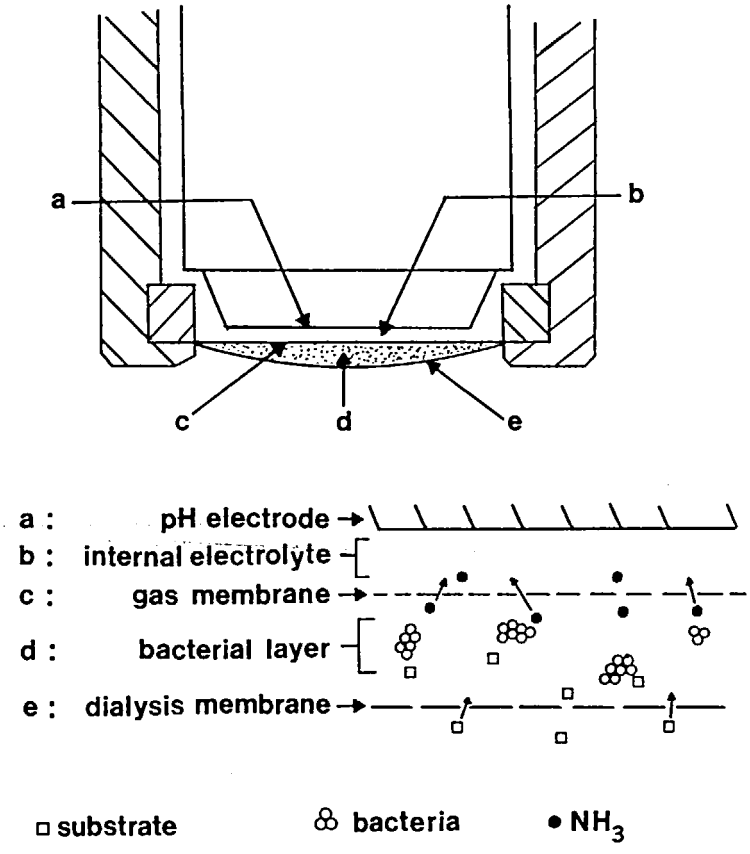


FIGURE 5. Construction of a bacterial electrode.

significantly, indicating that the cells possess deaminating activity for these two amino acids. This illustrates one of the limitations of bacterial-based sensors. However, by carefully choosing the bacterial strain and culturing it in deficient media, one can enhance an organism's production of specific enzymes. Also, genetic engineering may prove beneficial in tailoring a cell's biochemical make-up, thereby inducing cells to produce particular enzymes over others.

Another observation was that the L-arginine electrode was viable for at least 20 days, although with reduced response. Since the bacteria were stored in buffers, it was reasoned that some of the bacteria expired, resulting in reduced enzyme activity. It was demonstrated that the electrode could be regenerated by storage in the growth medium. The response characteristics were restored to those observed on the first day of testing.

This concept was taken full advantage of to form a bacterial electrode for L-aspartate.[96] The conventional enzyme electrode for this amino acid is active for only one day of operation since the enzyme is very labile. It was reasoned that if the enzyme were to remain in its natural environment the sensor may display a longer lifetime. The bacterial strain *Bacterium cadaveris* is the source for the isolated enzyme and was directly immobilized onto an ammonia gas-sensing electrode. The useful lifetime of the bacterial electrode was initially 2 days but was extended to 10 days by storage of the electrode in 1 m*M* dithiothreitol. This same stability is realized by storing the electrode in the growth medium at 30° C where it is thought that the bacteria regenerate and replenish the enzyme supply continuously.

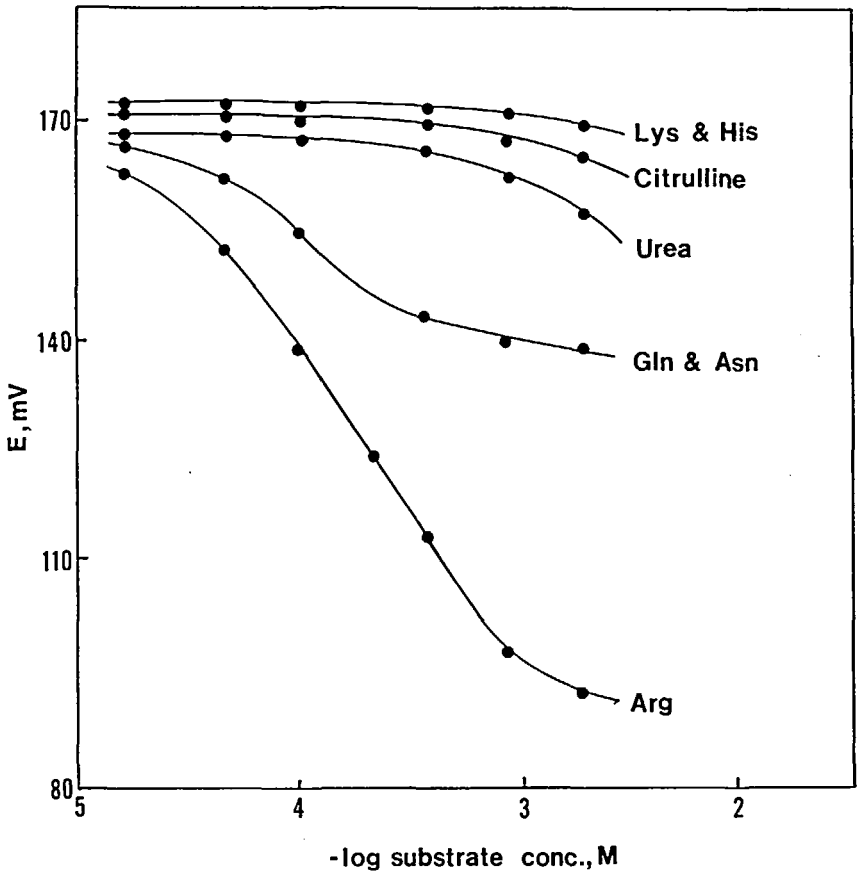


FIGURE 6. Selectivity studies of bacterial electrodes. (From Rechnitz, G. A., Kobos, R. K., Riechel, S. J., and Gebauer, C. R., *Anal. Chim. Acta*, 94, 357 (1977). With permission.)

Table 2
BACTERIAL ELECTRODES

Substrate	Bacterial strain	Sensing electrode	Ref.
Glutamine	*Sarcina flava*	NH_3	97
L-Cysteine	*Proteus morganii*	H_2S	98
NAD	*E. coli* plus NAD-ase	NH_3	99
Cephalosporins	*Citrobacter freundii*	pH	100
Glutamic acid	*E. coli*	CO_2	101
L-Histidine	*Pseudomonas*	NH_3	102
Nitriloacetic acid	*Pseudomonas*	NH_3 or CO_2	103

These experiments served to illustrate novel biocatalytic sensors which have lead to a host of publications which are increasing in number. Table 2 lists the more recent electrodes which have appeared and indicates the growing interest in this field.

The use of intact bacterial cells holds certain advantages over isolated enzymes when coupled with potentiometric sensors. The most obvious is the means of harvesting the biocatalytic agent for use. There are no extraction and purification steps to reduce the

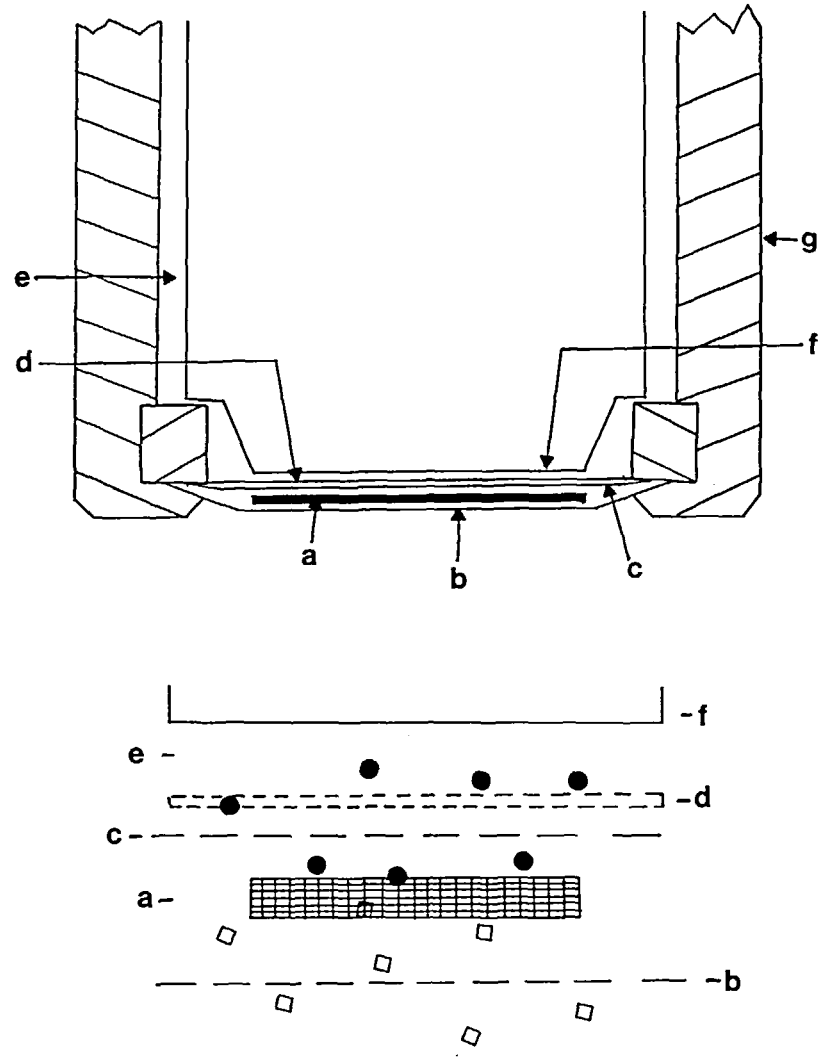


FIGURE 7. Top, schematic diagram of the kidney tissue-based membrane electrode for glutamine; (a) the slice of porcine kidney tissue, (b) a monofilament nylon mesh, (c) the protective dialysis membrane, (d) the gas-permeable membrane, (e) the internal electrolyte solution, (f) the pH sensing glass membrane, and (g) the plastic electrode body. (Components d-g represent the Orion model 95-10 ammonia gas sensing electrode.) Bottom, expanded view of the electrode sensing tip showing components and phases. (From Rechnitz, G. A., Arnold, M. A., and Meyerhoff, M. E., *Nature*, 278, 466 (1979). With permission.)

yield of enzyme. The enzyme remains in its natural environment optimized by evolutionary forces for that particular function. By not disturbing the cell, the enzyme function is left in its most intact state. Finally, the enzyme activity can be regenerated by simply growing more bacteria, even at the electrode surface.

There are, however, potential difficulties to overcome. Most bacteria possess a multitude of enzymes and metabolic pathways which can introduce numerous interferences. But by controlling the bacteria during its growth phase by nutrient deprivation or genetic engineering, enzyme activities can be enhanced or attenuated. It is

also possible to inhibit unwanted activities by choosing appropriate enzyme inhibitors to tailor the selectivity of the sensor to discriminate against the more serious interferants.

D. Tissue Electrodes

In the previous section, the concept of bacterial electrodes was developed as an extension of the enzyme electrode. Using analogous reasoning, it is known that mammalian tissue contains various enzymes and that this activity can be coupled to potentiometric electrodes to form biosensors.

The first demonstration of this novel type of sensor was accomplished by holding slices of porcine kidney tissue slices onto an ammonia gas-sensing electrode.[104] A diagram of this sensor and a depiction of the molecular events is illustrated in Figure 7.

The response of the electrode to glutamine reached a steady state value within 5 to 7 min. The electrode was tested for possible interference from urea, L-alanine, L-arginine, L-histidine, L-valine, L-serine, L-glutamic acid, L-asparagine, L-aspartic acid, D-alanine, D-aspartic acid, glycine, and creatinine. These response curves are shown in Figure 8 where the shaded envelope represents the responses from the interferents tested. The electrode displays excellent selectivity over the other substrates with good sensitivity and response slope. The tissue electrode was usable for at least 28 days with storage in simple buffer with sodium azide added to inhibit bacterial growth on the tissue slice.

This biosensor was used to measure glutamine levels in cerebrospinal fluid.[105] The level of this amino acid has been linked to various disorders such as hepatic coma and Reye's syndrome. The precision and accuracy of the tissue-based glutamine sensor was adequate to cover the clinically important range of glutamine. The construction of this type of sensor was recently patented[106] which serves to illustrate the novel nature of these types of sensors.

A comprehensive comparison of biocatalytic electrodes and the tissue electrode was performed with glutamine as the substrate.[107] Enzyme bacterial, mitochondrial, and tissue electrodes were prepared using the isolated enzyme glutaminase, the cells *Sarcina flava,* kidney mitochondria, and porcine kidney cortex cells, respectively.

Each electrode was tested under optimized conditions for each type of sensor. The operational response was characterized and compared. The response slope, linear range, limit of detection, response time, and minimum useful lifetimes were tabulated.

The isolated enzyme electrode proved to be the poorest sensor on all counts. The tissue and bacterial electrodes displayed the greatest linear range and had response slopes comparable to the mitochondrial electrode. The most striking difference between the electrodes is illustrated in Figure 9 which shows the minimum useful lifetimes of the sensors.

The tissue and bacterial electrodes offer the most useful sensors as compared to the enzyme and mitochondrial electrodes. When comparing the preparation of the tissue and bacterial electrodes, the tissue electrode would be the easiest to construct. The bacteria must be cultivated in sterile conditions, while the tissue slices do not. Moreover, the tissue slice contains its own connective tissue as a natural support structure which retains its integrity longer than the bacterial paste. It should be pointed out that this study is only concerned with glutaminase activity and further work should be carried out to characterize additional biochemical systems. However, this work clearly shows the significance and applicability of tissue-based sensors.

An excellent example of how the response characteristics of a tissue electrode can be manipulated has been described where mouse small intestine tissue was coupled with an ammonia gas-sensing electrode.[108] This particular tissue contains adenosine deaminase activity and can be used in the determination of adenosine. However, it was noted that the electrode displayed severe response interferences to the adenosine-containing

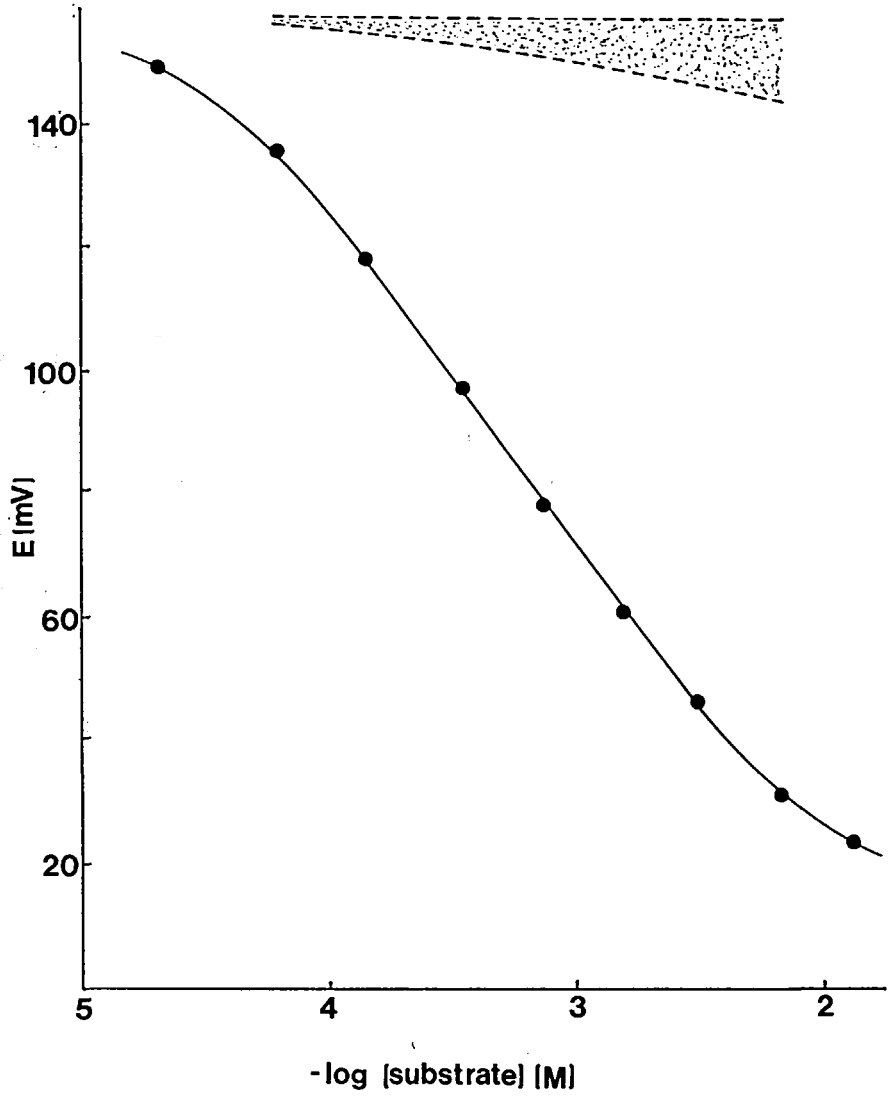


FIGURE 8. Potentiometric response and selectivity of the kidney-based electrode in 0.1 *M* phosphate buffer, pH 7.8, with 0.02% sodium azide preservative at 26°C. Solid line shows the electrode response as a function of glutamine concentration over the range 2×10^{-2} to 5×10^{-5} *M*. Dotted region shows the maximum response to the 13 possible interference compounds tested. (From Rechnitz, G. A., Arnold, M. A., and Meyerhoff, M. E., *Nature*, 278, 466 (1979). (With permission.)

nucleotides, AMP, ADP, and ATP. The response curves for these various substrates are shown in Figure 10.

The authors identified possible enzymatic pathways to describe the interfering effects for the adenosine nucleotides. A systematic study of activators, inhibitors, and pH optima revealed that the interfering activities could be sequentially eliminated. The results of the selectivity enhancement efforts resulted in the results shown in Figure 11.

