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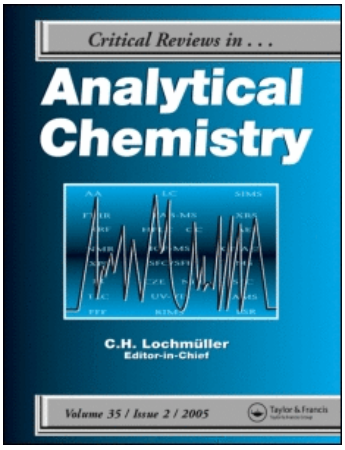

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Potentiometric Ion-, Gas-, and Bio-Selective Membrane Electrodes

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Potentiometric Ion-, Gas-, and Bio-Selective Membrane Electrodes

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ABSTRACT: Membrane electrodes are relatively simple electrochemical devices that can be used for the direct measurement of ions, gases, and biomolecules in complex samples. Selectivity for one species over another is determined by the nature and chemical composition of the membranes and associated reaction layers used to fabricate the devices. All membrane electrode probes employ at least one ion-selective membrane as the ultimate transduction element. This indicator membrane serves as an additional component of a classic two-electrode galvanic cell. The potential developed at the membrane/sample interface is directly or indirectly related to the activity or concentration of analyte in the sample. Because cell potentials are detected under essentially zero-current conditions, analytical measurements with these probes are generally not subject to the mass transfer and electron transfer kinetic limitations that often plague voltammetric or amperometric techniques.

In this report, we review the current state of the art with respect to development and application of potentiometric membrane electrode probes. As a starting point, we provide a brief introduction to the fundamentals of membrane-based galvanic cell potentials and their measurement. Subsequent sections summarize the various membranes now in use for direct ion sensing, the approaches taken to miniaturize these same ion-selective systems, and the use of such membranes in conjunction with secondary membranes to devise a variety of potentiometric gas sensing systems. Finally, we conclude with an overview of the approaches being employed presently to devise potentiometric bioprobes suitable for the selective, *in situ* detection of biologically important molecules.

KEY WORDS: ion-selective electrodes, potentiometry, gas sensors, *in vivo* probes, biosensors.

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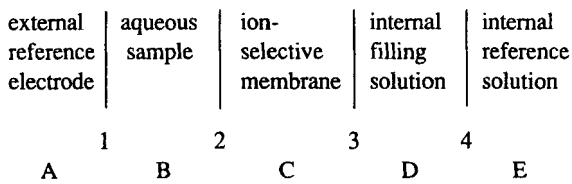
I. MEMBRANE-BASED ELECTROCHEMICAL CELLS

A. Classic Cell Arrangement

The experimental assembly required for ion-selective electrode (ISE) measurements is shown in Figure 1. The measurement principle is simple: two reference electrodes of fixed potential are separated by the ion-selective membrane. The solution on one side of the membrane is of known, constant composition and includes ions to which the internal reference electrode and ISE membrane respond. The sample solution contacts the other side. A common shorthand notation for this cell is



Each vertical line depicts an interface at which a phase-boundary potential can develop. In order for this cell to provide stable signals, all of the various interfaces must be "poised." This means that each electrode or membrane surface must contact a solution that contains an ion to which the electrode/membrane responds in a reversible manner. A cell properly designed for direct potentiometric measurements will maintain all of these

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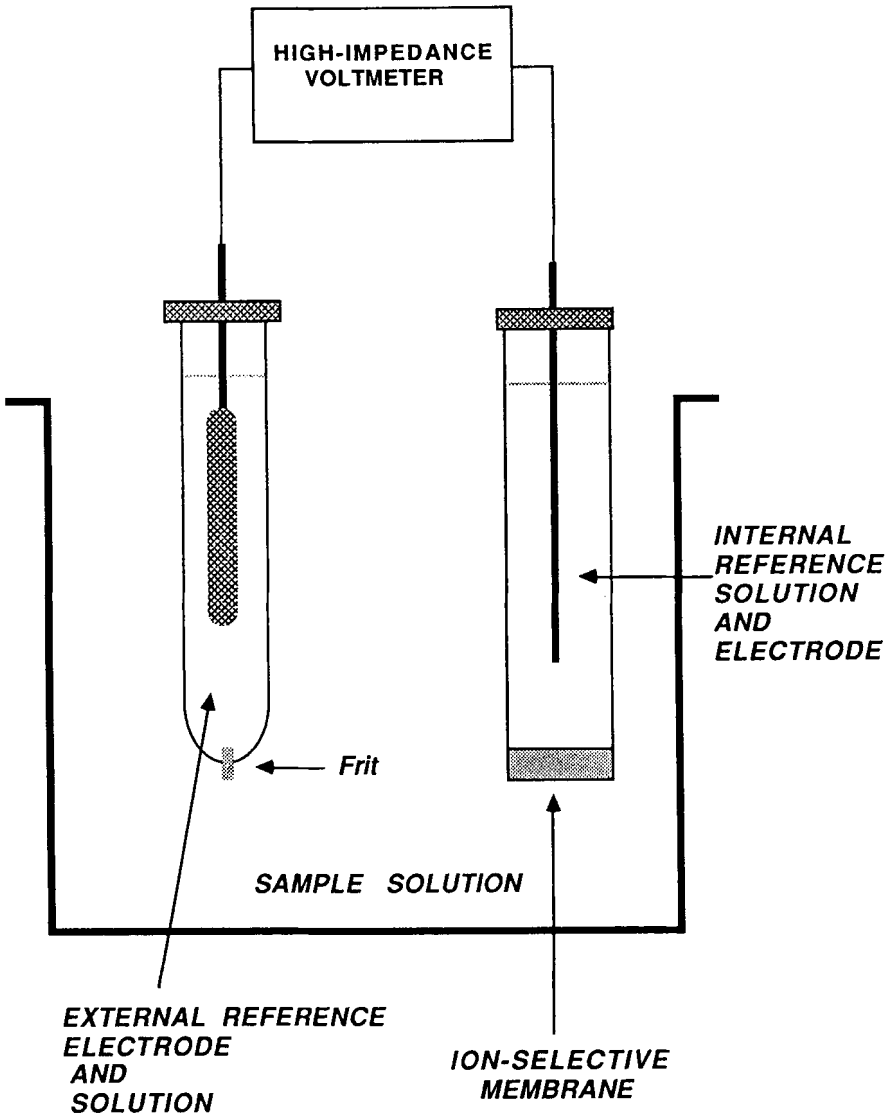

FIGURE 1. Schematic diagram depicting the experimental assembly required for making potentiometric ion-selective membrane electrode measurements.

potentials constant, except for the membrane potential ($\Delta\phi_M$), which is the difference in electrical potential between the internal filling solution and the sample solution ($\phi_{int} - \phi_{sam}$ or $\phi_D - \phi_B$). The variation in this membrane potential will be the indication of the activity of the analyte ion in the sample solution.