It can be clearly seen that this approach was completely successful in reducing the interferences to negligible levels. In this way, a relatively nonspecific biocatalyst was altered to enhance its response to a desired analyte. This should prove a fruitful area after

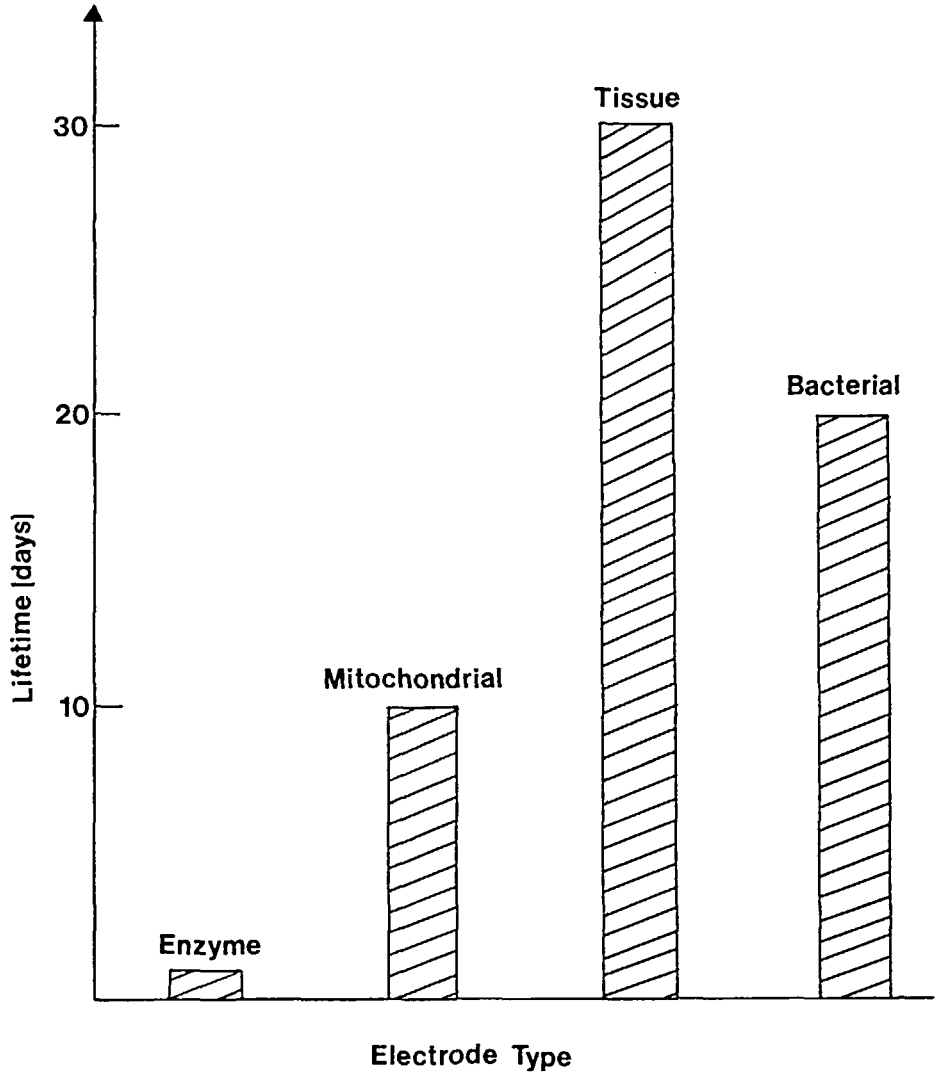


FIGURE 9. Comparison of minimum useful lifetimes. (From Arnold, M. A. and Rechnitz, G. A., *Anal. Chem.*, 52, 1170 (1980). With permission.)

further research as the animal kingdom has supplied numerous species and tissue types which can potentially offer improved methods of analysis.

One such tissue was recently identified and was used for the determination of antidiuretic hormone.[109] This hormone controls the transport of both water and sodium ions across the wall of the urinary bladder of a common toad (Bufo Marinus). A freshly removed bladder was mounted on a plastic former and held by a rubber O-ring. A sodium ion-selective electrode was placed inside the assembly and made contact with the inside surface of the bladder membrane. Changes in potential resulted when the electrode was exposed to solutions of varying antidiuretic hormone levels. Preliminary experiments demonstrated that the rate of potential change was related to the level of hormone present.

Other hormones also resulted in sodium and water transport changes in the toad

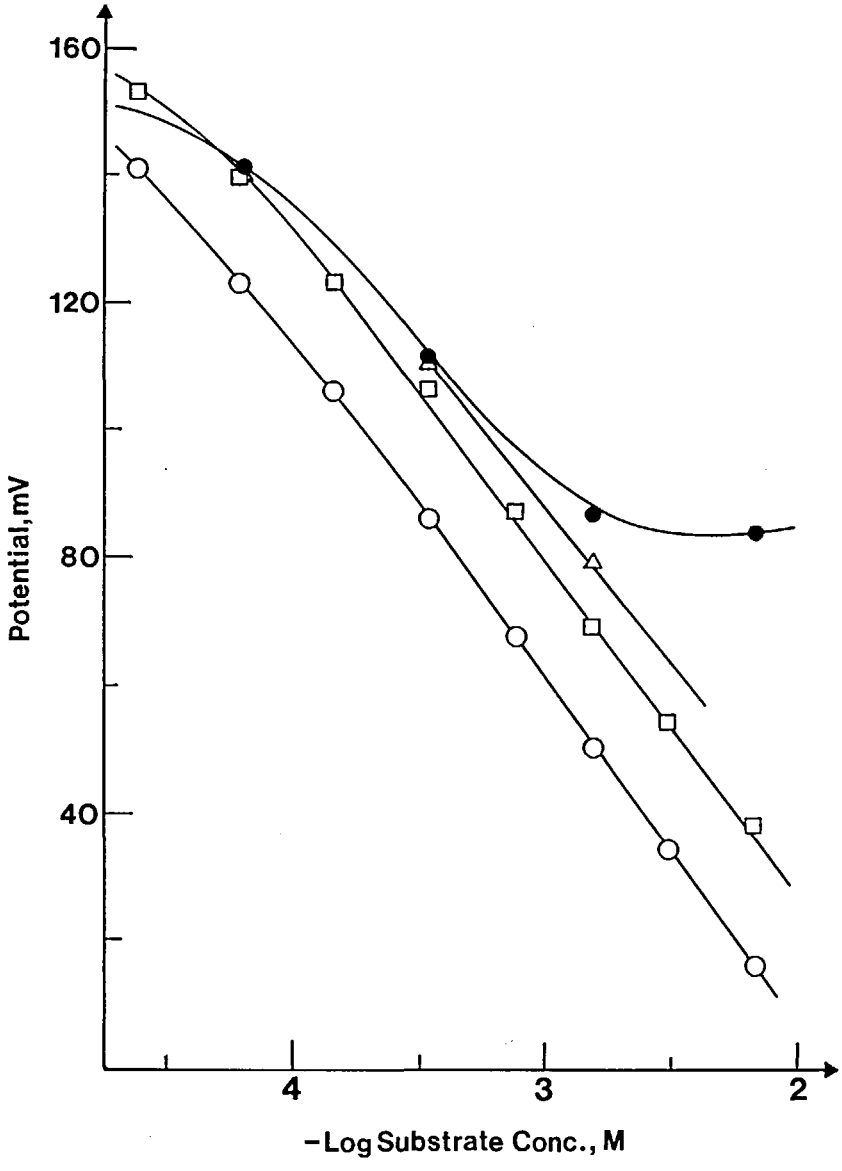


FIGURE 10. Response of the tissue-based adenosine electrode for adenosine (O), AMP (□), ADP (Δ), and ATP (●) using a 0.2 *M* Tris-HCl, 0.02% NaN_3, pH 8.2 buffer system (four electrodes tested). (From Arnold, M. A. and Rechnitz, G. A., *Anal. Chem.*, 53, 515 (1981). With permission.)

bladder electrode. In fact, vasopressin responded so well that it was suggested that this electrode be used in the bioassay of this material instead.

This work illustrates the possible assay schemes which can be devised by combining suitable tissues with ion-selective electrodes. Tissue samples are affected by a host of agents including hormones, drugs, inhibitors, activators, and many other chemical messengers. It should be feasible to assay any of these agents when the biochemical events are identified and exploited when used in conjunction with specific electrodes.

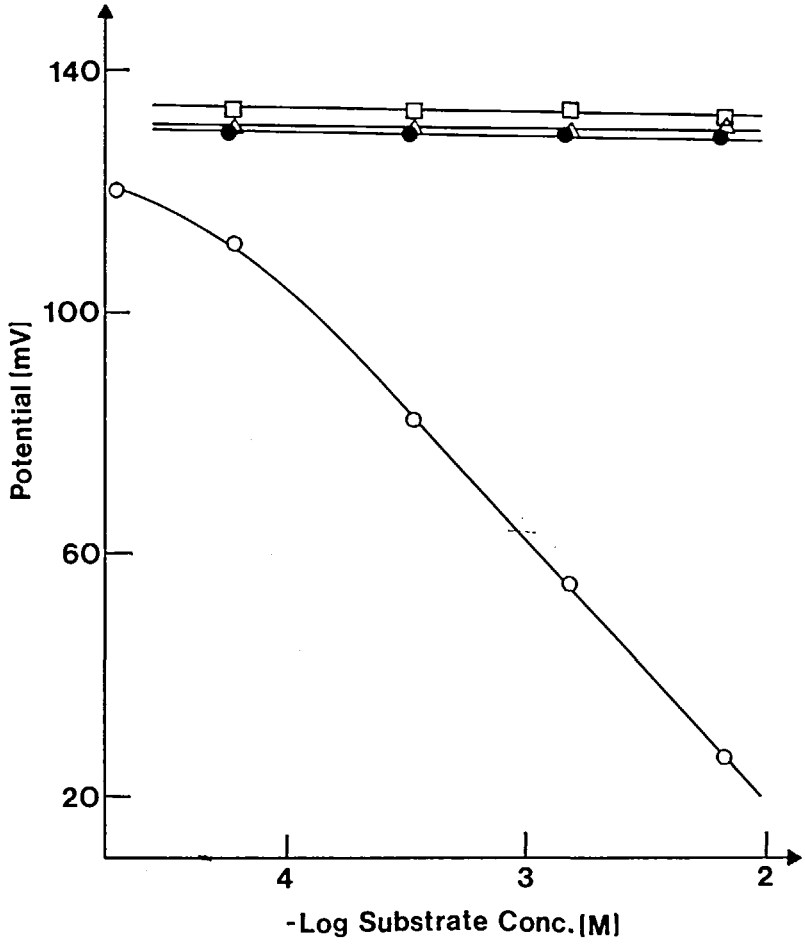


FIGURE 11. Response of the tissue-based adenosine electrode for adenosine (O), AMP (□), ADP (Δ), and ATP (●) using a 0.1 *M* Tris-HCl, 0.2 *M* K_2HPO_4, 0.02% NaN_3, pH 9.0 buffer system (two electrodes tested). (From Arnold, M. A. and Rechnitz, G. A., *Anal. Chem.*, 53, 515 (1981). With permission.)

V. IMMUNO-RESPONSIVE ELECTRODES

A. Indirect Measurements

Protein determinations can be accomplished using ion-selective electrodes by coupling a reaction which consumes ions to which the electrode responds. One such reaction takes advantage of the sulfhydryl content of proteins.[110] The sulfhydryl groups on proteins are made available to bind silver ions by denaturation in alkaline media. Human serum albumin (HSA) could be determined in the range 0.5 to 30 μg/mℓ with the addition of HSA antibody.[111] The HSA was serially diluted and incubated with its respective antibody. As in the classic precipitin test, a precipitate is formed in the region of the zone of equivalence. The precipitates are centrifuged and either the supernatant or the precipitate is denatured and mixed with silver ion. The difference in potential, as measured with a silver-selective electrode, between the sample and a blank can be plotted vs. the HSA concentration to form a standard curve.

An extension of this concept forms the basis for an automated immunoassay using ion-selective electrodes.[112] In this work, instead of using soluble antigens, a haptenic group is

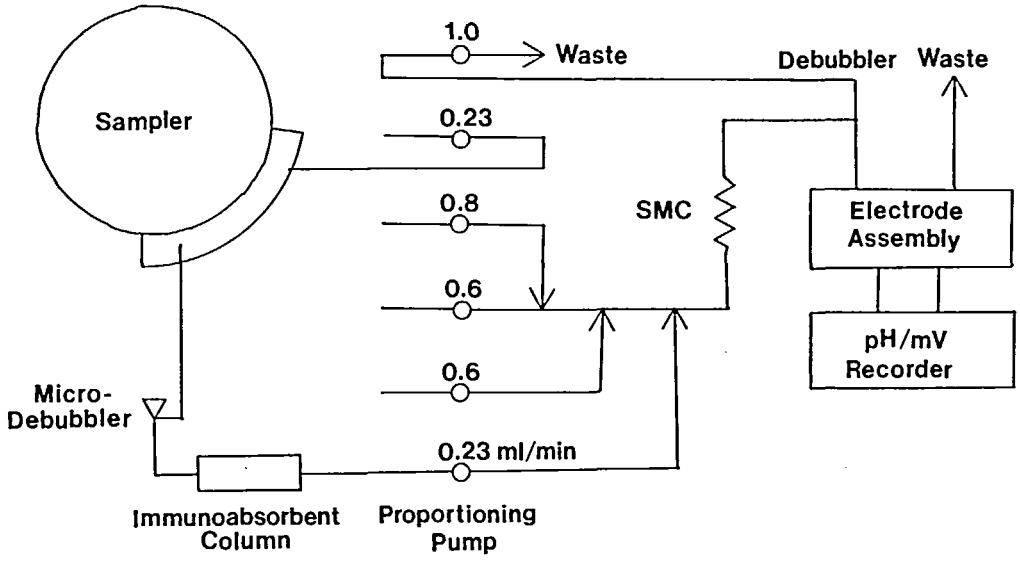


FIGURE 12. Schematic diagram of the continuous flow apparatus. Concentrations used throughout were: Ag^+ (6.0×10^{-5} *M*), urea (5.0 *M*), NaCl (0.9%). The cam was a modified 1:1 sample-to-wash at 20 samples per hour. (From Solsky, R. L. and Rechnitz, G. A., *Anal. Chim. Acta*, 99, 241 (1978). With permission.)

covalently linked to a solid support where the antigen-antibody reaction takes place. The antibody sample is applied, the specific antibody is retained in the immunoabsorbent column, and the nonspecific proteins are washed from the column. An eluting buffer is then passed through the column removing the bound antibody. This antibody is delivered to an alkaline denaturant containing silver ion. The sulfhydryl groups on the antibody bind the silver ion and the residual silver is sensed by a flow-through Ag/Ag_2S electrode. The experimental apparatus for this scheme is shown in Figure 12.

This flow-through automated immunoassay analyzer was capable of determining antibody in the low $\mu g/m\ell$ range while processing individual samples every 12 min. The analyzer could process whole serum and can be readily adapted for screening antibody titers as needed. By introducing a simple timed valve assembly, sample throughput should increase dramatically.

An innovative means of studying antigen-antibody reactions makes use of a hapten-selective electrode.[113] Direct antibody-hapten binding constant measurements were performed using this sensor. In this case, an electrode for the hapten trimethylphenyl ammonium ion was constructed and the equilibrium binding constant was determined homogeneously. No dialysis cell was required as the electrode could differentiate between free and bound hapten. This approach offers an attractive method for the titration of antibodies where an appropriate hapten electrode can be assembled.

B. Amplified Responses

1. Enzyme Immunoassay

In the previous section, indirect sensors were discussed which measure antibodies as a consequence of a direct chemical interaction between the antibody molecule and the ions the electrode senses. Amplified responses will be developed here starting with the enzyme immunoassay.

Radio-immunoassay is perhaps the grandfather of amplified assays. However, in recent years there has been a movement away from the radioisotopes needed for this

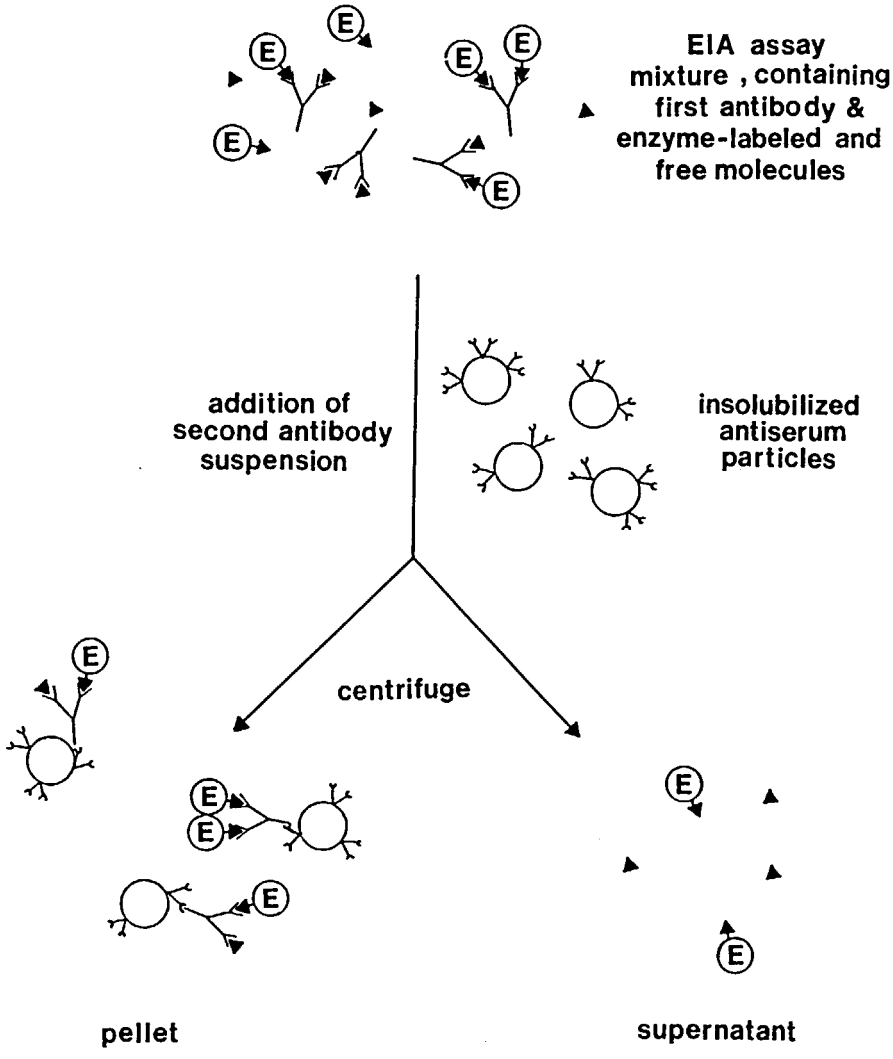


FIGURE 13. Schematic representation of double-antibody solid-phase EIA using cross-linked second-antibody particles. (From Meyerhoff, M. E. and Rechnitz, G. A., *Anal. Biochem.*, 95, 483 (1979). With permission.)

technique. Enzymes have been shown to be adequate substitutes in many cases. The enzyme label need only have a relatively high turnover number and produce or consume an ion which can be sensed by an electrode.

One of the first demonstrations of electrode-based enzyme immunoassay utilized an iodide electrode for the determination of hepatitis B surface antigen.[114] Antibodies to the surface antigen were immobilized on an artificial protein membrane which was held to the surface of an iodide ion-selective electrode. The assay for the surface antigen was performed according to the "sandwich" principle, i.e., the immobilized antibody membrane was incubated in a solution containing the hepatitis B surface antigen. The membrane was washed leaving bound antigen on the membrane. Peroxidase-labeled IgG antibody to the antigen was then used to treat the membrane. The sandwich structure is composed of the following:

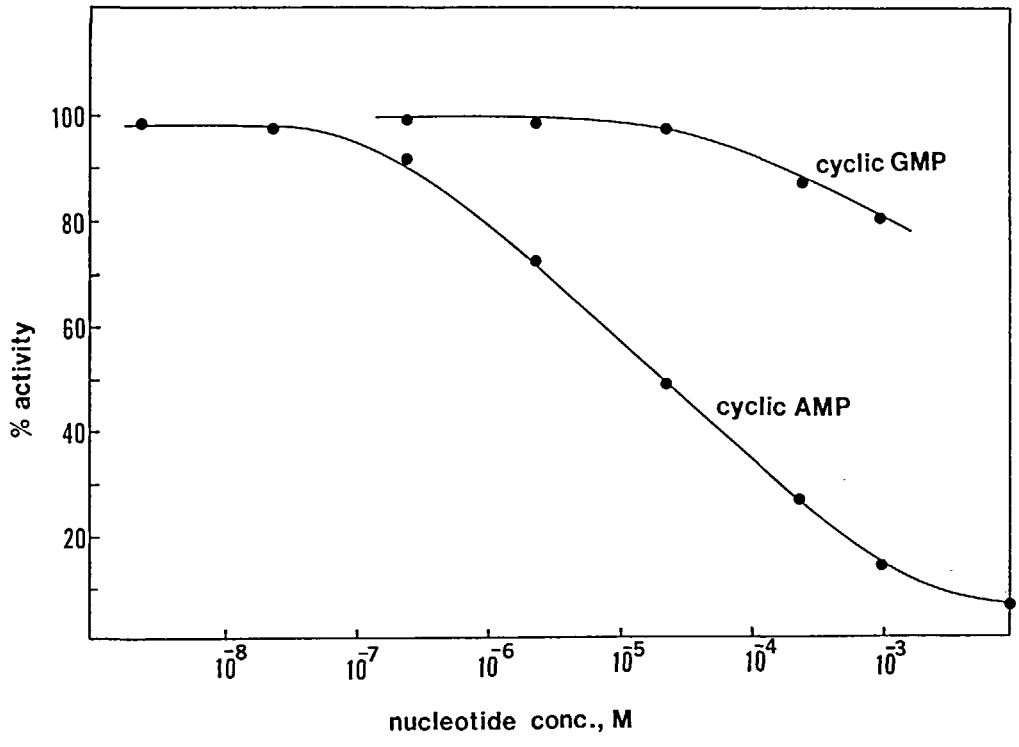


FIGURE 14. Calibration curves obtained for cAMP and cGMP using a urease-cAMP conjugate and cAMP antibody. Data obtained in 0.1 *M* Tris-HCl-EDTA, pH 7.5, using 1.0 mℓ of nucleotide standard, 30 μℓ 1:10 rabbit anti-cAMP antibody, 30 μℓ 1:10 urease-cAMP conjugate, and 300 μℓ of second-antibody suspension. (From Meyerhoff, M. E. and Rechnitz, G. A., *Anal. Biochem.*, 95, 483 (1979). With permission.)

Solid Support Membrane	Hepatitis B Antibody	Hepatitis B Antigen	Peroxidase-Labeled Hepatitis B Antibody

where the solid line represents a covalent bond while the dotted lines indicate antigen-antibody electrostatic interactions.

The treated membrane is fixed onto the surface of the iodide-sensing electrode and inserted into an optimized solution of hydrogen peroxide and iodide. The peroxidase-labeled IgG catalyzes the reduction of the peroxide while the iodide is consumed by oxidation. The decrease in iodide ion concentration expressed as a change in potential of the indicator electrode, is proportional to the amount of hepatitis B surface antigen.

Linear calibration curves were prepared in the range 0.5 to 50 μg/ℓ with a C.V. of 15 to 20%. Reproducibility was good but the length of incubation (6 to 7 hr) required for maximum signal output certainly reduces sample throughput.

Another example of electrode-based enzyme immunoassay is the determination of BSA or c-AMP.[115] The enzyme label is urease and an ammonia gas-sensing electrode is used as the detector.

The antigen is determined by a competitive-binding, solid-phase assay system which is depicted in Figure 13. A sample containing the analyte is mixed with antibody and enzyme-labeled analyte. After a suitable time, a solid-phase second antibody is added which removes the first antibody from solution. The mixture is sedimented and washed.

The pellet is resuspended and the level of enzyme activity is determined with the addition of substrate. The products of the enzymatic reaction are monitored with the electrode and the analyte is determined.

In the case of c-AMP, a typical inhibition calibration curve is shown in Figure 14. The % activity of the pellet is reduced at increasing c-AMP concentrations by the competition between enzyme-labeled and free c-AMP for the fixed amount of antibody.

Increased sensitivity was observed for c-AMP by using c-GMP or c-IMP conjugates with a concurrent loss of selectivity for c-AMP. This effect could very well be the result of different specific activities of the nucleotide-enzyme conjugates which would displace the calibration curve to either higher or lower analyte concentrations.

The combined time required for this assay was 3 hr followed by centrifugation and wash cycles. This would keep sample throughput low which seems to be a common feature of enzyme amplified assays.

2. Loaded Vesicle Immunoassay

An alternate immunoassay approach uses chemical amplification instead of enzyme amplification. In this method, an antigen-antibody reaction is revealed by the release of marker ions from vesicles.

Vesicles can be red blood cells ghosts which are viable red cells treated in a specific way to allow the incorporation of selected ionic species. These red cell ghosts, having specific antigenic determinants on their surface, are capable of binding corresponding antibodies and being lysed by complement. Complement is a series of enzymes which is capable of recognizing an antigen-antibody complex on a cell surface and subsequently lysing that cell. When the cell is lysed, its contents are released to the bulk medium.

An attractive immunoassay technique can be conceived by employing an ion-selective electrode with cells that have been loaded with a specific ion. This was demonstrated for the antibody, hemolysin, and loaded red cells containing the nonphysiologic ion, trimethylphenyl ammonium ion (TMPA).[116] The effects of antibody concentration, complement titer, and number of cells used in the assay were considered in optimizing the electrode response.

This work was further extended to the measurement of serum antibodies to bovine serum albumin (BSA) by utilizing the complement fixation test.[117] As stated before, complement can recognize antigen-antibody complexes. One of the first complement components binds to this complex and is "fixed". If this complex is free in solution, then that complement which is bound is unavailable for other complexes, say on the surface of a cell.

Thus, BSA antibody was determined by first mixing a fixed amount of BSA with the antiserum and then adding complement. A certain amount of the complement was tied up and upon addition of an aliquat of loaded red cells, an attenuation of the maximal lysis was observed. The BSA antibody could be quantitated in the low $\mu g/m\ell$ range. Actual analysis of commercial antisera was accomplished and correlated to within 3 to 4% for the stated bottle values.

The electrode detector offers several advantages over turbidometric means of analysis. The most obvious is that the electrode is oblivious to sample turbidity or color allowing whole blood samples to be run if desired. The marker ion can be chosen to best suit the selectivity of the electrode and the other constituents of the sample to avoid interferences. Lastly, the red cell ghosts are large vesicles capable of containing relatively large amounts of marker ion. Along this same line, the red cell is a large cell capable of carrying most any hapten or antigen regardless of size. This allows for a universal assay system composed of uniform reagents and electrode capable of monitoring a multitude of analytes.

Another type of vesicle which has been used in a similar way is the phospholipid liposome. Liposomes are artificial vesicles formed using phosphatidylcholines, cholesterol, and a variety of additional agents. The liposomes are most commonly formed as multilamellar structures which can trap ionic species as do the red cell ghosts.

In one example using liposomes, an intermediate of brain metabolism, ganglioside was incorporated into the wall of liposomes containing tetrapentyl ammonium ion (TPA).[118] Upon treatment with the specific antibody, complement lysed the vesicles releasing the trapped TPA.

In another publication, the authors used the same system where the liposomes were labeled with dinitrophenol (DNP).[119] Dinitrophenyl antiserum and complement were able to lyse the liposomes and the assay system was extended to include inhibition assays for free DNP. The free DNP was used to "titrate" a fixed amount of DNP antibody which was then mixed with sensitized liposomes and complement. The inhibition curve covered the range 10^{-7} to 10^{-8} molar of hapten and demonstrates the sensitivity of the assay. By coupling other haptens to the liposomes and increasing the loading level of trapped ion, inhibition assays for various drugs and hormones could become possible.

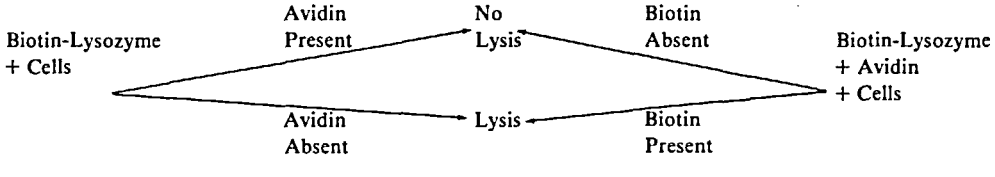
One potential drawback in these immunoassays is inherent in the antigen-antibody system itself. Some antibodies to haptens show severe cross-reactivity with metabolic byproducts. Also, the binding constant is variable from lot-to-lot of antiserum and can degrade the detection limit if the binding constant drops too low.

One way of avoiding these difficulties would be to use materials with binding constants that are relatively constant and reproducible. One such pair is the biotin-avidin couple. The binding constant is five to six orders of magnitude greater than hapten-antibody binding constants allowing for more sensitive assays to be considered. The use of avidin and biotin can be a tool when applied to a variety of molecular biological problems.[120]

One recent method estimates avidin or biotin using a lysozyme label and loaded vesicles.[121] In this instance, the vesicles are trimethylphenyl ammonium ion (TMPA) loaded *Micrococcus lysodeikticus* cells. This cell line was chosen for its high susceptibility to lysis by the action of lysozyme on its cell surface. The avidin takes the place of the antibody in traditional immunoassays. The lysozyme is labeled with biotin to form the other half of the lysis system.

The method of analysis is outlined below and depends on the inhibition of lysozyme activity by avidin binding. Avidin is a large protein and prevents the lysozyme from reaching the cell walls of the loaded *M. lysodeikticus*.

Avidin-Biotin Assay Scheme



Avidin was determined in the range 10 to 100 μg/mℓ and biotin could be assayed in an inhibition mode between 10^{-5} and 10^{-7} molar. The lysozyme label was homogeneously inhibited requiring no separation steps. The electrode detector allowed the analysis to be performed in turbid samples which enhance the potential use of this type of assay. The sensitivity of the assay could probably be improved through optimization of specific activities of the enzyme label and loading protocol of the cells.

C. Direct Sensors

The conception and demonstration of direct potentiometric electrodes which are capable of responding to immunoreagents is perhaps the most exciting and promising area of current research. Interest in this type of sensor was sparked by early work using bilayer lipid membranes which incorporated antigens or antibodies.[122-125] These thin, fragile membranes could be monitored for resistance changes upon the addition of the complementary immunopair. However, these membranes were difficult to prepare and proved very labile.

The first practical demonstration of a stable membrane response used the model immunosystem, Concanavalin A and yeast mannan.[126] Although Concanavalin A is not a true antibody, it does serve as a model as it selectively binds polysaccharides to form strong complexes. Concanavalin A was attached to a polyvinyl chloride covered platinum wire to form the indicating electrode and the cell was completed by using a Ag/AgCl electrode as reference. The response of this electrode to yeast mannan was observed in the concentration range 0.1 to 10 mg/mℓ at a pH of 3.5. However, it was noted that a polyvinyl chloride membrane not incorporating Concanavalin A also responded to yeast mannan nonselectively, indicating a high degree of nonspecific absorption.

This difficulty was overcome by preparing two identical electrodes, both containing Concanavalin A. One electrode was rendered a reference electrode by blocking the binding sites with D-(+)-glucosamine. This treatment prevents this electrode from binding yeast mannan but allows nonspecific absorption to parallel that for the indicating electrode. The use of this reference electrode allowed for the selective determination of yeast mannan. There is no response to agar, which is reported to be a nonbinding polysaccharide. Since this does not bind to Concanavalin A, one would not expect to observe a response.

When this model system was extended to include immobilized antigens and antibodies, no observable responses were noted. Thus, either the membrane conditions were not correct for a true immunoresponse or the antigen-antibody reaction did not take place. A possible explanation for the yeast mannan response may be found by inspection of its isoelectric point. Since the operating pH was so low (3.5), it may be the observed response arose from charge buildup at the membrane surface. This effect will be seen in several examples which follow.

For the remainder of this discussion, it is useful to divide electrodes into two types depending on whether or not their solution-membrane interfaces are blocked. There are several excellent descriptions of these interfaces so only a brief overview will be presented here.[26,30] A blocked interface is one where no ion conduction takes place and no net faradaic current flows over a given potential range. An example of this type of interface is that of certain metals placed in solution. An interface which is not blocked allows the passage of ions or electrons into and from the membrane to the solution phase. Examples of these interfaces include the various polymer membrane materials, polyvinyl chloride, cellulose acetates, silicon rubber, etc. which overlay electrochemically reversable metal compounds such as AgCl and oxidized platinum.

1. Blocked Interfaces

The first example of a blocked-interface, immuno-responsive electrode utilized a titanium wire coated with antibody to human chorionic gonadotropin (hCG).[127] The titanium wire was first oxidized then activated with cyanogen bromide. A reference electrode was prepared by coupling urea to a similarly prepared titanium wire. The electrode responded to hCG in a pH 8.5 buffer with no reported interferences from other proteins. The potential shifted in a positive direction, while when using an electrode

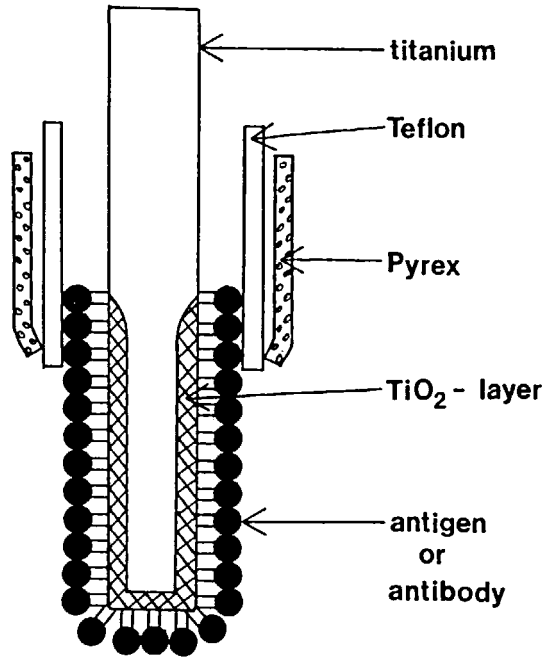


FIGURE 15. The schematic feature of the working electrode modified with proteins. (From Yamamoto, N., Nagasawa, Y., Sawai, M., Sudo, T., and Tsubomura, H. J., *Immunol. Methods*, 22, 309 (1978). With permission.)

coated with hCG, the potential shifted to more negative values upon anti-hCG additions. These findings can be explained by the buffer pH. The average isoelectric point for immunoglobulins (IgG) is below 7.0. Thus, at pH 8.5 the antibody should have a net negative change which would lead to the observed potential changes. The magnitude of potential response was low and the response did not reach a steady state value even after 1 hr.

Further work was performed on this system to investigate the operational characteristics of the electrode.[128,129] The electrode was prepared as before, but care was taken to standardize the active surface exposed to the test solution. The modified electrode is shown in Figure 15 and depicts the bonded immunoreagent and the protective layers to limit the surface area.

It was observed that the generated potential could be "fixed" by removing the hCG solution and replacing it with fresh buffer. The hCG could be dissociated from the bound antibody by treatment with dilute hydrochloric acid. In this way the electrode could be regenerated for reuse.

The pH dependency of hCG response was reported and reached a maximum at a pH of 8.7. Again this could be explained by the charge of the antibody. However, the electrode response fell off at pH values greater than 8.7. This could be due to a decreased affinity between the antibody and hCG. Just as low pHs can dissociate immune complexes, so can high pHs.

The low magnitude of potential response and long response times are most probably due to the surface area and geometry of the titanium wire. The wire does not present a large surface area and thus limits the maximum observable signal. The long response

times could be related to the kinetics associated with solid-phase reactants. If these two areas can be addressed, immunoelectrodes displaying both significant response and reasonable response times will become available.

Another example where a blocked interface is used is to assay human serum albumin (HSA).[130] A titanium wire was oxidized as before, and the dye, Cibacron Blue F3G-A, was physically absorbed to the wire. Another wire was prepared and used as reference by omitting the dye. The dye is able to interact with and bind HSA which alters the surface potential of the wire. The presence of HSA in physiological pH buffer decreases the potential of the electrode.

The affinity electrode responded linearly to HSA levels between 0 and 15 μg/mℓ but also responded equally to bovine serum albumin. Myoglobin and rabbit γ-globulin responded slightly while lysozyme and ovalbumin did not. The response to HSA was reversable and the electrode could be used for several months.

It should be cautioned that the use of relatively nonspecific binding phenomena may yield sensors of low specificity. However, this approach may prove very fruitful in the development of class-specific electrodes which could be used to monitor the presence of general classes of materials. This topic will be developed further in a later section.

2. Nonblocked Interfaces

This section will illustrate examples of immunoresponsive electrodes where one component of the immune pair is incorporated within a membrane which overlays a nonblocked electrode interface. The electrodes which are used are typically the saturated calomel electrode or the Ag/AgCl electrode. The membrane materials which are used in conjunction with these electrodes have been of only two types to date. The one that will be described first is triacetyl cellulose and the second is polyvinyl chloride.

The antibodies that are measured by a potentiometric immunosensor must, in some way, modify the surface characteristics of the sensing membrane to elicit the potentiometric response. Fundamental studies were performed using triacetyl cellulose as a membrane material to demonstrate the effects of chemical modification at the membrane surface on the transmembrane potential.[131] A series of derivatized membranes were prepared containing different functional groups. These groups consisted of bromide, amino, or epoxy residues which altered the surface nature of the membrane expressed as different transmembrane potentials.

The bromo- and epoxy-modified membranes exhibited slight negative charge densities while the amino-membrane displayed a strongly positive charge density on the membrane surface. These results are consistent with what would be expected for the respective groups.