Consider the example of a glass pH membrane, immersed in a dilute acid solution of fixed a_{Cl^-}, with Ag/AgCl internal and external reference wires. An appropriate internal solution might be 1 m*M* HCl because an Ag/AgCl wire is poised by chloride ions and the glass membrane is poised by H^+. By convention, the cell potential E_{cell} is calculated as:

$$E_{cell} = (\phi_B - \phi_A) + (\phi_C - \phi_B) + (\phi_D - \phi_C) + (\phi_E - \phi_D) \quad (1)$$

In practice, the internal reference wire, the internal solution, and the ISE membrane are often housed in one physical unit called the *indicator electrode*. In this case, the above equation becomes

$$E_{cell} = E_{indicator} - E_{ext.\ ref.} \quad (2)$$

where $E_{ext.\ ref.} = \phi_A - \phi_B$. The potentials at boundaries 1 and 4 are constant in this cell; the only variable potential occurs across the ISE membrane,

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and this potential is dependent on the pH of the sample solution (if the ISE membrane is pH sensitive). The above equation thus simplifies to

$$E_{cell} = K_{cell} + \Delta\phi_M \quad (3)$$

where E_{cell} is the sum of all the constant phase boundary potentials in the cell (i.e., $\Delta\phi_1$ and $\Delta\phi_4$), and $\Delta\phi_M = \Delta\phi_3 + \Delta\phi_2$ or $\phi_D - \phi_B$. Thermodynamic principles govern the magnitude of $\Delta\phi_M$. There are a number of ways to derive the value of $\Delta\phi_M$. The most rigorous approach involves treating $\Delta\phi_M$ as the sum of two phase boundary potentials, $\Delta\phi_3 + \Delta\phi_2$, plus an additional diffusion potential within the membrane phase. The same result, however, is obtained if the membrane is considered to be a very thin permselective junction between the sample and internal solutions. Thus, invoking the simplest of all junction potential expressions (type I junctions),

$$\Delta\phi_M + (t_+ - t_-)\frac{RT}{zF}\ln\frac{a_i(B)}{a_i(D)} \quad (4)$$

where t_+ and t_- are the transference numbers for the cation and anion species (at activities a) bathing both sides of the junction. If the membrane is truly cation selective for ion i, $t_+ = 1$ and $t_- = 0$. That is, if the current were to pass through the membrane, all current would be carried by the analyte cation, and

$$\Delta\phi_M = \frac{RT}{zF}\ln\frac{a_i(B)}{a_i(D)} \quad (5)$$

where R is the gas constant (8315 mV K^{-1} mol^{-1}), T is the temperature in K, z is the charge on the ion, F is the Faraday constant (96,485 coul mol^{-1}), and a_i is the activity of the analyte ion in solution. Thus, Equation 3 becomes

$$E_{cell} = K_{cell} + \frac{RT}{zF}\ln\frac{a_i(B)}{a_i(D)} \quad (6)$$

Because $a_i(D)$ is fixed by the composition of the internal solution of the electrode, Equation 6 can be rewritten as

$$E_{cell} = K'_{cell} + \frac{RT}{zF}\ln a_i(B) \quad (7)$$

where $a_i(B)$ is the activity of the analyte ion in the sample solution. At 25°C, for the pH electrode example above, this results in Equation 8:

$$E_{cell} = K'_{cell} - (59.2)pH) \quad (8)$$

Naturally, treatment of the membrane potential as a simple junction does not provide an accurate picture as to the processes that yield the charge separation and the measured ϕ_M. This is particularly true for pH-sensitive glass membranes where analyte ions do not diffuse into the bulk of the membrane phase, and an even more complex mechanism originating from five separate potential generating processes is involved.[1] Nonetheless, the simplistic junction potential derivation does hold true for all types of ISEs.

While Equation 4 predicts that the membrane potential should be zero when the activity of analyte ion is equal on both sides of the membrane, in practice this is rarely the case. Indeed, the equilibrium constant for the reaction of ions with the membrane surface is often slightly different on each side of the membrane, yielding a so-called *asymmetry potential*. Such asymmetry potentials can be caused by adsorption of sample components onto the outer surface of the ISE membrane (e.g., proteins) and nonhomogeneity in the membrane composition. The asymmetry potential is often considered to be constant for analytical measurements; however, this assumption may not always be valid, particularly when using ISEs in very complex samples where extraction and/or adsorption of various species on the outer surface of the membrane can occur.

Equation 7 is the general equation that provides the basis for electrode calibration curves. These should be obtained experimentally because the theoretical slope of 59.2/z mV per decade is, in practice, rarely attained exactly. The analyst wishing to determine ammonium with an ISE, for example, would record the electrode's millivolt signals for each of a series of NH_4Cl standards bracketing the sample's expected ammonium content. The familiar two-point calibration in common use for pH measurements is merely an instrumental means of correcting for this deviation from theoretical response.

In the above example of pH measurement, the external reference electrode was considered an Ag/AgCl wire immersed directly into the sample

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solution. This would be impractical for ordinary use because it would require that the chloride ion activity remain constant in all samples and standards. For this reason, the reference electrode is commonly housed in its own solution. This external reference solution is in electrical contact with the sample solution by means of a porous frit, wick, crack, or other junction in the housing. A saturated calomel electrode (SCE) is often used as the reference electrode of this configuration. However, the use of such a device introduces another factor into the calculation of E_{cell}. Because cations and anions migrate across this junction at different rates (the smaller cations usually faster than the larger anions), a separation of charge develops across the junction.[2] This extra *junction potential*, E_j, must be included in the overall cell potential, and Equation 7 becomes:

$$E_{cell} = K'_{cell} + \frac{RT}{zF} \ln a_{i,\,sample} + E_j \qquad (9)$$

The magnitude of E_j depends on the nature and activity of all ions on both sides of the frit (i.e., in the sample and external reference solutions). The analyst need not be concerned with the absolute magnitude of E_j itself, but it is imperative that it be kept constant during the analytical measurement in order for E_{cell} to reflect $a_{i,\,sample}$ accurately. The practical implication of this fact is that samples and standards must be of similar matrix, or at least similar ionic strength, to minimize variations in E_j when a reference electrode of this configuration is being used. For this reason, as well as those explained in Section I.B, the importance of considering the sample matrix in ISE-based analyses cannot be overemphasized.

In practice, the overall cell potential, E_{cell}, is measured with a high-impedance voltage meter. The necessary impedance can be estimated by considering that common ISE membranes exhibit resistances on the order of 1 to 250 MΩ. The cell assembly may be viewed as a simple voltage-divider circuit, in which

$$E_{measured} = (E_{cell}) \frac{R_x}{R_x + R_{mem}} \qquad (10)$$

where R_x is the resistance of the measuring meter itself. In order for $E_{measured}$ to equal the actual E_{cell}, R_x must be significantly larger than the resistance of the ISE membrane. It is common practice to use instruments with input impedances $\geq 10^{12}\Omega$. Modern laboratory pH/mV meters, which accept the electrode input with field-effect-transistor amplifiers, fill this requirement. The resistance of ISE membranes, however, increases dramatically as the size of the membranes decreases. Thus, for microelectrodes, specially designed equipment with even higher input impedances must be used. It is also common practice to shield connecting wires and to enclose ISE devices (including the external reference electrode) in a grounded metal container (a Faraday cage) to isolate them from external electrical noise.

B. Accounting for Selectivity

No membrane is as ideally permselective as the model used above. The Nernst equation for membrane electrodes (derived in the previous section) can be modified to account for this imperfect selectivity. By multiplying the activity of the interfering ion (a_j) by an experimentally determined coefficient, the interferent's effect on the electrode potential can be predicted.

$$E_{cell} = K_{cell} + \frac{RT}{zF} \ln\left[a_i + k_{ij}^{pot}\left(a_j\right)^{z/x}\right] \qquad (11)$$

where k_{ij}^{pot} is the *potentiometric selectivity coefficient* for the electrode when the electrode is used to measure a_i in the presence of some interferent ion *j* of charge *x*. This is known as the Nicolsky equation.[3,4] The selectivity coefficient should be determined under experimental conditions closely matching those to be used for the analytical measurement of *i*, because k_{ij}^{pot} can vary somewhat with conditions (i.e., ionic strength, protein content, etc.). Various procedures for evaluating k_{ij}^{pot} for a given electrode have been established.[4–6]

The analyst must be aware of these selectivity principles for two reasons. First, the lower limit of detection of *i* in the presence of *j* will be dictated by the electrode's k_{ij}^{pot}. Consider an analysis of an aqueous sample, 1 m*M* in *j*, with an electrode that displays a k_{ij}^{pot} of 10^{-3}. The lower

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limit of detection of i will be on the order of (10^{-3}) (1 mM) = 1 μM. At levels of a_i near 1 μM, the electrode potential will be severely affected by a_j and the slope will be significantly less than 59.2 mV per decade change in a_i. When a_i is substantially below k_{ij}^{pot} a_j (one or two orders of magnitude), changes in a_i will be impossible to detect. It should be obvious from this example that electrodes of lowest k_{ij}^{pot} are the most desirable.

The second aspect of selectivity considerations to be kept in mind is that of constant sample matrix. The selectivity term in the Nicolsky equation can also be written as $\sum_i k_{ij}^{pot} (a_j)^{z/x}$, that is, all the ions in the sample are likely to affect E_{cell} to a greater or lesser degree. As long as this selectivity term remains constant between standards and samples, the electrode potential reliably reflects a_i. When the activities of other interfering ions are variable, however, there will be variations in electrode potential unrelated to a_i, causing inaccurate results.

There is an additional reason for matching standard and sample matrices. Throughout this discussion, we have used the activity of the sample ion, not its concentration, in explaining electrode behavior. Activity is related to concentration, [i], by the activity coefficient, γ_i:

$$a_i = \gamma_i [i] \qquad (12)$$

The term γ_i is dependent in a nontrivial way on the ionic strength of the solution. Therefore, if an ISE is to be used to determine the concentration of an ion i, the matrix (in terms of ionic strength) of the calibration standards must match that of the samples as closely as possible.

II. CONSTRUCTION AND PERFORMANCE OF VARIOUS ION-SELECTIVE MEMBRANE ELECTRODES

The field of ion-selective membrane research is a vigorous and ever-expanding one. New developments in the design and application of various ISE membrane types are continuously being reported.[7–11] This section introduces the fundamental framework for the operation of various membrane types; the reader is invited to pursue the subject in more depth through available reviews, theoretical papers, and monographs.[9–20]

A. Glass Membranes

By far, the most commonly used ion-selective electrode is the glass pH electrode, the basic behavior of which was first described by Cremer in 1906.[21] The unusually high selectivity, wide dynamic range, and reliability of this electrode make it a paradigm for the design and performance of other ion-selective membranes. The pH response of this membrane arises from the presence of fixed SiO^- ion exchanger sites in the hydrated surface of the glass, which are highly sensitive to hydrogen ion activity. As shown in Table 1, the membrane exhibits extraordinary selectivity coefficients over other cations; this is due to the favorable equilibrium for the reaction involved,

$$SiO^- + H^+ \rightleftarrows SiOH$$

as well as the relative mobility of H^+ vs. other cations in the hydrated outer layer of the glass.[22] These k_{ij}^{pot} values vary with the exact composition of the glass. For example, the so-called "sodium electrode" is a glass membrane of increased Al_2O_3 content (see Table 1). It is important to realize that while such an electrode does show enhanced sensitivity to Na^+, it is still primarily a pH electrode because $k_{Na,A}^{pot} > 1$. Thus, any sample to be analyzed for alkali metal ion content with a glass electrode must be at a neutral or alkaline pH. The glass Na^+ electrode has, however, found wide use in clinical chemistry analyzers for measuring sodium in whole blood, serum, or plasma, giving reliable results in the presence of varying potassium and calcium concentrations.