Further experiments demonstrated the effects of immobilized proteins on the transmembrane potential. Egg albumin and lysozyme were covalently attached to the epoxy-modified membrane and the membrane potentials were measured. The transmembrane potentials, charge densities, and protein isoelectric points are listed in Table 3 to illustrate the membrane properties.

The pH during measurements was not given but is assumed to be 5 to 6 as unbuffered saline was used. As one would predict, the albumin membrane is negatively charged and the lysozyme membrane is positively charged.

This work serves as a model for what is required of an immunosensor. It demonstrates how the properties of specific proteins result in a predictable potentiometric response when that protein is attached to the surface of a membrane. In fact, an immunoresponsive membrane was demonstrated by blending the cardiolipin antigen complex in a cellulose triacetate membrane.[132] This membrane was shown to be responsive to the

Table 3
CHEMICALLY MODIFIED MEMBRANE PROPERTIES

Membrane modification	Isoelectric point	Charge density (eq./L)	Transmembrane[a] potential (mV)
Bromo	N/A	-3.54×10^{-3}	−1.3
Amino	N/A	1.86×10^{-1}	16.0
Expoxy	N/A	-1.40×10^{-3}	1.3
Egg albumin	4.6	-1.0×10^{-2}	−4.3
Lysozyme	11.0	4.1×10^{-2}	4.7

[a] Membrane separating 0.01 and 0.005 *M* NaCl.

Wasserman antibody. However, nonspecific absorption of proteins interfered which required a lengthy (30 min) incubation with sample followed by extensive washing.

This assay system was investigated further to demonstrate an immunosensor for syphilis.[133] Again, the triacetyl cellulose membrane was used to attach the antigen. In these examples, reversibility and reproducibility data are not given which, when combined with the nonspecific response in whole serum samples, lowers the expectations for practical application of these devices. However, one of these drawbacks was addressed with the discussion of possible reuse of a sensor for ABO blood typing.[134]

The sensors which have been described above all operate on the perturbation of surface charge density of the membrane. A common feature is interference from nonspecific protein absorption. Also, the response times are rather lengthy and the irreversibility precludes accurate calibration and reuse of the devices.

An entirely different approach in the preparation of immunoresponsive electrodes was recently described where the past difficulties seem to be minimized.[135,136] Neutral ion carriers, such as those described in Section III are employed as one of the active agents in the membrane. The membranes become immunologically active when a hapten or antigen is covalently coupled directly to the ion carrier. The incorporation of this conjugate into an appropriate polymer matrix produces electrodes which respond to their respective antibodies in a reproducible and specific manner.

The first immunogen system to be demonstrated involved the use of the hapten, dinitrophenol, which was covalently linked to the cyclic crown ether, dibenzo-18-crown-6. This conjugate was dispersed in a plasticized polyvinyl chloride membrane and the membrane was mounted in a commercial electrode body. Since the conjugate contains a cation-selective complexing agent, it is necessary to control the concentrations of susceptible ion species in the sample in order to observe only the antibody contribution to the measured potential.

The response of the electrode to dinitrophenyl (DNP) antibodies was rapid and ranged from 6 to 16 min for a steady-state signal, depending on antibody titer. The electrode was also tested for bovine serum albumin (BSA) antibody response since the DNP antiserum was raised using DNP-BSA conjugates. Figure 16 shows the response of this electrode to the different antisera and clearly illustrates the selective response of the sensor to the specific antibody.

It should be noted that the antisera being tested is whole serum containing the full spectrum of serum proteins. No washing was required between sample application and measurement, and there was a complete lack of nonspecific protein absorption on the membrane. The specific response to the DNP antibody could be inhibited by the presence of free DNP in the test serum.

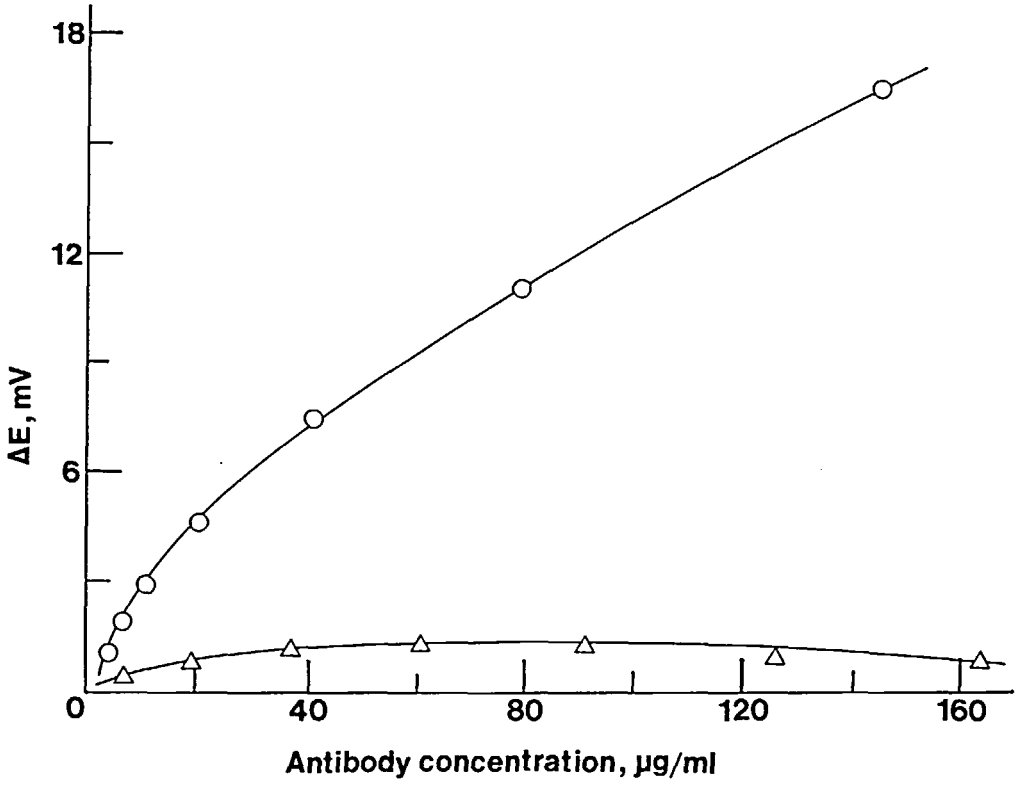


FIGURE 16. Antibody response of the electrode at pH 7.5; (O) DNP antibodies; (Δ) BSA antibodies. (From Solsky, R. L. and Rechnitz, G. A., *Anal. Chim. Acta*, 123, 135 (1981). With permission.)

The membrane was further tested for nonspecific response by comparing a complete immunoelectrode to one which contained the crown ether less the conjugated hapten. Figure 17 illustrates that the crown ether and hapten act together when conjugated to mediate the specific antibody response. There is negligible response of the electrode which does not contain the hapten.

The proposed mechanism of this immunoelectrode involves an apparent shift in the selectivity factors towards various cations. Figure 18 displays the measured selectivity constants, as defined by the Nicolsky equation in Section II, and how they are shifted when the membrane is in contact with sample containing the specific antibody. Although no quantitative results were given, the change in selectivity constants could explain for the direction and general magnitude of observed potential changes.

This concept shows great promise in the development of practical immunoelectrodes. Much work still must be done before routine measurements will commence in serum, but these first investigations are paving the way for future sensors.

VI. BIOCHEMICAL APPLICATIONS

In Section IV, several types of sensors were described which are responsive to a variety of biochemicals. These electrodes were either based on isolated enzymes or bacterial or tissue biocatalytic layers which enzymatically degrade the substrate to an electrode-measureable product. The ultimate sensing element was one of the basic electrode types.

In this section, several electrodes which sense biochemicals directly will be described.

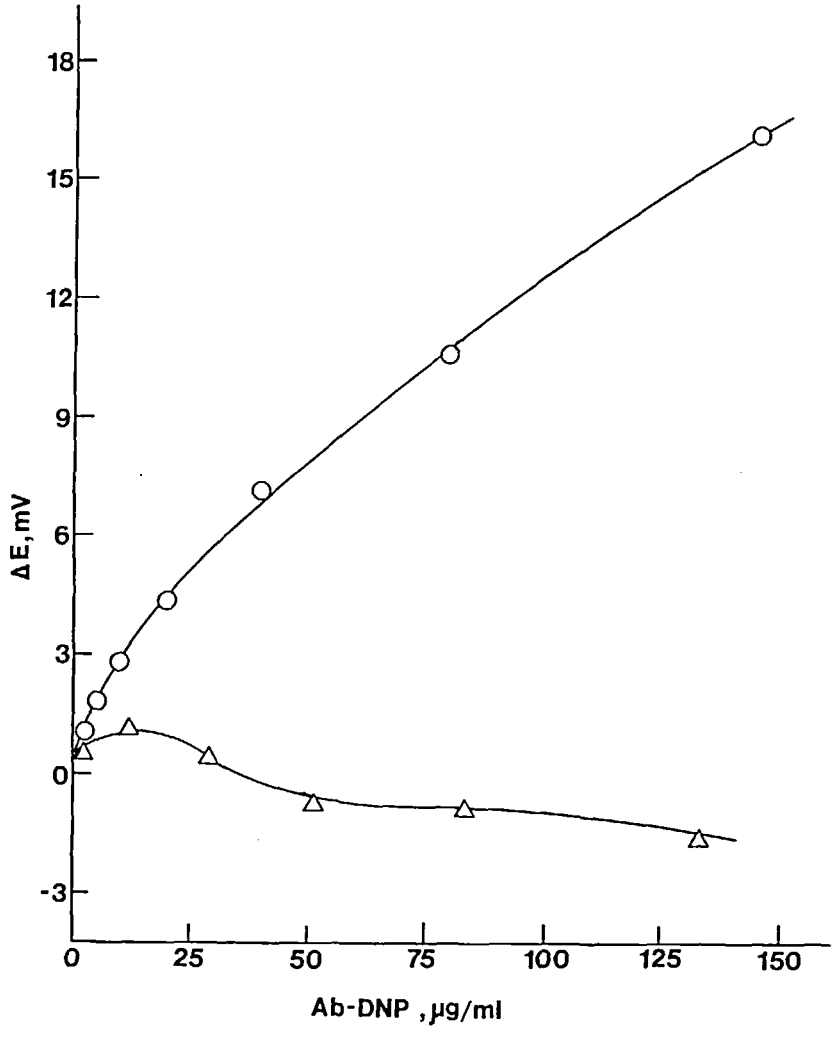


FIGURE 17. Antibody response of membrane electrodes containing (O) the DNP-carrier conjugate, and (Δ) the carrier alone. (From Solsky, R. L. and Rechnitz, G. A., *Anal. Chim. Acta*, 123, 135 (1981). With permission.)

These sensors operate on fundamentally different principles than the sensitized electrodes. In fact, they may be considered basic sensors of the type described in Section III. These include the liquid membrane electrodes of which many of the following electrodes are based.

Alternate techniques are also described where biochemical systems are investigated using titration methods. Furthermore, additional applications to fundamental studies are outlined where several types of electrodes are used to illustrate the diverse disciplines where these sensors can be utilized.

A. Pharmaceutical Assays

Ion-selective electrodes have been demonstrated which are responsive to several drug and pharmaceutical preparations. Many such sensors have been described in review articles[19,58] and will illustrate the diverse nature of materials which can be determined.

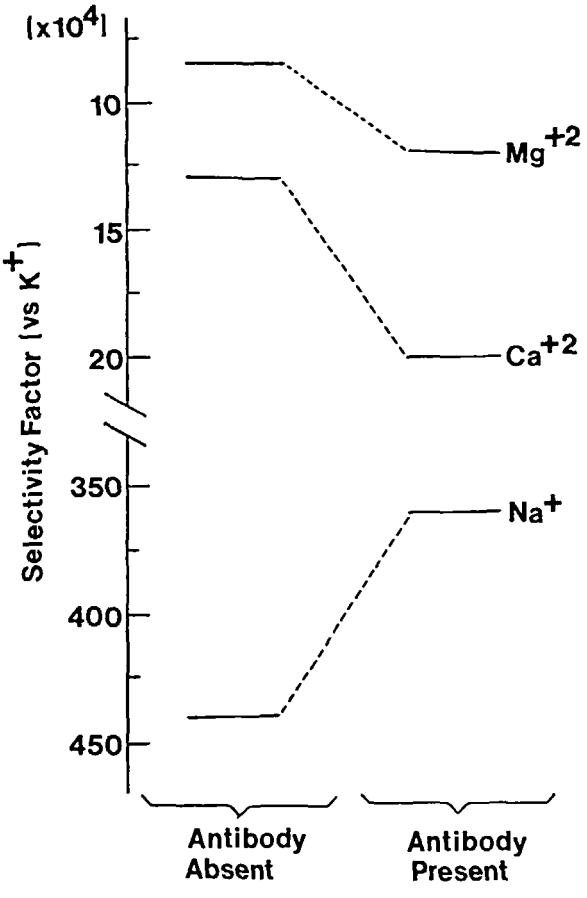


FIGURE 18. Comparison of electrode selectivity patterns in the presence and absence of DNP antibody. (From Solsky, R. L. and Rechnitz, G. A., *Anal. Chim. Acta*, 123, 135 (1981). With permission.)

The sensors that will be discussed here have been divided into two sections which distinguish the different modes of operation.

1. Direct Sensors

There have been a number of electrodes which have been constructed that respond to various drugs and compounds of pharmaceutical interest. These electrodes are fundamentally similar to the liquid exchanger membranes which have been previously described. Since the basic operating principles are not new for these sensors, they will be briefly listed with their applications and uses.

Electrodes can be made to respond to selected drug compounds if a highly insoluble salt can be formed. This salt usually includes a counter-ion which is lipophilic in nature and allows the salt to be dissolved in an organic solvent. The drug salt is dissolved in such solvents as 1,2-dichloroethane, nitrobenzene, 2-nitrotoluene and other common solvents, and is dispersed within a membrane matrix, such as filter paper, or within appropriate polymers (polyvinyl chloride, cellulose acetates, etc.). The drug salt is a selective ion-exchanger which produces the response of the drug electrode to aqueous test samples of the drug. The finite equilibrium dissociation of the salt at the membrane-

Table 4
LIQUID MEMBRANE DRUG-SENSING ELECTRODES

Drug sensed	Counter-ion	Ref.
Propanolol	Diodecylnaphthalenesulfonate	137
Strychnine, papaverine, quinine, cocaine	Picrate	138
Codeine	Dipicrylaminate	139
Phencyclidine	Dinonylnaphthalensulfonate	140
Papaverine, procaine, pyridoxine, ethacrine, quinidine, amylocaine, chloropromazine, ephedrine, amphetamine, thiamine, and emetine	Tetraphenyl borate, hexadecyl sulfate, octadecyl sulfate, and 1,4-azimino dibenzenesulfonate	141
Codeine, morphine and dionin	Dipicrylaminate	142
Vitamin B_6	Dipicrylaminate	143
Novocaine	Tetraphenylborate or dipicrylaminate	144
Phenobarbital	Aliquat 336S	145

solution interface is altered by the presence of the drug ion in the aqueous sample and results in the observed potential signal.

A representative listing of these types of electrodes is found in Table 4 which also includes the typical counter-ions that are commonly used. A great number of these electrodes show poor selectivity over interfering compounds. This is a result of the nature of the sensor and the choice of the counter ion which forms the ion-exchanger salt. However, when appropriately applied, these electrodes can be very useful when the samples are carefully chosen.

An active area of current research centers on bilayer lipid membranes as biosensors for biological substrates. These membranes have been studied for a number of years, but a recent development is worth mention here. The bilayer lipid membrane is a very unstable but useful model of biological membrane systems. Moreover, this membrane can be utilized to assay various materials, such as antibiotics, by monitoring transmembrane electrical parameters. Thus, amphotericin B and nystatin were determined by observing decreased membrane resistance in the presence of the antibiotics.[146] The bilayer membranes are good for only one measurement and eventually break. The limit of detection at 10^{-9} molar drug still makes the application of these sensors attractive when compared with traditional electrodes.

One possible scheme to increase the durability of these fragile membranes is to support them on another matrix. This was recently demonstrated by depositing a lipid monolayer membrane on both sides of a polyamide polymer matrix.[147] This "extended bilayer lipid membrane" is composed of two monolayer films covering the polymer membrane forming a "fat" bilayer membrane. The hydrophobic nature of the polyamide membrane mimics the hydrophobic nature of the interior of the bilayer lipid membrane. This stabilized membrane structure is practical and will withstand rugged use. It is also easier to form reproducible membranes in this fashion. The membranes were tested for response to the antibiotics, valinomycin, amphotericin B, and gramicidin S to demonstrate applications for this new type of supported structure.

Since bilayer lipid membranes have been shown to be useful for the determination of various proteins and immunoagents, this new approach may allow for the routine use of these potentially sensitive devices. Other support materials besides nylon may prove to be more appropriate and may extend the application of lipid films to additional substrates.

2. Indirect Measurements and Titrations

Alternate techniques exist to assay pharmaceutical products using ion-selective

Table 5
DETERMINATION OF ATROPINE AND NOVATROPINE IN PHARMACEUTICAL PREPARATIONS[148]

	Atropine sulfate or novatropine (mg $m\ell^{-1}$)		
	Present method	Reference method	Nominal
Atropine sulfate injection (aqueous)	0.92	0.93	1
0.5% Atropine sulfate (eye-drops)	6.06	5.64	5
1% Atropine sulfate (eye-drops)	9.8	9.3	10
Novatropine solution (aqueous)	4.08	3.97	4
Novalumine solution	1.27	1.29	1.3
Novatropine tablets	2.27	2.35	2.5

electrodes. These approaches are potentially more useful than the direct sensors especially in the areas of process and quality control. These methods do not employ more selective sensors but combine available electrode technology with appropriate chemical reactions to successfully assay the drug material.

One such coupling of technologies is the long-familiar titration which is most commonly associated with acid-base determinations. Traditionally, the acid-base chemical reaction is coupled with another reaction (involving phenolphthalein) to give a visual indication of the progress of the analysis. Modern instrumentation can replace the visual indicator with a selective electrode to perform potentiometric titrations.

The electrode is chosen to respond to one of the reactants of the chemical reaction. In this way, several compounds of pharmaceutical interest can be analyzed when either a relatively nonspecific drug sensor is coupled with a selected complexing agent or an electrode to the complexing agent itself is used.

One recent description of this type of assay employs drug electrodes for atropine and novatropine.[148] The sensors are of the ion-exchanger types using tetraphenylboron as the counter ion. These electrodes displayed severe interference from other alkaloids such as codeine. However, a viable analytical method was devised using the drug electrode and performing potentiometric titrations on pharmaceutical preparations.

The indicating electrode was an atropine electrode and the sample was titrated with sodium tetraphenylboron. The inflection point of the titration curve is taken as the end point. Atropine and novatropine were both assayed in pharmaceutical dosage forms by the electrode method and an accepted reference method. The agreement between the two was good and the comparison is shown in Table 5.