The major limitations of glass membrane electrodes are their high resistance and fragility. These factors create severe practical problems when trying to devise miniaturized sensors, for example, for continuous *in vivo* or intracellular measurements of pH and sodium. Owing to the higher resistivity (compared with other ISE membranes), miniature and microglass electrodes often require specialized amplifier systems with even higher input impedances.

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TABLE 1
Membrane Compositions and Selectivities of Important Ion-Selective Electrodes

Analyte	Membrane type	Membrane composition	Linear range ($-\log a_i$)	$\log K^{pot}_{i,j}$
H^+	Glass	72.2% SiO_2, 6.4% CaO, 21.4% Na_2O (mol%)	2–12	Na^+: –11 K^+: –11
H^+	Polymer	Tri-*n*-dodecylamine, PVC, *bis*-2-ethylhexylsebacate, tetraphenylborate	3–12	Na^+: –10.4 K^+: –9.8 Ca^{2+}: –11
Na^+	Glass	11% Na_2O, 18% Al_2O_3, 71% SiO_2	1–6	K^+: –2 Ag^+: +2.6 NH_4^+: –4.2 H^+: 1–2.5
Na^+	Polymer	ETH 227, PVC, 2-nitrophenyloctyl ether, tetraphenylborate	1–3	Li^+: +0.3 K^+: –1.7 Ca^{2+}: –0.4 Mg^{2+}: –2.4
K^+	Polymer	Valinomycin, PVC, dioctyladipate	0–5	Na^+: –4.2 NH_4^+: –1.9 Ca^{2+}: –3.3 Mg^{2+}: –4.4
Li^+	Polymer	ETH 1810, PVC, 2-nitrophenyloctyl ether, tetraphenylborate	<2–4	Na^+: –2.2 K^+: –2.3 NH_4^+: –2.3 Ca^{2+}: –2.7 Mg^{2+}: –4 H^+: +1
Li^+	Polymer	Dodecylmethyl-14-crown-4, PVC, 2-nitrophenyloctyl ether, tetraphenylborate	0–5	K^+: –1.8 Na^+: –2.1 NH_4^+: –2.8 Ca^{2+}: –4.2 Mg^{2+}: –4.6 H^+: –3.2
Ca^{2+}	Polymer	ETH 1001, PVC, 2-nitrophenyloctyl ether, tetraphenylborate	2–7	Na^+: –5.5 K^+: –5.4 Mg^{2+}: –4.9
Ca^{2+}	Polymer	Ca-di-(*n*-decyl)phosphate, PVC, di-(*n*-octylphenyl)phosphonate	0–5	Na^+: –4.4 K^+: –4.5 Mg^{2+}: –4.9
Mg^{2+}	Polymer	ETH 5282, PVC, 2-nitrophenyloctyl ether, KtpCIPB	<2–5	Na^+: –3.8 K^+: –1.4 Ca^{2+}: –2.4
Ag^+	Solid state	Ag_2S	1–7	Cu^{2+}: –6 Pb^{2+}: –6 to –9 H^+: –5 Hg^{2+}: –2

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TABLE 1 (continued)
Membrane Compositions and Selectivities of Important Ion-Selective Electrodes

Analyte	Membrane type	Membrane composition	Linear range ($-\log a_i$)	$\log K^{pot}_{i,j}$
Acetylcholine	Liquid	Tetra(*p*-chlorophenyl)borate, 3-nitro-*o*-xylene	1–5	Na^+: –4 NH_4^+: –3 K^+: –3
F^-	Solid-state	Single LaF_3 crystal	0–6	OH^-: –1 Br^-: –4 Cl^-: –4 HCO_3^-: <–3
Cl^-	Liquid	Tri-*n*-octylpropylammonium chloride, decanol	1–3	OH^-: +0.4 Br^-: –0.4 F^-: –0.2 AcO^-: +0.7
I^-	Solid-state	50 mol% Ag_2S, 50 mol% AgI	<4–8	Cl^-: –4 Br^-: –7 SCN^-: –4 S^{2-}: >10
S^{2-}	Solid-state	Ag_2S	0–5	Br^-: –25 I^-: –18 Cl^-: –30

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B. Solid-State Membranes

Solid-state membrane electrodes utilize sparingly soluble inorganic salts as membrane materials. For example, the fluoride-selective electrode membrane in use today is a single crystal of lanthanum fluoride doped with europium to reduce resistance.[23] This crystal acts as a pure ionic conductor for fluoride ions. When the crystal is embedded in an electrode arrangement, it responds to fluoride ion activity in the expected manner:

$$E_{cell} = K_{cell} - (59.2\,mV)\log a_{F^-}\,(at\ 25°C) \quad (13)$$

The electrode can be used to measure pF levels of 0 to 6, with excellent selectivity over other common anions.[24] The principal interferent is hydroxide, due to the insolubility of $La(OH)_3$, which fouls the surface of the crystal. This interference can be controlled simply by buffering the samples to moderate pH. Low pHs also should be avoided because fluoride protonates below pH 5, decreasing the activity of free fluoride ions. For ISE measurements of F^-, a "total ionic strength adjustment buffer" is usually added to the sample. This solution buffers the pH at around 5 to 6 and contains complexing agents (e.g., citrate) to remove cations such as aluminum and iron that would otherwise complex fluoride.[25]

Other widely used solid-state membrane electrodes are based on pressed pellets of silver sulfide (Ag_2S). This membrane acts as a conductor of silver cations and has been used to measure Ag^+ to extremely low levels.[26] The Ag_2S electrode displays sensitivity for sulfide ions as well, because changes in $a_{S^{2-}}$ affect the available a_{Ag^+} at the electrode surface:

$$Ag_2S_{(s)} \rightleftarrows 2Ag^+ + S^{2-}$$

This membrane has been put to wider use by the inclusion of more soluble silver or sulfide salts. These so-called *mixed precipitate membranes* can be designed for sensitivity to a wide range of ions.[27] For example, an Ag_2S/CuS membrane is sensitive to Cu^{2+}; an $Ag_2S/AgCN$ pellet responds to CN^-.

The selectivity pattern of such electrodes is dictated by the relative solubilities of analyte vs. interferent ions with Ag^+ or S^{2-}. For example, an Ag_2S/AgBr pellet used for Br^- measurements shows considerable selectivity over Cl^- ($\log k_{Br^-,Cl^-} = -2.6$); however, because AgI is less soluble than AgBr, it influences the Ag^+ activity at the membrane surface more than AgBr, making I^- a serious interferent ($\log k_{Br,I} = 3.7$). The mixed crystal or precipitate Ag_2S^- based pellet membranes are always more responsive to Ag^+ and S^{2-} than to the analyte ion itself. Unfortunately, species that complex Ag^+, such as thiols (or biological molecules containing sulfhydryl groups), will degrade the membrane's performance.[28] Mercury(II) is always a serious interferent, regardless of the other components in the membrane, as HgS is less soluble than Ag_2S. Membranes incorporating HgS instead of Ag_2S have been proposed,[29] with performance reportedly comparable to or somewhat improved over the Ag_2S-based pellets. Theoretical models have been proposed to explain the selectivity behavior of solid-state membranes.[30,31]

The reader may be aware that an Ag/AgCl wire also can be used to determine Ag^+ or Cl^- activities, behaving similarly to the Ag_2S/AgX membranes described above. Although these wires are prepared more easily than the pressed solid-state pellets, the potential of such a metal wire is affected by the presence of redox species in the solution into which it is placed. The metal sulfide membranes are largely impervious to this effect because there is no exposed metal on the electrode. This fact accounts for their more universal application.

C. Liquid/Polymer Membranes

The liquid/polymer electrode membranes in wide use today (particularly in biomedical/clinical chemistry instrumentation) find their origin in the ion transport phenomena of biological cells. Agents present in the lipophilic cell walls can transport particular ions between the aqueous solutions inside and outside the cell.[32] From the discussion in Section I, it can be seen that such separation of ions would give rise to an electrical potential, and this is in fact the principle behind liquid/polymer membrane electrodes. In such devices, an appropriate lipophilic ionophore or ion exchanger is dissolved in an organic phase and placed between two aqueous phases, the sample solution and an internal reference solution. The membrane potential that develops is indicative of the activity of analyte ion in the sample.

In early designs, the organic phase of such a membrane was simply a liquid organic solvent, situated between the two aqueous phases either in bulk[33] or with the support of a thin, porous cellulose sheet, sintered glass,[34] or the like. Membranes of this design were reported as being selective toward calcium[35] and potassium[36] ions. As work with these membranes proceeded, it was found that more durable polymer supports, most often poly(vinyl chloride) (PVC) or silicone rubber, served as satisfactory replacements for the delicate cellulose. Owing to its ease of construction, stability in use, and similarity in response to the true "liquid" membranes, this configuration is now used almost universally for fabrication of this type of electrode.[37] In practice, the ion transfer agent is dissolved in tetrahydrofuran (THF) along with the PVC and a suitable plasticizer; this mixture can then be applied to a variety of supports (typically flat or tubular) from which the THF is left to evaporate, leaving a plasticized PVC ion-selective membrane. Because the plasticized polymer behaves like a viscous liquid (the ion carriers exhibit diffusion coefficients of 10^{-8} to 10^{-6} cm^2s^{-1}), the properties of the electrode are very similar to those of the original wet membranes.[38] They thus are called "liquid/polymer" or "solvent/polymeric" membranes.

In recent years, there have been considerable efforts to develop alternate polymer matrices for construction of solvent/polymeric membrane electrodes. These efforts have focused primarily on enhancing the rather poor adhesion of ion-selective polymer membranes to the silica substrates of solid-state ion sensors (see Section IV.B). Equal interest has been aimed at producing functionalized polymer matrices that may enable the covalent attachment of enzymes, antibodies, and other biomolecules with the goal of producing new biosensors and/or improving the biocompatibility of these devices. The introduction of more hydrophilic functionalities in the PVC matrix (e.g., that of hydroxyl substituents) has resulted in a substantial reduction in the asymmetry potential commonly associated with PVC

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membranes when in contact with protein-containing solutions.[39] The same PVC-OH matrix, used in conjuction with polyurethane, as well as certain room-temperature vulcanized silicone rubbers have exhibited improved adhesion to silicon nitride and less overall nonspecific protein adsorption than the PVC or hydroxylated PVC membranes.[40] Aminated-PVC (NH_2-PVC) also has offered attractive advantages. While this membrane in itself (without the addition of ion-selective species) displays significant potentiometric pH response, most exciting is the possibility of immobilizing bioreagents on the surface of ion sensors prepared with this polymeric material by direct attachment via amide bonds to the free amino groups on the surface.[41]

The ion-selective species used within these various polymer membranes fall into three main classes — the *ion exchangers, neutral carriers,* and *charged carriers*. These three types are discussed next.

1. Ion Exchangers

A lipophilic cation such as tricaprylmethylammonium ("Aliquat 336") dissolved in an organic membrane provides an attractive counterion site for anions in the adjacent aqueous phase. Similarly, an organic anion such as tetraphenylborate serves as a suitable membrane counterion for cations. By effectively lowering the chemical potential of these respective analyte ions in the membrane, these ionic agents induce a partitioning of sample ions across the sample/membrane interface, generating the usual phase-boundary potential that can be related to the activity of the aqueous sample ion. These types of membranes have been used to prepare electrodes for a wide variety of species. Their primary advantage of wide applicability also is their primary disadvantage: the ion exchanger exerts roughly the same attractive force on all the various counterions present in the sample. The selectivity of such a membrane is thus dictated solely by the ability of these various ions to partition from the aqueous to the organic phase. Therefore, the specific plasticizer used in such a membrane also affects the final membrane selectivity to a limited degree.[42] As would be expected, an ion exchanger-based membrane generally exhibits the greatest response to organic ions, followed by inorganic ions in increasing order of their hydration energies. For anions, this selectivity pattern is known as the Hofmeister[43] series, which follows the general sequence:

$$ClO_4^- > SCN^- > I^- > NO_3^- > Br^- > Cl^- > HCO_3^- > F^-$$

An ion exchanger-type anion electrode based on Aliquat 336 will characteristically display higher selectivity for the lipophilic anions (perchlorate, thiocyanate) over the strongly hydrated anions (such as fluoride).[44] Mechanistic and other background information on anion exchanger membranes have been presented.[45,46] A similar effect is seen for the cation electrodes, (often proposed with tetraphenylborate as the cation exchanger) which are most selective for organic cations such as long-chain quaternary ammonium ions. These cation electrodes have been used frequently as sensors for clinically important molecules that exist as cations at low pH (e.g., drugs[47,48]); it should be realized, however, that these electrodes are likely to respond similarly to all such species in the sample, and a prior separation step is usually required to gain true selectivity and accuracy in such an analysis.