This type of analysis is typical of potentiometric titrations of organic compounds. Many drugs and intermediates can be assayed in the same way. Several different electrodes can be used in combination with selected titrants to make the determination of pharmaceutical compounds both possible and attractive to perform. Potentiometric titrations can be reduced to practice readily and are appropriate for the quality control testing of products to the measurement of drugs in physiological fluids. Table 6 lists several drugs which have been assayed by potentiometric titrations from a number of sources.

One of the more practical applications has been the determination of methadone in urine using a hydrophobic cation-selective electrode.[154] A potentiometric titration was performed since, when using a standard curve and direct potentiometry, relative accuracies of only ±15% could be attained. Acidified urine was titrated with tetraphenylboron, and the level of free methadone was monitored with the electrode. In this mode, the methadone could be determined with better than 2 % accuracy.

Table 6
DRUGS ASSAYED BY POTENTIOMETRIC TITRATION

Drug	Indicating electrode	Titrant	Ref.
Tetracycline	pH	CV^{+2}	149
Procyclidine, Cyclizine, Diethylcarbamazine	Amine responsive PVC	Tetraphenylboron	150
Chloramphenicol	Cadmium	EDTA	151
Thiobarbiturates	Silver	$AgNO_3$	152
Potassium Isobutylxanthogenate	Copper	$CvSO_4$	153
6-Carbethoxy-as-triazine-3,5(2H,4H)-dithione	Silver	$AgNO_3$	153

Table 7
ELECTRODE DETERMINATIONS OF PHARMACEUTICAL PREPARATIONS

Analyte	Electrode used	Significance	Ref.
Drugs	Various	Review — analytical control — drugs	155
Inorganic ions in drugs	Various	Review — inorganic ion analysis in drug preparations	156
Pharmaceutical compounds	Bromide	Bromide-containing drugs assayed	157
Pharmaceutical preparations	Chloride, bromide	Quality control applications	158
Al, NH_3, pH	Various	Automated drug analysis	159
Pharmaceuticals	Various	Review — electrode applications	160
Various drugs	Halide	Combustion — hydrolysis splits off halides	161
Antibiotics	Rubidium	Rb^+-loaded yeast cells	162

There are a variety of other electrode methods for assaying drugs and pharmaceutical preparations. Some of these approaches are listed in Table 7 to illustrate several examples of alternate electrode methods.

B. Fundamental Studies

In previous sections, many electrodes have been described which respond to biochemicals. The enzyme and substrate electrodes are directly applicable to monitor materials of interest in many biochemical pathways. Since these electrodes have been discussed earlier, no attempt will be made to duplicate their description.

There are, however, some rather novel applications of ion-selective electrodes to basic studies which are noteworthy. One of the more common tests for proteins is the determination of nitrogen content by Kjeldahl digest. Ion-selective electrodes have been utilized to determine this protein nitrogen as ammonia.[163-165] Both ammonia gas and ammonium ion electrodes have been used with good results. The application of the standard addition method further simplified the analysis since the electrode need not be calibrated prior to use.

Electrodes can be applied to enzymatic analysis as well. The ion-selective electrode is well suited for this purpose since it can operate in a variety of matrices even if they are turbid or colored. One such application is the kinetic analysis of cholinesterase activities by a choline-selective electrode.[166] The operating principles and mode of use were reviewed stressing the advantages over existing technologies. Another example describes how electrodes are used to study enzyme reactors[167] which can be used in flow-through applications. Urease was immobilized in a column and the kinetic parameters, K_m and

V_{max}, were evaluated under a series of reaction conditions. The results were extended to cofactor-dependent enzyme systems to illustrate how more complicated enzyme reactions can be monitored.

Protein conformation and functional group analysis is also possible with ion-selective electrode detection.[168] The response of the Ag_2S crystal-membrane electrode to sulfur-containing proteins was shown for a series of representative proteins. At given pH values, the sulfhydryl groups are available to directly interact with the sensing element. Denaturation and refolding can be followed by monitoring the appearance of buried groups. Furthermore, the disulfide linkages can be determined by breaking them and subtracting the background sulfhydryl content. Lysozyme, ovalbumin, human serum albumin, and RNase A were characterized by their sulfhydryl and disulfide content as a function of conformational change to demonstrate the applicability of this technique.

A rather novel use for gas-sensing electrodes is the analysis of cigarette smoke. The levels of nitrogen oxides in cigarette smoke were recently determined using a commercially available electrode.[169] Nitrogen dioxide was trapped in a buffer and directly determined. Nitrogen monoxide was determined by oxidation as the sum, NO + NO_2, and calculated by difference. The results of the gas electrode were in agreement with those of an accepted reference method.

There are a great number of additional examples where ion-selective electrodes have been applied to solving biochemical problems or elucidating pathways of biochemical interest. These can be found in general review articles or bibliographies where this information is properly located. Since these are predominantly application notes, they have not been emphasized here.

VII. CLINICAL APPLICATIONS

We have seen how the basic electrodes function and how they can be altered to monitor substances of biological interest. Ion-selective electrodes can be made to respond to a large number of analytes and their selectivity can be tailored for specific substrates to render them useful in complex samples. An area where ion-selective electrodes have recently been applied is one of the more complicated and interesting to work in, the field of biomedical analysis.

The full-operational advantages of ion-selective electrodes are realized with their use in biological fluids analysis. The biological activity of materials is closely tied to that substance's chemical activity. This is the quantity to which an electrode responds. The range of applications where ion-selective electrodes have been used is outlined in the following sections. Attention will be focused on the use of basic electrode types and where they are applied to illustrate the present status of these devices in a clinical setting.

A. Physiological Studies

Ion-selective electrodes have been readily accepted by physiologists who are eager for sensors which can be used to monitor processes occurring in living systems. The ability to measure ion activities within and between cells is highly significant since many processes depend on the potential differences across cell membranes. Many of these applications require the miniaturization of the electrode as we have seen in Section IV with the micro NH_3 electrode.

The field has advanced to the stage where several books[170-172] and review articles[173-177] are available which describe the art and useage of ion-selective microelectrodes.

One of the earlier microelectrodes to be made was the pH sensor since the glass could be readily formed using pipet pullers. The micro-pH electrodes have been used to

measure local pH values in brain tissue during hyper- and hypoventilation in cats.[178] The pH adjacent to the pial arteries was monitored and changed in proportion to the diameter of the artery during ventilation changes. The electrodes were found to be very useful and displayed near-Nerstian response slopes (56 mV/pH), rapid response times, and low drift.

The pH electrode has also found use in elucidating fundamental processes in the kidney.[179] Renal tubule fluid was assayed with a pH electrode to study the differentiation of H^+ secretion from bicarbonate reabsorption. The proximal and distal nephron were independently measured during the application of various treatments to accentuate the processes under study.

These types of studies need not be limited to microsize electrodes. Large, flat-surface pH electrodes have been used to study cerebral surface pH changes in dogs during asphyxia, hypotension, and circulatory arrest.[180] It was found that the cerebral pH decreased more rapidly than arterial pH during asphyxia and circulatory arrest. The metabolism rate of the intracellular cortex area could be effectively monitored since the blood-brain barrier is impermeable to lactate.

Another organ which has been studied with electrodes illustrates the unique measurement capabilities of these sensors. The estimation of skin metabolism and blood flow was performed using a transcutaneous CO_2 electrode.[181] Following arterial occlusion, the transcutaneous CO_2 level rose two to three times more rapidly as the skin became hypoxic. After restoration of circulation, the CO_2 level returned to normal in a semi-exponential fashion. The blood flow was estimated by the behavior of the CO_2 diffusion.

Electrolytes have also been studied with micro ion-selective electrodes. The electrodes for sodium and potassium are of the liquid membrane type where an inert micro pipet is pulled and filled with the complexing agent dissolved in an organic solvent. Valinomycin is used for the potassium electrode and a synthetic, neutral ion carrier is used for sodium.[182]

These electrodes have been used for recording sodium and potassium levels in the proximal renal tubules of frogs.[183] They have also been applied to the extracellular measurement in heart ischemia in dogs.[184] Local areas were monitored and the potassium changes were correlated with the pathologic condition. Another application of these microelectrodes is the resting potassium levels in single neurons.[185] The ability to make these measurements is highly significant for the understanding of neurologic function as well as for the effects of blocking agents.

A potential problem with the use of these small electrodes is resistive artifacts which can degrade the electrodes sensitivity. These effects can be caused by improper electrical shielding or by the use of incompatable materials. This was recently shown for sodium microelectrodes where the exchange resin had a resistance comparable to the resistance of the glass wall itself.[186] This leads to resistance shunts which results in artificially high potential readings. With proper attention to construction details, this problem can be minimized.

Ion-exchange and neutral-carrier liquid membrane microelectrodes have also been constructed for calcium and magnesium ion measurements.[187-188] The calcium electrode was made using phosphoric acid esters and was applied to the determination of intracellular ionized calcium levels in ganglia. Ten different measurements were taken and the level of error approached 100%. However, it should be noted that the concentrations were at the submicromolar levels and magnesium could very well interfere. The neutral carrier for the magnesium electrode allowed intracellular measurements when the magnesium activity was greater than 0.4 mM but suffered from calcium interference above 1 μM.

Table 8
ELECTRODE ANALYTES OF CLINICAL INTEREST

Analyte	Normal range[a]	Electrode system
pH	7.31—7.45	Glass, neutral carrier
NH_3	0.04—0.12	Glass (pH), neutral carrier
HCO_3^-	22—30	Glass (pH), ion exchanger
Ca^{+2}	2.4—2.7	Neutral carrier, ion exchanger
Cl^-	98—109	Crystal
Mg^{+2}	1.3—2.1	Neutral carrier
K^+	3.6—5.5	Neutral carrier
Na^+	135—155	Glass, neutral carrier

[a] Concentrations are millimolar except for pH.

B. Clinical Assays

Ion-selective electrodes are rapidly becoming the technology of choice for the analysis of biological fluids. Many new electrodes have been developed which have overcome the difficulties experienced in the past. The advances made and the techniques of using electrodes in clinical analysis have been discussed in several textbooks[1,6,9,13,15,189,190] and review articles.[16-18,191-195]

The following sections will review the current status of the use of ion-selective electrodes with clinical samples. Much has been learned concerning how electrodes function in complex media such as blood, and there still remains some difficulty in establishing acceptable standards of operation. These concerns and others will be illustrated by describing specific applications that have been studied.

1. Electrolytes ($Na/K/Cl/CO_2$)

Biological fluids are complex mixtures containing a wide variety of diverse substances. Out of this soup, quantitative measurements must be made to establish a patient's metabolic condition to facilitate the diagnosis of altered states or to monitor a treatment regimen. The determination of the ionic composition of blood serum has long been practiced to partially fulfill this role. There are several elements which are assayed and these are listed in Table 8. The electrode systems are indicated for the respective ions and represent the sole class of sensors which can be used for all of these important ions. It is important to reemphasize the fact that these electrodes respond rapidly to ion activities, and we will see examples where this becomes important when handling abnormal serum samples and whole blood.

There is such a staggering volume of literature describing individual electrolyte assays that the simple applications will be overlooked and emphasis will be placed on the particular features and difficulties of the electrode systems as a whole. In order to get a better perspective of the availability of these electrolyte analyzers, Table 9 lists a partial compilation of the more predominant analyzers. There are numerous other analyzers which appear sporadically as model lines change and new manufacturers enter the field. This activity further serves to describe the ever changing nature of the field.

One of the more hotly debated controversies centers around the mode of measurement itself. As has been stressed throughout this paper, ion-selective electrodes respond to activities and not concentrations. This debate has grown from a slow but steady trend towards replacing the conventional flame photometric methods for sodium and potassium with potentiometric methods. The electrodes are much easier to work with

Table 9
COMMERCIALLY AVAILABLE ELECTROLYTE ANALYZERS

Analyzer	Ions assayed	Manufacturer
Abalyte®	Na, K	Abbott
Electrion®	Ca	AMT
Clin Ion	Na, K, Cl, HCO_3, Ca	
AVL-980®	Na, K, Ca	AVL
Astra 4®	Na, K	Beckman
Astra 8®	Na, K	
Ektachem Electrolyte Analyzer®	Na, K, Cl, CO_2	Eastman Kodak
Ektachem 400®	Na, K, Cl, CO_2	
Hitachi 702®	Na, K, Cl	Hitachi
Hitachi 772®	Na, K, Cl	
IL 502®	Na, K	Instrumentation Laboratory
IL 504®	Na, K	
Nova-1®	Na, K	Nova Biomedical
Nova-2®	Ca	
Nova-3®	Cl, CO_2	
Nova-4®	Na, K, Cl, CO_2	
SS-20®	Ca	Orion Research
SS-30®	Na, K	
Space Stat 30®	Na, K	
Space Stat®	Na, K, Cl, CO_2, pH, Ca_T, Ca_I	
PVA-4®	Na, K, Cl, CO_2	Photovolt Corp.
Biol. Alkali Micro-Analyzer®	Na, K	Radelkis
ICA-1®	Ca	Radiometer
SMAC®	Na, K	Technicon
Stat/Lyte®	Na, K, Cl, CO_2	
Stat/Ion®	Na, K, Cl, CO_2	

and present fewer difficulties to laboratory personnel. However, many instances have been noted where the two methods do not correlate.[196-201] This effect disappers when the samples are diluted with buffer which brings the activity coefficient closer to unity.[202-203]

The techniques have been labeled "direct" vs. "indirect" measurements since the former uses unaltered sample while the latter dilutes the sample to effectively control the ionic strength and thus the activity coefficients. Early experiments indicated that there was a discrepancy between direct electrode measurements in undiluted serum for sodium and potassium when compared to flame photometry.[204] Several hundred patient samples were processed and there appeared to be a 7% and 4% bias in the values for sodium and potassium, respectively. Similar studies dealt with the relationship between potassium activities and concentrations in plasma samples.[205] A difference was noted in the activity between whole blood and separated plasma. At this time it was assumed that the bias was a result of the effect of red blood cells on the liquid junction of the reference electrode.

A more intensive investigation was undertaken to explain these effects.[206] The response of a sodium electrode was monitored in solutions with varying albumin content. These test solutions served as models for measurements that are taken in whole blood or plasma samples. The effect of the albumin on the potential can be traced to variations in the activity of sodium modulated by the different water content of the solutions. The albumin occupies space in the sample and affects the total volume in the concentration calculations. These results are illustrated in Figure 19 and clearly indicate the bias occurring using this model system.

An evaluation of one of the commercial sodium/potassium analyzers was reported, and a discrepancy was noted similar to the prior work.[207] Other manufacturers immediately

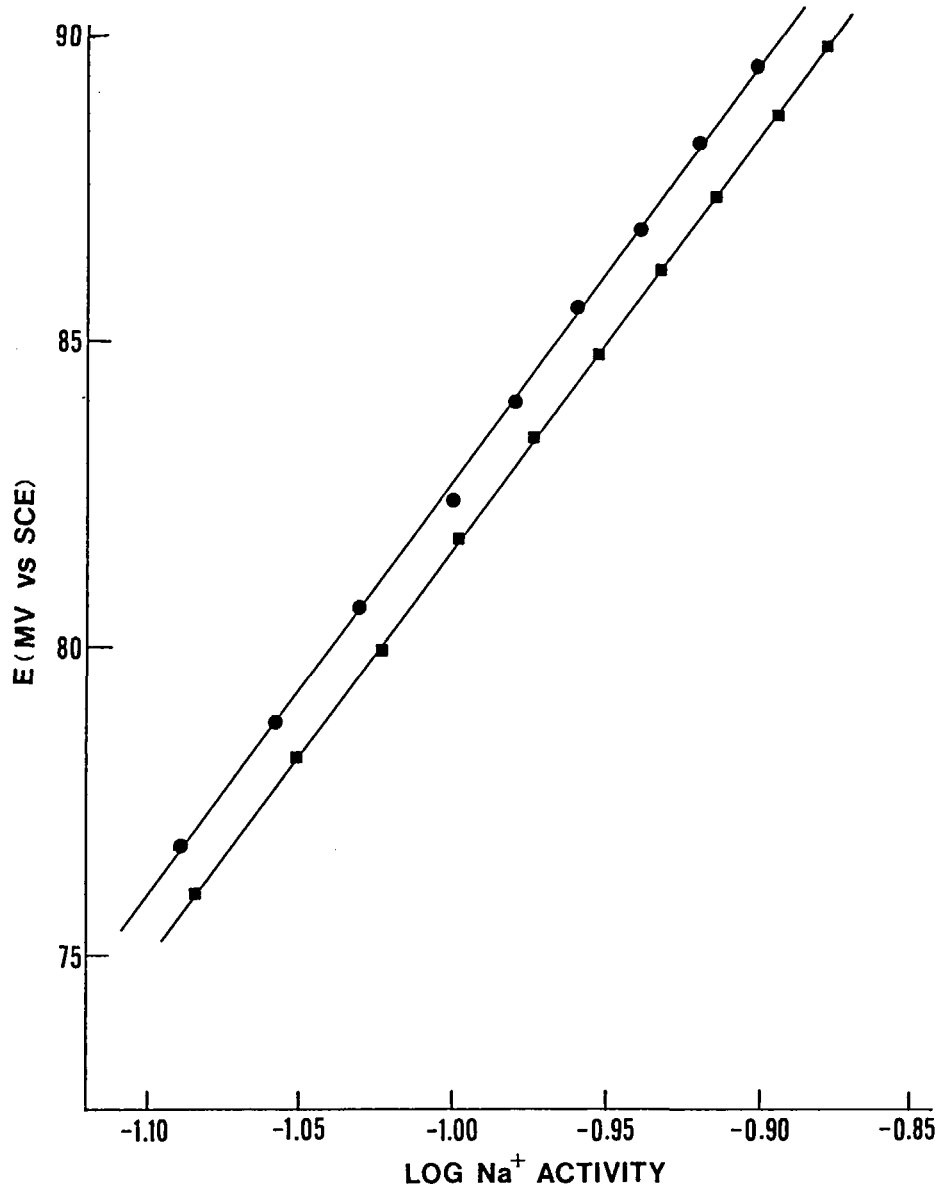


FIGURE 19. Sodium response curves in the presence (O) and absence (□) of albumin. (From Mohan, M. S., Bates, R. G., Hiller, J. M., and Brand, M. J., *Clin. Chem.*, 24, 580 (1978). With permission.)

responded that this effect was not inherent to ion-selective electrode analyzers and precipitated a flood of letters, replies, and responses to replies.[197,198,208-214] A recent publication summarizes these events and discusses the problem of "direct" vs. "indirect" measurements.[215] The various ways in which the sample can be handled as well as the results that are reported to the clinician were examined. The author chose the indirect or diluted methodology as most appropriate since the calibrators can be exactly defined and traced to the NBS. It was felt that the physician is entirely capable of mentally correcting the values when cases involve multiple myeloma and similar disorders. Another opinion which was expressed stated that this decision should be made by the clinician and not by the instrument manufacturers.[216]

There are other considerations to be aware of besides the plasma water issue. For instance, the technique of sample collection directly affects the measurement of potassium. The sample should be venous rather than capillary to avoid hemolysis which results in abnormally high levels.[217] There are also significant changes when the measurements are made when certain drugs are present in the sample. Nystatin, amphotericin, and oxytocin[218] increase the measured potassium level while procaine or lidocaine[219] reduce the observed potentials.