2. Neutral Carriers

There exists a wide range of neutral ion-complexing agents that can be incorporated into polymeric membranes. Figure 2 illustrates the structures of some of these. These compounds have been discovered, or designed, to exhibit selective complexation of a particular ion (usually a cation) in the presence of other ions of the same charge. Valinomycin and nonactin, for example, are complexing agents that show particular affinity for potassium and ammonium cations, respectively. An arsenal of neutral ionophores have been developed for use in membranes selective for common cations (e.g., Li^+, Na^+, Ca^{2+}, Mg^{2+}, and H^+).[49–54]

The methods by which such ionophores achieve their selectivities vary. Crown ethers and other macrocyclic compounds, such as valinomycin and nonactin, are believed to discriminate among cations on the basis of size: cations that fit well in the complexing site will be most strongly complexed.[55,56] Long-chain amines serve well as H^+ sensors due to their propensity to protonate at suitably low pH;

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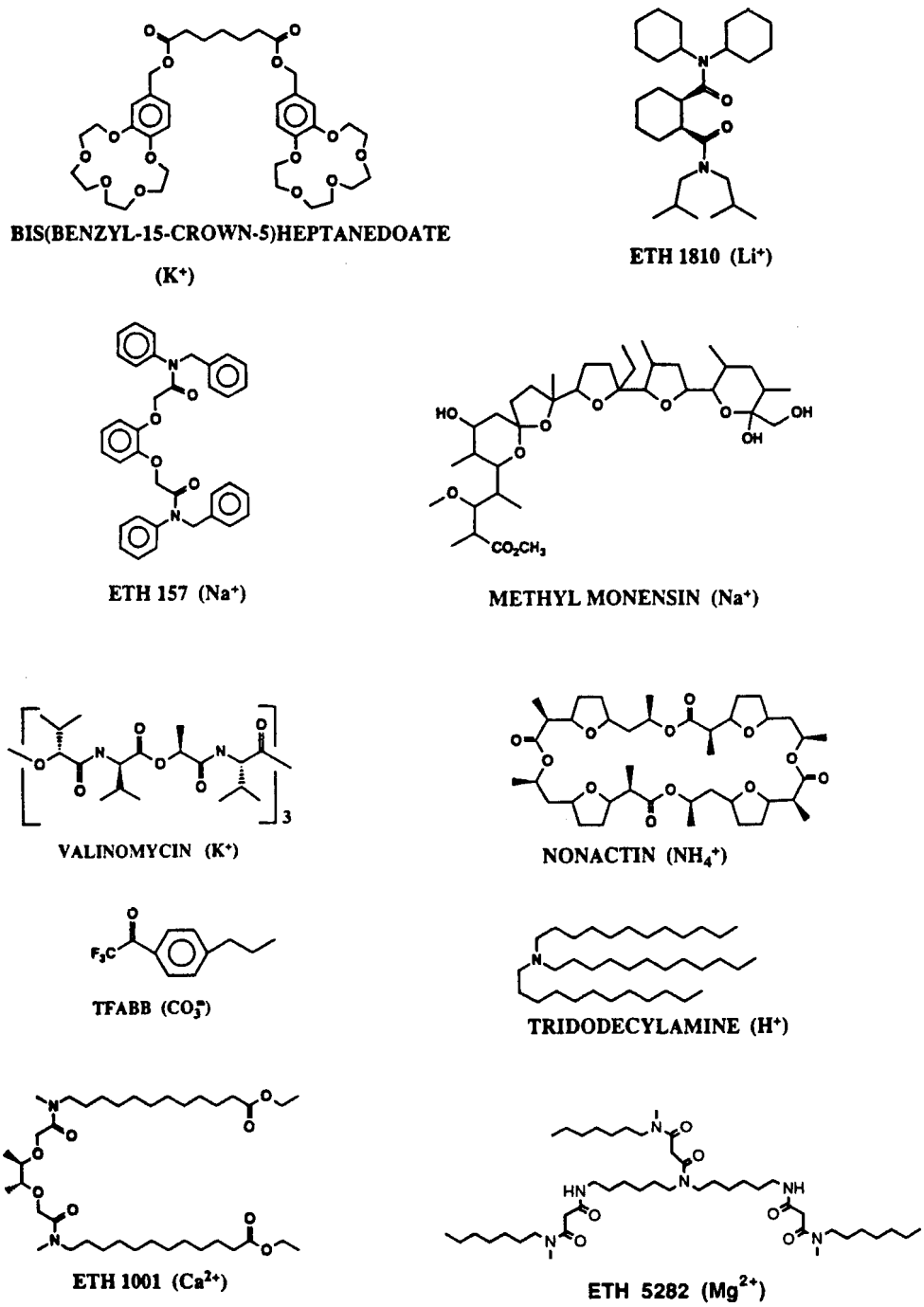

FIGURE 2. Structures of several neutral-carrier ionophores used to fabricate liquid or polymer membrane ISEs.

their attraction to other cations is minimal.[57] Neutral carriers are most commonly applied to the sensing of cations, with some notable exceptions. Molecules (trifluoroacetophenone derivatives) have been found that complex carbonate anions due to this anion's unique participation in an ion/ionophore reversible adduct reaction.[58] In addition, certain organometallic species have been used to take advantage of a metal's coordination affinity to a particular anion. Indeed, certain metalloporphyrin-doped membranes with metal(II) centers [e.g., octaethyl porphine ruthenium(II) carbonyl] exhibit anion selectivities that are significantly different from the Hofmeister pattern.[59] Organotin compounds

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also have been investigated[60–63] and appear promising as neutral-carrier ionophores for anion-selective membranes (e.g., for phosphate, chloride, etc.).

The exact mechanism by which neutral-carrier membranes operate is currently a matter of extensive research.[64–69] The investigation centers on determining the existence and role of fixed anionic sites within cation-selective membranes, the extent of diffusion within the membrane of the charged complexes, verifying the origins of the membrane's respective selectivity patterns, elucidating the role of the membrane plasticizer, and other fundamental questions.

3. Charged Carriers

A third class of ion-selective carriers consists of the so-called *charged carriers* or *associated ion exchangers*. These organic species are ionic within the membrane phase, as are the ion exchangers described above. Unlike the simple ion exchangers, however, the selectivity of charged carriers is dictated by both the degree of association of the analyte ion with the carrier as well as the partitioning of the analyte ion into the membrane solvent. The most notable examples of such ion-selective agents are the alkyl phosphates originally used to construct calcium-selective electrodes.[35,70–72] In this case, calcium selectivity is further enhanced by employing alkyl phosphonates as membrane solvents. More recently, a lipophilic derivative of vitamin B_{12} was shown to exhibit high selectivity for nitrite.[73,74] The mechanism for this selectivity has been traced to the high coordination affinity of nitrite as an axial ligand to a positively charged Co(III)-corrin complex. Similarly, the unique selectivity of some metalloporphyrin-based electrodes in which the metal center is in the +3 or +4 oxidation state [e.g., dichloro(5,10,15,20,-tetraphenylporphyrinato) tin(IV) for salicylate[75] and chloro(octaethylporphyrinato) indium(III) for Cl^- ions[76]] seems to be based on the coordination of these ions as axial ligands to their respective positively charged metal-ligand complexes.

III. GAS-SENSING PROBES

Prompted by the need for an accurate method to determine CO_2 in blood, Severinghaus and Bradley developed a new technique in 1958 for the potentiometric measurement of gases.[77] In what has become known as the *Severinghaus design* (Figure 3), a thin electrolyte film is held between a gas-permeable membrane and a glass pH electrode. The outer gas-permeable barrier is typically a thin silicon rubber or microporous Teflon® membrane. The thin electrolyte film is in contact with a bulk reservoir in which the reference electrode is placed. Because both electrodes are contained in a single unit, the complete device is termed a "sensor" rather than an electrode. When the device is placed in a sample containing dissolved CO_2, the gas diffuses across the membrane into the electrolyte layer until the partial pressure of the gas in the recipient electrolyte equals that of the sample. The acidic (in the case of CO_2, NO_x, or SO_x) or basic (in the case of NH_3) gas changes the recipient's pH. This change in pH is sensed by the glass electrode and can be related to the partial pressure of analyte gas in the sample.

For the CO_2 sensor, the pH of the recipient layer is determined by the Henderson-Hasselbach equation, derived from the chemical equilibrium between solvated carbon dioxide and its ions:

$$pH = pK_1 + \log \frac{[HCO_3^-]}{[H_2CO_3]} \qquad (14)$$

If the recipient solution contains a large background concentration of bicarbonate, the pH will be logarithmically proportional to only one variable, $[H_2CO_3]$, which is in turn dependent on the amount of CO_2 that has diffused across the gas-permeable membrane. Thus, for a CO_2 sensor,

$$E_{cell} = K_{cell} + S \log p_{CO_2} \qquad (15)$$

where S should approach 59.2 mV per decade, and p_{CO_2} is the partial pressure of CO_2 in the aqueous sample. For this reason, gas sensors of this design contain a recipient layer that is buffered in the ionic form of the analyte gas: HCO_3^- for CO_2 sensors, or NH_4^+ for NH_3 sensors. (The recipient solution may also contain a reference ion, such as Cl^-, to poise the reference electrode.) Gas sensors for NO_x and SO_x have been developed that operate on the same principle.[78] Theoretical treatment of the response times,

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selectivity, and detection limits of these devices have appeared in the literature.[79–83]

The selectivity of Severinghaus-style gas sensors is quite high. Simple inorganic ions do not permeate the outer gas membrane and thus do not interfere with gas measurements. Furthermore, concerns regarding variation in liquid junction potentials between samples and standards are eliminated because the reference half-cell is not placed directly into the sample solution (i.e., E_j is constant).

It should not be inferred from the above comments that this gas-sensing design is without interferences. The most obvious of these are the acidic or basic gases (or their ions) that coexist in the sample with the analyte. A CO_2 sensor of the Severinghaus design is likely to display sensitivity to nitrite, sulfite, and other ions that form gases in acidic solution, permeate the membrane, and decrease the pH of the recipient solution. Similarly, volatile amines are interferents in ammonia-gas-sensor analyses.[84,85] In addition to gaseous interferents, neutral lipophilic species in the sample also can migrate through a silicone rubber membrane, reaching the recipient layer; if these are acidic (e.g., salicylic or benzoic acids), they will dissociate to produce an interfering pH change.[86]

A newer gas-sensing design eliminates these selectivity problems. When the glass pH membrane