The precision of measurements for the typical electrode analyzers is usually $\leqslant 1\%$ for the monovalent ions and from 1 to 3% for calcium.[197,220-223] The chloride electrodes have traditionally performed poorly with significant interferences from bromide and iodide. This interference has been virtually eliminated with the introduction of surface-treated electrodes.[224]

A significant deviation from the traditional electrolyte analyzer format has been announced by Eastman Kodak.[225] A new type of disposable, thin-film electrode technology has been developed which is traditional electrode construction in an unconventional package. A schematic diagram of this device is shown in Figure 20 and illustrates the many layers that contribute to the entire electrode structure. The disposable ion-selective electrodes are responsive to the electrolytes, $Na/K/Cl/HCO_3$, and use only 10 $\mu\ell$ of sample. Reference solution of known ionic concentration is applied to the reference side of the slide and a differential measurement is made. The paper bridge spans the two ion-selective membranes and acts as a salt bridge after the sample and reference solutions are applied.

The analyzer is undergoing field trials at present thus limiting available performance data at this time. However, since valinomycin and methyl monensin are used as active agents for the potassium and sodium electrodes, respectively, the response and selectivity should be excellent.[226] The chloride electrode is based on a silver halide base overlayed with a scavenging layer for bromide and iodide.[227] The bicarbonate electrode uses a liquid ion exchanger for carbonate, and buffer layers that lie over the membrane adjust the pH of the sample appropriately.

A difficulty experienced with these devices is the inability to calibrate individual sensors. Through tight manufacturing control, this can be addressed if the clinician is willing to sacrifice precision with greater error limits. However, the convenience and small sample requirements of this instrument may offset the reduced precision when compared to other electrode-based analyzers.

2. Ionized Calcium

Calcium electrodes have been available for more than 10 years and have been applied to the determination of plasma ionized calcium. In this case, it is universally accepted that the calcium activity is the more relevant information required for the diagnosis of disturbances of calcium homeostasis.

Improvements in the design and performance of calcium electrodes have been steadily reviewed and updated.[228-232] As was the situation with the sodium/potassium analyzers, measured values differ for different instrumentation.[233,234] These differences can be attributed to electrode construction differences as well as tubing pathlength considerations and pH adjusted samples.[235-239]

When the normal range of ionized calcium is considered, an electrode which is operating in a Nerstian fashion would only display a 3 mV change over this range. The precision of the newer calcium electrodes have been reported at C.V.s of 1.1% for serum and 2.3% for whole blood.[240] This is the equivalent of measuring pH values with a precision of ± 0.002 pH units.[1]

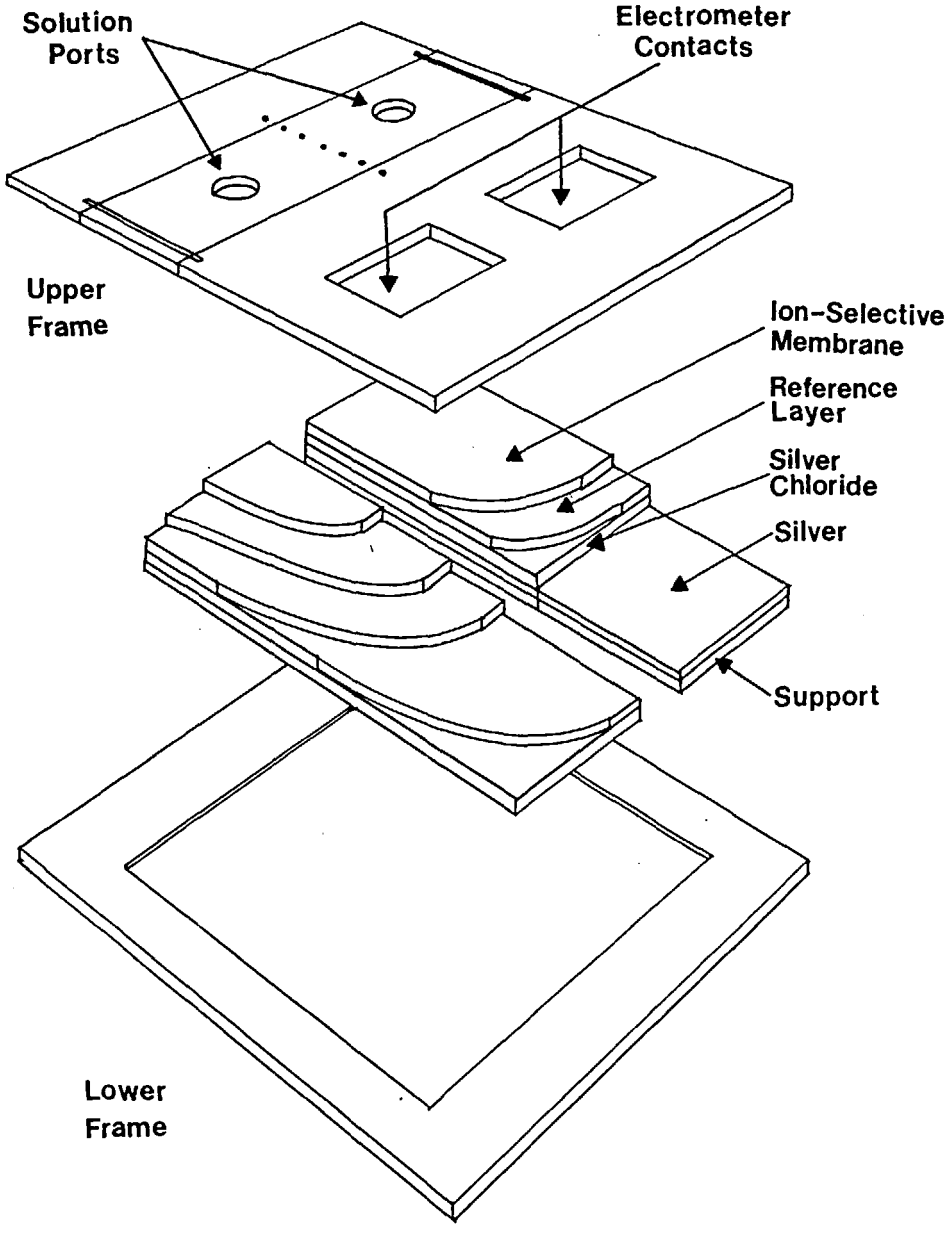


FIGURE 20. Kodak's single use, disposable electrode slide.

3. Halides

The importance of the determination of fluoride in blood has been recently reviewed.[241] The technique is relatively simple since the LaF_3 crystal electrode is extremely selective for fluoride over the other halides. Fluoride exists in both free and bound states in blood.[242] The bound fluoride is associated with proteins which can be released by calcination in the presence of an alkaline fixative.

The effects of industrial exposure to fluorine were investigated by the determination of fluoride in urine and bone tissues.[243] Workers suffering from industrial fluorosis displayed urinary fluoride levels 15 times higher than unexposed controls. The level of

fluoride in serum can also be used to establish the existence of the condition as well.[244] Chloride testing for cystic fibrosis with combination chloride electrodes has been evaluated and the techniques of measurement described.[245] This is a moderately precise method (rel. std. dev. <10%), but depends on many variables which include the duration and quantity of sweat collected.[246,247] The history of the technique and its present status was reviewed in depth and gives a perspective on the use of electrodes for this diagnostic method.[248]

The iodide electrode has been used in the analysis of milk to levels of 100 μg/ℓ.[249] The standard deviation was 3.3% and was in good agreement with a standard reference method. The technique of standard additions was also demonstrated for this assay as a means to increase accuracy.[250] Neither of these methods was applied to human breast milk to assess its nutrient quality. However, the sodium electrode was used for this purpose and demonstrates the feasibility to assay this type of sample.[251] The iodide electrode has also been used for the quantitation of iodide in thyroid tissue.[252] An excised tissue sample is ashed in pure oxygen and the iodide is dissolved and determined by direct potentiometry. This technique was used to correlate an experimental X-ray fluorescence procedure.

4. Miscellaneous

There are several other analytes which are assayed in serum besides the electrolytes that can be sensed by electrodes. Ammonia is one of these species and the determination of plasma ammonia is important for the diagnosis of liver diseases and Reye's syndrome. The ammonia levels can be determined in perchloric acid supernatants using commercially available electrodes.[253,254] The range of blood ammonia levels was 13 to 73 μg/dℓ with within run and between run precisions of 2.1 and 3.5%, respectively. Recovery experiments demonstrated excellent performance of the assay (98 to 100%).

It is important to consider the anticoagulant used when collecting the blood sample. The plasma ammonia level was found to be 46% greater when using oxalate instead of heparin as the anticoagulant.[255] The choice of anticoagulant influenced the distribution of ammonia between plasma and the red blood cells.

Whole blood can be assayed for ammonia using flow-through electrodes as well.[256] The electrode used here incorporates the flowing internal electrolyte concept as depicted in Figure 4. Samples were analyzed at the rate of 30 per hour with precision better than 4%. The apparatus yielded recoveries of 105% and compares favorably with standard reference methods. The ammonia level in plasma averaged 50% higher than in whole blood and was attributed to the hematocrit effect.

Since most ammonia is disposed as urea, it is important to measure this material as well. Urea levels in blood (BUN) can be correlated with the proper functioning of the kidneys. Urea is typically measured by coupling urease to either an ammonia[257] or ammonium[258,259] electrode or to a pH electrode as was described in Section IV. The analysis can be easily automated, and by using the flow-injection method and soluble urease, sample rates of 60 per hour are attainable with reproducibility better than 0.5%.[260]

A more reliable indication of renal function is possible by measuring serum creatinine instead of BUN. Creatinine can also be assayed by using deaminating enzymes, but these are often contaminated with urease and interfere with the measurement. An alternate technique was recently introduced which takes advantage of the conventional standard method. The Jaffe reaction combines the creatinine with picrate ions in an alkaline medium to form an orange-red complex which is determined photometrically. A picrate ion-selective electrode is used instead, and the creatinine is determined kinetically by observing the decrease in free picrate concentration.[261] The accuracy of the method was

excellent with recovery experiments averaging 101%. The C.V. of the technique was 8.1% and was not affected by bilirubin.

The same approach was used to demonstrate the determination of albumin in serum, also using the picrate electrode.[262] Albumin complexes picrate at a pH of 6.0 and excess picrate is determined by the electrode. The pH is far enough removed from 8.0 so that creatinine does not interfere. The accuracy and precision of the method were excellent with within run and day-to-day CVs of 2% and recoveries averaging 102%. The reaction and detection were automated allowing sampling rates of 50 per hour.

C. Continuous Monitoring

Ion-selective electrodes are ideally suited for the continuous measurement of electrolytes in blood. The response times of most electrodes are fast enough to produce immediate, real-time results which can assist the clinician's ability to begin corrective therapy in a timely manner. These measurements may be taken in one of two ways, either the electrode can be implanted within a vein or a catheter can withdraw blood to a bedside analyzer.

1. Extracorporeal Devices

The need for rapid and frequent measurements does not apply to the majority of testing performed today. However, there is a definite need to be able to monitor certain ions in special circumstances, such as the potassium ion levels during and after open heart surgery. The potassium level must be within the physiological range prior to reactivation of the heart to ensure normal heart function. This measurement has traditionally been performed using flame photometers, but this procedure requires centrifugation of the sample which results in delays of up to 10 min before the results are reported.

The determination of potassium in whole blood during open heart surgery was compared with flame photometric analysis performed immediately and following a 2 to 5-hr delay.[263] Whole blood samples were continuously withdrawn by tapping into the venous line of the heart-lung machine. The length of the sampling lines varied from 40 to 100 cm depending on how close the instrument could be placed to the patient. The potassium levels were reported within 2 min, depending on the sample line length. There were no statistical differences noted when comparing the electrode response to the flame photometer. The most significant result of this demonstration illustrated the ability of the electrode analyzer to rapidly display the potassium levels with minimum delay. Other analyzers have been reported for the intraoperative measurement of sodium, calcium, and potassium levels as well.[265] In this case, heparin was added to the sample line immediately after blood was withdrawn from the patient to prevent clotting in the analyzer manifold.

The use of bedside monitors is also important for a number of applications besides surgery. A number of patients suffering from severe diabetes can benefit from on-line monitoring. The levels of sodium, potassium, calcium, and glucose were measured continuously with an electrode-based analyzer to demonstrate this application.[265] Another instrument that measures potassium, calcium, and glucose is the Biostator® from Miles Labs.[266] This apparatus uses a double lumen catheter for the infusion of heparin into the sample during withdrawal. The dilution of the sample by the anticoagulant has to be calculated out, but does not seem to affect the results. The day-to-day CVs for both potassium and calcium determinations averaged slightly higher than 1%. The potassium electrode displayed a slightly negative bias when compared with a flame photometer while the calcium sensor showed no bias vs. a commercial calcium analyzer. The Biostator® consumes small quantities of blood (2 mℓ/hr) while offering rapid electrolyte determinations in whole blood samples.

Another application of real-time monitoring is for those patients undergoing renal dialysis therapy. The importance of maintaining electrolyte balance in the serum is of utmost importance. Of equal importance is the determination of the removal of toxic substances. This can be either performed in the patients blood or in the expended dialysis fluid. An apparatus has been described which measures the urea content of dialysate effluent from the dialyzer unit.[267] The fluid is continuously sampled and is passed through an immobilized urease reactor which converts the urea into ammonia and carbon dioxide. The ammonia is determined using an ammonium ion-selective electrode and is continuously recorded to display the progress of the dialysis treatment.

In all of the above analyzers great care must be taken to insure a stable reference electrode liquid junction potential. Since the total potential change over the normal ranges for the electrolytes is small (sodium: 2.4 mV, potassium: 11.4 mV and calcium: 3.0 mV), the liquid junction has to be highly stable and reproducible.[1] Several junctions have been described and are available for use in continuous monitoring analyzers.

2. In Vivo Sensors

Since ion-selective electrodes provide immediate measurements in whole blood samples, the placement of the electrode directly in the patient is an obvious extension of the extracorporeal analyzer. However, the continuous monitoring of specific ions is still predominantly experimental in nature. The concepts and difficulties of in vivo measurements have been reviewed[1] and specific applications have been discussed for continuous monitoring during anesthesia[268] and for obstetrics.[269]

One of the principle difficulties in making these measurements is selecting a stable reference electrode. Many of the reference elements that are acceptable for the extracorporeal monitors either fail or are inappropriate for indwelling sensors. Liquid junction fluctuations and streaming potentials are often at fault which lead to spurious readings. The principles governing in vivo reference use have been reviewed and a suggestion is made for a more stable junction.[270] This reference element is a charged palladium hydride electrode which appears to be more stable than conventional reference electrodes. A decreased resistance and a reduced susceptibility to protein poisoning indicate that this type of reference electrode may prove valuable for future in vivo applications.

Potassium electrodes have been fabricated as catheters for venous and myocardial monitoring.[271] The catheters were 1.5 mm and 0.6 mm in diameter, respectively, and used valinomycin-PVC membranes as the active element. The potassium levels in the bloodstream were measured at normal and artificially elevated levels and correlated well with samples withdrawn for flame photometric analysis. The electrodes displayed drift rates of about 1 mV/hr and could not be used for extended periods of time. However, the capability to monitor potassium levels instantaneously is a significant demonstration for future applications.

Implantable pH electrodes have also been studied with emphasis on the design of solid state devices to overcome materials problems.[272-274] Ceramic substrates coated with metals or conductive metal oxides replace the conventional internal electrolyte filling solution. Glasses, sensitive to pH, are silk-screened and fired on the coated substrates to form the solid state, implantable sensors. The stability and response of many of these devices are comparable to commercial pH electrodes. There even exists a commercially available electrode for the continuous measurement of tissue pH which uses a 1 mm-long glass tip in conjunction with an integrated capillary-type liquid junction reference electrode.[275]

A particular problem which may interfere with in vivo measurements is the interaction of specific constituents in whole blood which alters the performance of the electrodes.

Fibrin deposition is a specific example of one of these concerns and its effect on the response of the pH electrode was investigated.[276] Fibrin was deposited on a pH electrode in vitro using thromboplastin and fibrinogen or with thromboplastin and whole blood. The presence of a clot on the electrode surface significantly influenced the time required for the pH value to stabilize. However, the ultimate value was not altered indicating that these types of deposited materials will not affect the usefulness of implanted electrodes.

VIII. CONCLUSIONS

Ion-selective electrodes have very exciting possibilities for biochemical applications. Their use has been extended to medicine and biomedical research through an understanding of the basic properties and limitations of the sensing devices. The difficulties concerning proper calibration standards should be resolved by agreement reached by both instrument manufacturers and clinicians to result in the most appropriate results.

Much still has to be learned before the widespread use of implantable devices becomes a reality. However, through the efforts of clinicians and bioanalytical chemists together, it is only a matter of time before this comes to pass.

ACKNOWLEDGMENTS

The author is grateful for the continuing support of the DuPont Photo Products Department during the preparation of this manuscript. Thanks are also extended to Dr. Richard S. Schifreen for reviewing the paper and to Maria J. Gonzalez and Anne Y. Koiv for preparing the manuscript.

REFERENCES

1. **Band, D. M. and Treasure, T.,** Ion-selective electrodes in medicine and medical research, in *Ion-Selective Electrode Methodology*, Vol. 2, Covington, A. K., Ed., CRC Press, Boca Raton, Fla., 1979, chap. 3.
2. **Pungor, E. and Buzas, I., Eds.,** *Ion-Selective Electrodes,* Akademiai Kiado, Budapest, 1978.
3. **Freiser, H., Ed.,** *Ion-Selective Electrodes in Analytical Chemistry,* Vol. 1, Plenum Press, New York, 1978.
4. **Freiser, H., Ed.,** *Ion-Selective Electrodes in Analytical Chemistry,* Vol. 2, Plenum Press, New York, 1980.
5. **Covington, A. K., Ed.,** *Ion-Selective Electrode Methodology,* Vol. 1, CRC Press, Boca Raton, Fla., 1979.
6. **Koryta, J., Ed.,** *Medical and Biological Applications of Electrochemical Devices,* John Wiley & Sons, New York, 1980.
7. **Cheung, P. W., Fleming, D. G., Ko., W. H., and Neuman, M. R., Eds.,** *Theory, Design and Biomedical Applications of Solid State Chemical Sensors,* CRC Press, Boca Raton, Fla., 1978.
8. **Bailey, P. L.,** *Analysis with Ion-Selective Electrodes,* Heyden, London, 1976.
9. **Kessler, M., Clark, L. C., Lubbers, D. W., Silver, I. A., and Simon, W., Eds.,** *Ion and Enzyme Electrodes in Biology and Medicine,* University Park Press, University Park, Md., 1976.
10. **Fuchs, C.,** *Ion-Selective Electrodes in Medicine,* Thieme, Stuttgart, 1976.
11. **Lakshminarayaniah, N.,** *Membrane Electrodes,* Academic Press, New York, 1976.
12. **Vesely, J., Weiss, D., and Stulik, K.,** *Analysis with Ion-Selective Electrodes,* John Wiley & Sons, New York, 1977.
13. **Lubbers, D. W., Acker, H., and Buck, R. P., Eds.,** *Progress in Enzyme and Ion-Selective Electrodes,* Springer-Verlag, Berlin, 1981.
14. **Pungor, E. and Buzas, I., Eds.,** *Ion-Selective Electrodes,* Akademiai Kiado, Budapest, 1977.
15. **Durst, R. A., Ed.,** *Blood pH, Gases and Electrolytes,* National Bureau of Standards Special Publ. No. 450, U.S. Government Printing Office, Washington, D.C., 1977.

16. **Wescott, C. C.,** *Am. Lab.,* 10, 131 (1979).
17. **Moody, G. J. and Thomas, J. D. R.,** *Prog. Med. Chem.,* 14, 51 (1977).
18. **Moody, G. J. and Thomas, J. D. R.,** *Proc. Anal. Div. Chem. Soc.,* 14, 340 (1977).
19. **Fricke, G. H.,** *Anal. Chem.,* 52, 259R (1980).
20. **Covington, A. K.,** *CRC Crit. Rev. Anal. Chem.,* 3, 355 (1974).
21. **Koryta, J.,** *Anal. Chim. Acta,* 61, 329 (1972).
22. **Koryta, J.,** *Anal. Chim. Acta,* 91, 1 (1977).
23. **Koryta, J.,** *Anal. Chim. Acta,* 111, 1 (1979).
24. **Buck, R. P.,** *Anal. Chem.,* 50, 17R (1978).
25. IUPPAC Analytical Chemistry Division, *Pure Appl. Chem.,* 48, 127 (1976).
26. **Buck, R. P.,** *CRC Crit. Rev. Anal. Chem.,* 5, 323 (1976).
27. **Van der Linden, W. E.,** Ion-selective electrodes, in *Comprehensive Analytical Chemistry,* Vol. 11, Svehla, G., Ed., Elsevier, New York, 1981, chap. 3.
28. **Rechnitz, G. A.,** in Trace Organic Analysis: A New Frontier in Analytical Chemistry, National Bureau of Standards Special Publ. No. 519, U.S. Government Printing Office, Washington, D.C., 1979, 525.
29. **Pace, S. J.,** *Sensors Actuators,* 1, 475 (1981).
30. **Buck, R. P.,** Theory and principles of membrane electrodes, in *Ion-Selective Electrodes in Analytical Chemistry,* Vol. 1, Freiser, H., Ed., Plenum Press, New York, 1978, chap. 1.
31. **Eisenman, G.,** in Ion-Selective Electrodes, Durst, R. A., Ed., National Bureau of Standards Special Publ. No. 314, U.S. Government Printing Office, Washington, D.C., 1969, chap. 1.
32. **Srinivasan, K. and Rechnitz, G. A.,** *Anal. Chem.,* 41, 1203 (1969).
33. **Van der Linden, W. E.,** Ion-selective electrodes, in *Comprehensive Analytical Chemistry,* Vol. II, Svehla, G., Ed., Elsevier, New York, 1981, pg. 367.
34. **Ebel, S., Glaser, E., and Seuring, A.,** *Fresenius Z. Anal. Chem.,* 291, 108 (1978).
35. **Durst, R. A.,** Sources of error in ion-selective electrode potentiometry, in *Ion-Selective Electrodes in Analytical Chemistry,* Vol. 1, Freisier, H., Ed., Plenum Press, New York, 1978, chap. 5.
36. **Horvai, G. and Pungor, E.,** *Anal. Chim. Acta,* 113, 287 (1980).
37. **Horvai, G. and Pungor, E.,** *Anal. Chim. Acta,* 113, 295 (1980).
38. **Midgley, D.,** *Analyst,* 104, 248 (1979).
39. **Midgley, D.,** *Analyst,* 105, 1002 (1980).
40. **Thomas, J. D. R.,** *Hung. Sci. Instrum.,* 49, 33 (1980).
41. **Eisenman, G., Ed.,** *Glass Electrodes for Hydrogen and other Cations: Principles and Practice,* Marcel Dekker, New York, 1966.
42. **Durst, R. A., Bates, R. G., Mattlock, G., and Friedman, S. M.,** in *The Glass Electrode,* Interscience, New York, 1966.
43. **Sollner, K.,** *Ann. N.Y. Acad. Sci.,* 148, 154 (1968).
44. **Pioda, L. A. R., Stankova, V., and Simon, W.,** *Anal. Lett.,* 2, 655 (1969).
45. **Rechnitz, G. A. and Eyal, E.,** *Anal. Chem.,* 44, 370 (1972).
46. **Moody, G. J., Oke, R. B., and Thomas, J. D. R.,** *Analyst,* 95, 910 (1970).
47. **Mascini, M. and Pallozzi, F.,** *Anal. Chim. Acta,* 73, 375 (1974).
48. **Wipf, H. K., Olivier, A., and Simon, W.,** *Helv. Chim. Acta,* 53, 1605 (1970).
49. **Craggs, A., Moody, G. J., Thomas, J. D. R., and Willcox, A.,** *Talanta,* 23, 799 (1976).
50. **Thoma, A. P., Nauer, A. V., Arranitis, S., Morf, W. E., and Simon, W.,** *Anal. Chem.,* 49, 1567 (1977).
51. **Wipf, H. K.,** *Diss. ETHZ,* 4492 (1970).
52. **Morf, W. E. and Simon, W.,** Ion-selective electrodes based on neutral carriers, in *Ion-Selective Electrodes in Analytical Chemistry,* Vol. 1, Freiser, H., Ed., Plenum Press, New York, 1978, chap. 3.
53. **Meier, P. C., Ammann, D., Morf, W. E., and Simon, W.,** Liquid-membrane ion-selective electrodes and their biomedical applications, in *Medical and Biological Applications of Electrochemical Devices,* Koryta, J., Ed., John Wiley & Sons, New York, 1980, chap. 2.
54. **Oesch, U. and Simon, W.,** *Anal. Chem.,* 52, 692 (1980).
55. **Rechnitz, G. A.,** *Chem. Eng. News,* Jan. 27, 1975, 29.
56. **Ross, J. W., Riseman, J. H., and Krueger, J. A.,** *Pure Appl. Chem.,* 36, 473 (1973).
57. **Riley, M.,** Gas-sensing probes, in *Ion-Selective Electrode Methodology,* Vol. 2, Covington, A. K., Ed., CRC Press, Boca Raton, Fla., 1979, chap. 1.
58. **Buck, R. P., Thompsen, J. C., and Melroy, O. R.,** A compilation of ion-selective membrane electrode literature, in *Ion-Selective Electrodes in Analytical Chemistry,* Vol. 2, Freiser, H., Ed., Plenum Press, New York,1980, chap. 4.
59. **Pui, C. P., Rechnitz, G. A., and Miller, R. F.,** *Anal. Chem.,* 50, 330 (1978).
60. **Durst, R. A.,** *Anal. Lett.,* 10, 961 (1977).
61. **Scott, W. J.,** in *Electroanalysis in Hygiene, Environmental, Clinical and Pharmaceutical Chemistry,* Smyth, W. F., Ed., Elsevier, Amsterdam, 1980, 47.

62. **Fraticelli, Y. M. and Meyerhoff, M. E.,** *Anal. Chem.,* 53, 992 (1981).
63. **Bailey, P. L. and Riley, M.,** *Analyst,* 102, 213 (1977).
64. **Mascini, M. and Cremisini, C.,** *Anal. Chim. Acta,* 97, 237 (1978).
65. **Van der Pol, F.,** *Anal. Chim. Acta,* 97, 245 (1978).
66. **Guilbault, G. G. and Montalvo, J. G.,** *J. Am. Chem. Soc.,* 91, 2164 (1969).
67. **Guilbault, G. G. and Montalvo, J. G.,** *Anal. Lett.,* 2, 283 (1969).
68. **Kobos, R. K.,** Potentiometric enzyme methods, in *Ion-Selective Electrodes in Analytical Chemistry,* Vol. 2, Freiser, H., Ed., Plenum Press, New York, 1980, chap. 1.
69. **Clark, L. C.,** The future of enzyme electrodes, in *Theory, Design, and Biochemical Applications of Solid States Chemical Senses,* Cheung, P. W., Fleming, D. G., Neuman, M. R., and Ko, W. H., Eds., CRC Press, Boca Raton, Fla., 1978, 183.
70. **Guilbault, G. G.,** Fugure of enzyme electrodes, in *Theory, Design, and Biomedical Applications of Solid State Chemical Sensors,* Cheung, P. W., Fleming, D. G., Neuman, M. R., and Ko, W. H., Eds., CRC Press, Boca Raton, Fla., 1978, 193.
71. **Guilbault, G. G.,** Enzyme electrodes, in *Biomedical Applications of Immobilized Enzymes and Proteins,* Vol. 2, Chang, T. M. S., Ed., Plenum Press, New York, 1977, 163.
72. **Barker, A. S. and Somers, P. J.,** Enzyme electrodes and enzyme-based sensors, in *Topics in Enzyme and Fermentation Biotechnology,* Vol. 2 Wiseman, A., Ed., Ellis Horwood Limited, Chichester, 1978, chap. 3.
73. **Guilbault, G. G.,** Enzyme electrodes in analytical chemistry, in *Comprehensive Analytical Chemistry,* Vol. 8, Svehla, G., Ed., Elsevier, Amsterdam, 1977, chap. 1.
74. **Cammann, K.,** *Fresenius Z. Anal. Chem.,* 281, 1 (1977).
75. **Gray, D. N., Keyes, M. H., and Watson, B.,** *Anal. Chem.,* 49, 1067A (1977).
76. **Gray, D. N. and Keyes, M. H.,** *Chemtech.,* 7, 642 (1977).
77. **Scheller, F. and Pfeiffer, D.,** *Z. Chem.,* 18, 50 (1978).
78. **Guilbault, G. G. and Sadar, M. H.,** *Acc. Chem. Res.,* 12, 344 (1979).
79. **Fishman, M. M.,** *Anal. Chem.,* 52, 185R (1980).
80. **Ngo, T. T.,** *Int. J. Biochem.,* 11, 459 (1980).
81. **Guilbault, G. G.,** *Methods Enzymol.,* 44, 579 (1976).
82. **Brady, J. E. and Carr, P. W.,** *Anal. Chem.,* 52, 977 (1980).
83. **Mascini, M. and Guilbault, G. G.,** *Anal. Chem.,* 49, 795 (1977).
84. **Riechel, T. L. and Rechnitz, G. A.,** *Biochem. Biophys. Res. Commun.,* 74, 1377 (1977).
85. **D'Orazio, P., Meyerhoff, M. E., and Rechnitz, G. A.,** *Anal. Chem.,* 50, 1531 (1978).
86. **Mascini, M. and Palleschi, G.,** *Anal. Chim. Acta,* 100, 215 (1978).
87. **Mascini, M. and Palleschi, G.,** *Ann. Chim.,* 69, 249 (1979).
88. **Liu, C. C., Wingard, L. B., Wolfson, S. K., Yad, S. J., Drash, A. L., and Schiller, J. G.,** *Bioelectrochem. Bioenerg.,* 6, 19 (1979).
89. **Wingard, L. B., Schiller, J. G., Wolfson, S. K., Liu, C. C., Drash, A. L., and Yao, S. J.,** *J. Biomed. Mater. Res.,* 13, 921 (1979).
90. **Mascini, M.,** Protein determination by gas-sensing electrodes, in *Ion-Selective Electrodes,* Pungor, E. and Buzas, I., Eds., Elsevier, Amsterdam, 1978, 463.
91. **Mascini, M. and Giardini, R.,** *Anal. Chim. Acta,* 114, 329 (1980).
92. **Meyerhoff, M. E. and Rechnitz, G. A.,** *Anal. Lett.,* 12, 1339 (1979).
93. **Canh, T. M. and Beaux, J.,** *Anal. Chem.,* 51, 91 (1979).
94. **Ogren, L. and Johansson, G.,** *Anal. Chim. Acta,* 96, 1 (1978).
95. **Rechnitz, G. A., Kobos, R. K., Riechel, S. J., and Gebauer, C. R.,** *Anal. Chim. Acta,* 94, 357 (1977).
96. **Kobos, R. K. and Rechnitz, G. A.,** *Anal. Lett.,* 10, 751 (1977).
97. **Rechnitz, G. A., Riechel, T. L., Kobos, R. K., and Meyerhoff, M. E.,** *Science,* 199, 440 (1978).
98. **Jensen, M. A. and Rechnitz, G. A.,** *Anal. Chim. Acta,* 101, 125 (1978).
99. **Riechel, T. L. and Rechnitz, G. A.,** *J. Membr. Sci.,* 4, 243 (1978).
100. **Suzuki, S. and Karube, I.,** *ACS Symp. Ser.,* 106, 221 (1979).
101. **Hikuma, M., Obana, H., Yasuda, T., Karube, I., and Suzuki, S.,** *Anal. Chim. Acta,* 116, 61 (1980).
102. **Walters, R. R., Moriarty, B. E., and Buck, R. P.,** *Anal. Chem.,* 52, 1680 (1980).
103. **Kobos, R. K. and Pyon, H. Y.,** *Biotechnol. Bioeng.,* 23, 627 (1981).
104. **Rechnitz, G. A., Arnold, M. A., and Meyerhoff, M. E.,** *Nature,* 278, 466 (1979).
105. **Arnold, M. A. and Rechnitz, G. A.,** *Anal. Chim. Acta,* 113, 351 (1980).
106. **Arnold, M. A., Meyerhoff, M. E., and Rechnitz, G. A.,** U.S. Patent 4,216,065, 1980.
107. **Arnold, M. A. and Rechnitz, G. A.,** *Anal. Chem.,* 52, 1170 (1980).
108. **Arnold, M. A. and Rechnitz, G. A.,** *Anal. Chem.,* 53, 515 (1981).
109. **Updike, S. and Treichel, I.,** *Anal. Chem.,* 51, 1643 (1979).
110. **Alexander, P. W. and Rechnitz, G. A.,** *Anal. Chem.,* 46, 256 (1974).

111. Alexander, P. W. and Rechnitz, G. A., *Anal. Chem.*, 46, 1253, (1974).
112. Solsky, R. L. and Rechnitz, G. A., *Anal. Chim. Acta*, 99, 241 (1978).
113. Meyerhoff, M. E. and Rechnitz, G. A., *Science*, 195, 494 (1977).
114. Boitieux, J. L., Desmet, G., and Thomas, D., *Clin. Chem.*, 25, 318 (1979).
115. Meyerhoff, M. E. and Rechnitz, G. A., *Anal. Biochem.*, 95, 483 (1979).
116. D'Orazio, P. and Rechnitz, G. A., *Anal. Chem.*, 49, 2083 (1977).
117. D'Orazio, P. and Rechnitz, G. A., *Anal. Chim. Acta*, 109, 25 (1979).
118. Shiba, K., Watanabe, T., Umezawa, Y., Fujiwara, S., and Momoi, H., *Chem. Lett.*, 1980, 155 (1980).
119. Shiba, K., Umezawa, Y., Watanabe, T., Ogawa, S., and Fujiwara, S., *Anal. Chem.*, 52, 1610 (1980).
120. Bayer, E. A. and Wilchek, M., *Methods Biochem. Anal.*, 26, 1, 19 (1979).
121. Gebauer, C. R. and Rechnitz, G. A., *Anal. Biochem.*, 103, 280 (1980).
122. del Castillo, J., Rodriguez, A., Romero, C., and Sanchez, V., *Science*, 153, 185 (1966).
123. Barfort, P., Arquilla, E. R., and Vogelhut, P. O., *Science*, 160, 1119 (1968).
124. Toro-Goyco, E., Rodriguez, A., and del Castillo, J., *Biochim. Biophys. Res. Commun.*, 23, 341 (1966).
125. Wodschall, D. and McKeon, C., *Biochim. Biophys. Acta*, 413, 317 (1975).
126. Janata, J., *J. Amer. Chem. Soc.*, 97, 2914 (1975).
127. Yamamoto, N., Nagasawa, Y., Shuto, S., Sawai, M., Sudo, T., and Tsubomura, H., *Chem. Lett.*, 245, 1978.
128. Yamamoto, N., Nagasawa, Y., Sawai, M., Sudo, T., and Tsubomura, H., *J. Immunol. Methods*, 22, 309 (1978).
129. Yamamoto, N., Nagasawa, Y., Shuto, S., Tsubomura, H., Sawai, M., and Okumura, H., *Clin. Chem.*, 26, 1569 (1980).
130. Lowe, C. R., *FEBS Lett.*, 106, 405 (1979).
131. Kato, S., Aizawa, M., and Suzuki, S., *J. Membr. Sci.*, 3, 29 (1978).
132. Aizawa, M., Kato, S., and Suzuki, S., *J. Membr. Sci.*, 2, 125 (1977).
133. Aizawa, M., Suzuki, S., Nagamura, Y., Shinohara, R., and Ishiguro, I., *J. Solid-Phase Biochem.*, 4, 25 (1979).
134. Aizawa, M., Kato, S., and Suzuki, S., *J. Membr. Sci.*, 7, 1 (1980).
135. Solsky, R. L. and Rechnitz, G. A., *Science*, 204, 1308 (1979).
136. Solsky, R. L. and Rechnitz, G. A., *Anal. Chim. Acta*, 123, 135 (1981).
137. Yamada, T. and Freiser, H., *Anal. Chim. Acta*, 125, 179 (1981).
138. Diamandis, E. P. and Hadjiioannou, T. P., *Anal. Chim. Acta*, 123, 341 (1981).
139. Hopirtean, E. and Kormos, F., *Chem. Anal.*, 25, 209 (1980).
140. Martin, C. R. and Freiser, H., *Anal. Chem.*, 52, 1772 (1980).
141. Luca, C., Baloescu, C., Semenescu, G., Tolea, T., and Semenescu, E., *Rev. Chim.*, 30, 72 (1979).
142. Goina, T., Hobai, S., and Rozenberg, L., *Farmacia*, 26, 141 (1978).
143. Goina, T. and Hobai, S., *Rev. Med.*, 23, 70 (1977).
144. Hopartean, E. and Kormos, F., *Stud. Univ. Babes-Bolyai, (Ser.) Chem.*, 22, 35 (1977).
145. Carmack, G. D. and Freiser, H., *Anal. Chem.*, 49, 1577 (1977).
146. Thompson, M., Worsfold, P. J., Holuk, J. M., and Stubley, E. A., *Anal. Chim. Acta*, 104, 195 (1979).
147. Thompson, M., Krull, U. J., and Worsfold, P. J., *Anal. Chim. Acta*, 117, 133 (1980).
148. Diamandis, E. P., Athanasiou-Malaki, E., Papastathopoulos, D. S., and Hadjiioannou, T. P., *Anal. Chim. Acta*, 128, 239 (1981).
149. An, T. T. and Khanh, N. T., *Tap Chi Duoc Hoc*, 1980, 21 (1980).
150. Campbell, M. J. M., Demetriou, B., and Jones, R., *Analyst*, 105, 605 (1980).
151. Hassan, S. S. M. and Eldesouki, M. H., *Talanta*, 26, 531 (1979).
152. Cosofret, V. V. and Bunaciu, A. A., *Anal. Lett.*, 12, 617 (1979).
153. Cosofret, V. V. and Zugravescu, P. G., *Rev. Chim.*, 28, 785 (1977).
154. Srianujata, S., White, W. R., Higuchi, T., and Sternson, L. A., *Anal. Chem.*, 50, 232 (1978).
155. Cosofret, V. V., *Ion-Sel. Elec. Rev.*, 2, 159 (1980).
156. Ionescu, M. and Cosofret, V. V., *Rev. Chim.*, 31, 1088 (1980).
157. Rakias, F., Toth, K., and Pungor, E., *Anal. Chim. Acta*, 121, 93 (1980).
158. Vinz, W. and Jahn, V., *Pharm. Prax*, 35, 8 (1980).
159. Finnerty, W. A., Luttrell, H., and Zudeck, S., An Evaluation of some pharmaceutical applications of an ISE-auto analyzer II system, in *Advances in Automated Analysis, Technicon International Conference, 1976*, Vol. 2, Barton, E. C., Ed., Mediad, Inc., Tarrytown, 1977, 210.
160. Martens, J., Declercq, H., Massart, D. L., Michotte, Y., Van den Winkel, P., Dryon, L., and Henrion-Boeckstijns, A., *Actual. Chim. Anal., Org., Pharm. Bromatol.*, 23, 33 (1975).
161. Goina, T., Hobai, S., and Rodeanu, A., *Farmacia*, 24, 89 (1976).
162. Cosgrove, R. F. and Beezer, A. E., *Anal. Chim. Acta*, 105, 77 (1979).
163. Varela Rial, J., Valdes Gonzalez, J., and Gonzalez Carrero, J., *Acta Cient. Compostelana*, 14, 439 (1977).

164. **Nube, M., Van den Aarsen, C. P. M., Giliams, J. P., and Hekkens, W. T. J. M.,** *Clin. Chim. Acta,* 100, 239 (1980).
165. **Roy, R. B., Jansen, J., and Sahn, M.,** *J. Assoc. Off. Anal. Chem.,* 63, 931 (1980).
166. **Baum, G.,** An automated kinetic analysis of cholinesterase activity by a substrate-selective ion-exchange electrode, in *Ion and Enzyme Electrodes in Biology and Medicine, International Workshop,* Kessler, M., Clark, L. C., and Lubbers, D. W., Eds., University Park Press, Baltimore, 1976, 193.
167. **Johansson, G. and Ogren, L.,** Application of ion-selective electrodes to enzymatic analysis, in *Ion-Selective Electrodes,* Pungor, E. and Buzas, I., Eds., Akademiai Kiado, Budapest, 1977, 93.
168. **D'Orazio, P. and Rechnitz, G. A.,** *Anal. Chem.,* 49, 41 (1977).
169. **Lionetti, G., Carugno, N., and Neri, M.,** *Riv. Merceol.,* 17, 239 (1978).
170. **Thomas, R. C.,** *Ion-Sensitive Intracellular Microelectrodes. How to Make and Use Them,* Academic Press, London, 1978.
171. The application of ion-selective microelectrodes, Zeuthen, T. Ed., in *Research Monographs in Cell and Tissue Physiology,* Vol. 4, Dingle, J. T. and Gordon, J. L., Eds., Elsevier, Amsterdam, 1981.
172. **Sykova, E., Hnik, P., and Vyclicky, L., Eds.,** *Ion-Selective Microelectrodes and Their Use in Excitable Tissues,* Plenum Press, New York, 1981.
173. **Walker, J. L. and Brown, H. M.,** *Physiol. Rev.,* 57, 729 (1977).
174. **Zeuthen, T.,** *Curr. Top. Membr. Transp.,* 13, 31 (1980).
175. **Armstrong, W. M. and Garcia-Diaz, J. F.,** *Fed. Proc.,* 39, 2851 (1980).
176. **Armstrong, W. M.,** The use of ion-selective microelectrodes to measure intracellular ionic activities, in *Epithelial Ion and Water Transport,* Macknight, A. D. C. and Leader, J. P., Eds., Raven Press, New York, 1981, 85.
177. **Fujimoto, M., Kotera, K., Kajino, K., and Aoki, S.,** *Sogo Rinsho,* 29, 2699 (1980).
178. **Schneider, W., Wahl, M., Kuschinsky, W., and Thurau, K.,** *Pfluegers Arch,* 372, 103 (1977).
179. **DuBose, T. D., Pucacco, L. R., and Carter, N. W.,** *Am. J. Physiol.,* 240, F138, 1981.
180. **Young, A. E., Fencl, V., Woods, M., Dmochowski, J., and Couch, N. P.,** *Anesth. Analg.,* 56, 817 (1977).
181. **Severinghaus, J. W., Stafford, M., and Thundstrom, A. M.,** *Acta Anaesthesiol. Scand., Suppl.,* 68, 9 (1978).
182. **Steiner, R. A., Oehme, M., Ammann, D., and Simon, W.,** *Anal. Chem.,* 51, 351 (1979).
183. **Honda, M.,** *Osaka Ika Daigaku Zasshi,* 38, 202 (1979).
184. **Gueggi, M., Kessler, M., Greitschus, F., Wiegand, V., and Meesmann, W.,** *Front. Biol. Energ.,* 2, 1427 (1978).
185. **Wong, B. and Woody, C. D.,** *Exp. Neurol.,* 61, 219 (1978).
186. **Lewis, S. A. and Wills, N. K.,** *Biophys. J.,* 31, 127 (1980).
187. **Owen, J. D., Brown, H. M. and Pemberton, J. P.,** *Anal. Chim. Acta,* 90, 241 (1977).
188. **Lanter, F., Erne, D., Ammann, D., and Simon, W.,** *Anal. Chem.,* 52, 2400 (1980).
189. **Siggaard-Andersen, O.,** Analytical procedures and instrumentation. Section III. Electrochemistry, in *Fundamentals of Clinical Chemistry,* 2nd ed., Tietz, N. W., Ed., W. B. Saunders, Philadelphia, 1976, 135.
190. **Simon, W., Ammann, D., Osswald, H. F., Meier, P. C., and Dohner, R. E.,** Applicability of ion-selective electrodes in automated systems for clinical analysis, in *Advances in Automated Analysis, Technicon International Conference, 1976,* Vol. 1, Barton, E. C., Ed., Mediad, Tarrytown, 1977, 59.
191. **Griswold, K. E.,** *Bayou Tech.,* 18, 11 (1974).
192. **Fatt, I.,** *Proc. Anal. Div. Chem. Soc.,* 14, 329 (1977).
193. **Buck, R. P.,** *Proc. Anal. Div. Chem. Soc.,* 14, 332 (1977).
194. **Treasure, T. and Band, D. M.,** *Proc. Anal. Div. Chem. Soc.,* 14, 334 (1977).
195. **Vallon, J. J. and Pegon, Y.,** *Pharm. Biol.,* 14, 531 (1980).
196. **Iwata, M., Takahashi, Y. and Kushiro, J.,** *Eisei Kensa,* 26, 883 (1977).
197. **Patel, S. and O'Gorman, P.,** *Clin. Chem.,* 24, 1856 (1978).
198. **Annan, W., Kirwan, N. A., and Robertson, W. S.,** *Clin. Chem.,* 25, 643 (1979).
199. **Preuss, C. J. and Fuchs, C.,** *J. Clin. Chem. Clin. Biochem.,* 17, 639 (1979).
200. **Jenny, H. B., Ammann, D., Doerig, R., Magyar, B., Asper, R., and Simon, W.,** *Mikrochim. Acta,* 2, 125 (1980).
201. **Jenny, H. B., Riess, C., Ammann, D., Magyar, B., Asper, R., and Simon, W.,** *Mikrochim. Acta,* 2, 309 (1980).
202. **Marsoner, H. J. and Harnoncourt, K.,** *Aerztl. Lab.,* 23, 327 (1977).
203. **Schindler, J. G.,** *Biomed. Tech.,* 24, 203 (1979).
204. **Ladenson, J. H.,** *J. Lab. Clin. Med.,* 90, 654 (1977).
205. **Band, D. M., Kratochvil, J., Poole Wilson, P. A., and Treasure, T.,** *Analyst,* 103, 246 (1978).
206. **Mohan, M. S., Bates, R. G., Hiller, J. M., and Brand, M. J.,** *Clin. Chem.,* 24, 580 (1978).

207. Ladensen, J. H., *Clin. Chem.*, 25, 757 (1979).
208. Levy, G. B., *Clin. Chem.*, 25, 1516 (1979).
209. Coleman, R. L., *Clin. Chem.*, 25, 1865 (1979).
210. Annan, W., Kirwan, N. A., and Robertson, W. S., *Clin. Chem.*, 25, 1865 (1979).
211. Shyr, C. and Young, C. C., *Clin. Chem.*, 26, 1517 (1980).
212. Czaban, J. D. and Cormier, A. D., *Clin. Chem.*, 26, 1921 (1980).
213. Coleman, R. L., Young, C. C., and Sidoni, L., *Clin. Chem.*, 26, 1922 (1980).
214. Czaban, J. D. and Cormier, A. D., *Clin. Chem.*, 26, 1923 (1980).
215. Levy, G. B., *Clin. Chem.*, 27, 1435 (1981).
216. Baron, D. N., *Clin. Chem.*, 27, 642 (1981).
217. Kiszel, J. and Havas, J., Experiences collected in the field of neonatology with potentiometric potassium and sodium determinations, in *Ion-Selective Electrodes,* Pungor, E. and Buzas, I., Eds., Elsevier, Amsterdam, 1978, 435.
218. Szabo, A., Velosy, G. A., and Koranyi, L., *Fres. Z. Anal. Chem.*, 301, 190 (1980).
219. Greenwood, R. S., Dodson, W. E., and Goldring, S., *Brain Res.*, 165, 171 (1979).
220. Osswald, H. F., Dohner, R. E., Meier, T., Meier, P. C., and Simon, W., *Chimia,* 31, 50 (1977).
221. Truchaud, A., Hersant, J., Glikmanas, G., Fievet, P., and Dubois, O., *Clin. Chem.*, 26, 139 (1980).
222. Slaunwhite, D., Clements, J. C., and Reynoso, G., *Clin. Biochem.*, 10, 44 (1977).
223. Schindler, J. G., Duerr, H. K., Riemann, W., Braun, H. E., and Kellner, V., *Biomed. Techn.*, 23, 45 (1978).
224. Baker, C., Kahn, S. E., and Bermes, E. W., *Ann. Clin. Lab. Sci.*, 10, 523 (1980).
225. Battaglia, C. J., Chang, J. C., and Daniel, D. S., U.S. Patent 4,214,968, 1980.
226. Battaglia, C. J., Chang, J. C., and Daniel, D. S., *Res. Discl.*, 176, 15 (1978).
227. Battaglia, C. J., Kim, S. H., and Secord, D. S., U.S. Patent 4,199,412, 1980.
228. Thomas, J. D. R., *Lab. Pract.*, 27, 857 (1978).
229. Moody, G. J., Nassory, N. S., and Thomas, J. D. R., *Proc. Anal. Div. Chem. Soc.*, 16, 32 (1979).
230. Simon, W., Ammann, D., Oehme, M., and Morf, W. E., *Ann, N.Y. Acad. Sci.*, 307, 52 (1978).
231. Robertson, W. G. and Marshall, R. W., *CRC Crit. Rev. Clin. Lab. Sci.*, 11, 271 (1979).
232. Moody, G. J. and Thomas, J. D. R., *Ion-Sel. Elec. Rev.*, 1, 3 (1979).
233. Ohman, S. and Larsson, L., *Clin. Chem.*, 24, 2070 (1978).
234. Demetriou, L., *Clin. Chem.*, 24, 2071 (1978).
235. Fuchs, C. and McIntosh, C., *Clin. Chem.*, 23, 610 (1977).
236. Schwartz, H. D., *Clin. Chem.*, 23, 610 (1977).
237. Larsson, L. and Ohman, S., *Clin. Chem.*, 26, 1761 (1980).
238. Coleman, R., *Clin. Chem.*, 26, 1762 (1980).
239. Larsson, L. and Ohman, S., *Clin. Chem.*, 26, 1762 (1980).
240. Fogh-Anderson, N., Christiansen, T. F., Komarmy, L., and Siggaard-Andersen, O., *Clin. Chem.*, 24, 1545 (1978).
241. Guy, W. S., *AAAS Sel. Symp.*, 11, 125 (1979).
242. Chiba, K., Tsunoda, K., Haraguchi, H., and Fuwa, K., *Anal. Chem.*, 52, 1582 (1980).
243. Irlweck, K., Czitober, H., and Machata, G., *Acta Med. Austriaca,* 6, 99 (1979).
244. Paez, D. M., De Bianchi, L. P., Gil, G. A., Dapas, O., and Coronato, R. G., *Fluoride,* 13, 65 (1980).
245. Bray, T., Clark, G. C. F., Moody, G. J., and Thomas, J. D. R., *Clin. Chim. Acta,* 77, 69 (1977).
246. Price, C. P. and Spencer, K., *Ann. Clin. Biochem.*, 14, 171 (1977).
247. Warnick, W. J. and Hansen, L. G., *Clin. Chem.*, 24, 381 (1978).
248. Moody, G. J. and Thomas, J. D. R., *Ion-Sel. Elec. Rev.*, 2, 73 (1980).
249. Craven, G. S. and Griffith, M. C., *Aust. J. Dairy Technol.*, 32, 75 (1977).
250. O'Reilly, J. E., *J. Chem. Ed.*, 56, 297 (1979).
251. Hall, J. and Pearson, J. T., *J. Pharm. Pharmacol.*, 32, 69P (1980).
252. Puttemans, F., Deconinck, F., Jonckheer, M., Vandeputte, M., and Massart, D. L., *Clin. Chem.*, 25, 1247 (1979).
253. Moses, G. C., Thibert, R. J., and Draisey, T. F., *J. Clin. Pathol.*, 31, 1207 (1978).
254. Liu, C. H., Chon, K., and Ho, P. M., *Chung-hua I Hsueh Chien Yen Tsa Chih,* 2, 114 (1979).
255. Davidson, J. S. D. and Jennings, D. B., *Can. J. Physiol. Pharmacol.*, 58, 550 (1980).
256. Fraticelli, Y. M. and Meyyerhoff, M. E., *Anal. Chem.*, 53, 992 (1981).
257. Georges, J., *Clin. Chem.*, 25, 1888 (1979).
258. Klein, E., Montalvo, J. G., Wawro, R., Holland, F. F., and Lebeouf, A., *Int. J. Artif. Organs,* 1, 116 (1978).
259. Klein, E. and Montalvo, J. G., *Int. J. Artif. Organs,* 1, 175 (1978).
260. Ruzicka, J., Hassen, E. H., Ghose, A. K., and Mottola, H. A., *Anal. Chem.*, 51, 199 (1979).
261. Diamandis, E. P. and Hadjiioannou, T. P., *Clin. Chem.*, 27, 455 (1981).

262. **Diamandis, E. P., Papaststhopoulos, D. S., and Hadjiioannou, T. P.,** *Clin. Chem.*, 27, 427 (1981).
263. **Osswald, H. F., Asper, R., Dimai, W., and Simon, W.,** *Clin. Chem.*, 25, 39 (1979).
264. **Schindler, J. G., Stork, G., Dennhardt, R., Schael, W., Braun, H. E., Karaschinski, K. D., and Schmid, W.,** *J. Clin. Chem. Clin. Biochem.*, 17, 573 (1979).
265. **Schindler, J. G., Dennhardt, R., and Simon, W.,** *Chimia*, 31, 404 (1977).
266. **Fogt, E. J., Eddy, A. R., Clemens, A. H., Fox, J., and Heath, H.,** *Clin. Chem.*, 26, 1425 (1980).
267. **Klein, E. and Walthen, R. L.,** U.S. Patent 4,244,787, 1981.
268. **Wong, K. C. and Jordan, W. S.,** The potential uses of solid state chemical sensors in patient monitoring during anesthesia, in *Theory, Design, and Biomedical Applications of Solid State Chemical Sensors*, Cheung, P. W., Fleming, D. G., Ko, W. H., and Neuman, M. R., Eds., CRC Press, Boca Raton, Fla., 1978, 263.
269. **Neuman, M. R.,** Applications of chemical sensors in obstetrics, in *Theory, Design, and Biomedical Applications of Solid State Chemical Sensors*, Cheung, P. W., Fleming, D. G., Ko, W. H., and Neuman, M. R., Eds., CRC Press, Boca Raton, Fla., 1978, 277.
270. **Tseung, A. C. C. and Goffe, R. A.,** *Med. Biol. Eng. Comput.*, 16, 677 (1978).
271. **Hill, J. L., Gettes, L. S., Lynch, M. R., and Hebert, N. C.,** *Am. J. Physiol.*, 235, H453 (1978).
272. **Peter, D. M., Afromowitz, M. A., and Yee, S. S.,** *Proc. Int. Microelectron. Symp.*, 166, 1976.
273. **Nichols, M. F., Watts, V., Whiteside, F. A., and Hahn, A. W.,** *Biomed. Sci. Instrum.*, 17, 97 (1981).
274. **Afromowitz, M. A. and Yee, S. S.,** *J. Bioeng.*, 1, 55 (1977).
275. **Mindt, W., Maurer, H., and Moeller, W.,** *Arch. Gynecol.*, 226, 9 (1978).
276. **Lofgren, O.,** *Arch. Gynecol.*, 226, 17 (1978).