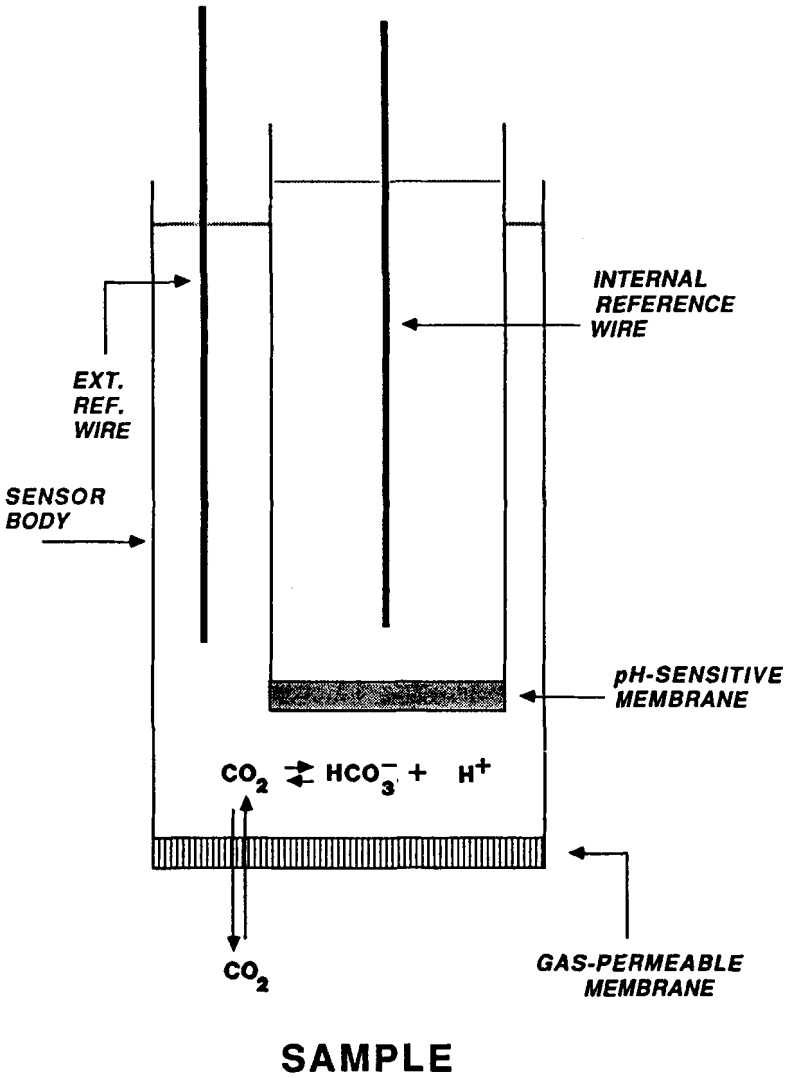

FIGURE 3. Schematic diagram of a Severinghaus-type gas sensor. In actual gas sensors, the glass membrane is pressed tightly against the gas-permeable membrane; the gas is received in a very thin film of solution between the two membranes, changing the film's pH.

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is replaced with a polymer membrane responsive to the ionic form of the analyte gas, a more specific sensor results. For example, a NH_3 gas sensor can incorporate a nonactin-based ammonium-responsive polymeric membrane (Figure 4).[87] The signal from this sensor is not dependent on the pH of the recipient film (in fact, the film is pH buffered), but rather on the equilibrium concentration of ammonium acquired from the sample. The signal from the sensor is not affected by the presence of volatile amines as the nonactin membrane exhibits only slight response to the protonated forms of these species.[88] Similarly, NO_x sensors that use an inner nitrate- or nitrite-responsive polymer membrane ISE will be impervious, to a large extent, to other acidic gases in the sample.[89,90]

As mentioned, the pH of the recipient film in these new sensors is fixed. This provides a buffer-trap effect; that is, at equilibrium with a solution of given ammonia content, a much higher level of ammonium is reached in a pH-buffered recipient than in one whose pH is allowed to rise as ammonia is collected. Therefore, these new gas-sensing designs are more sensitive as well as more selective than the original Severinghaus-style devices.[91]

A further advance in this gas-sensing configuration is the development of flowing recipient systems.[92-94] Here, as shown in Figure 5, the gas permeation step and the ion-sensing step are separated. After migration across the gas-permeable membrane, the analyte gas dissolves in the recipient electrolyte that continuously flows through an appropriate ISE detector (and reference). The ISE may be responsive either to pH or to some other ion, as explained above. While a static gas sensor may take several minutes to equilibrate, this flowing system allows more rapid analyses because (1) complete equilibration is not actually necessary for quantitation and (2) exposed recipient is continuously being replaced. This latter feature greatly enhances the recovery times of the gas sensors, especially the transition from a high-gas to a low-gas measurement.

This flow-through gas-sensing arrangement has been employed for the automated analysis of a variety of samples.[95,96] As with the static sensor, selective measurements may be made even in complex matrices. The idea has been expanded recently to the continuous monitoring of atmospheric gases.[97,98] The principle of the sensor is the same described above, but rather than accepting the analyte gas from a liquid sample, a continuously flowing recipient solution is exposed directly to the gaseous atmospheric sample. Careful design of the recipient solution and gas sampling apparatus are required to obtain the necessary detection limits, while the system's selectivity, as usual, depends in large measure on the choice of the indicator ISE.

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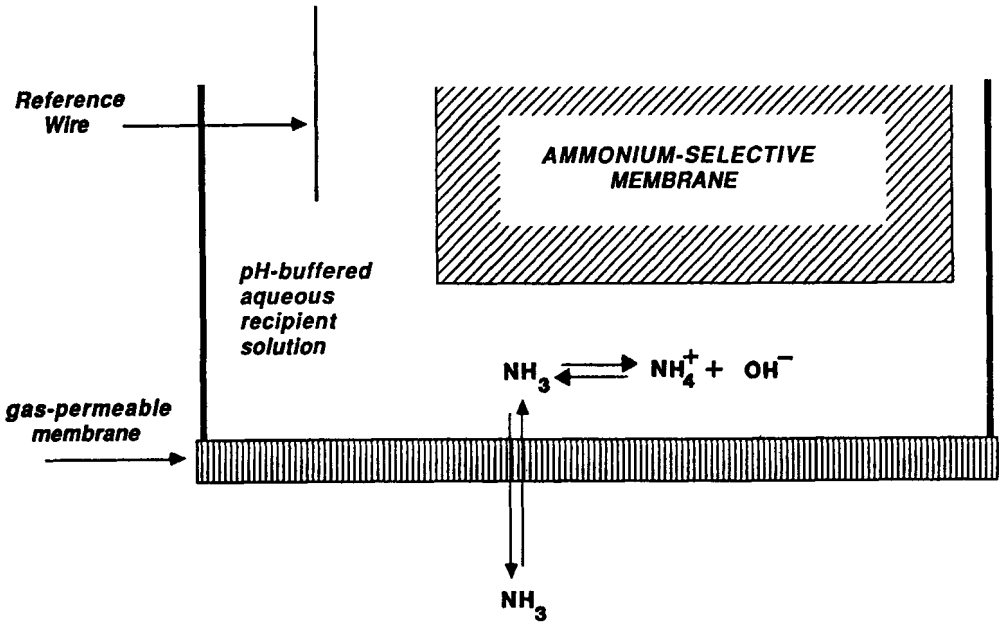

FIGURE 4. Expanded view of sensing region of improved ammonia gas sensor, which detects an ionic form (NH_4^+) of the analyte gas (NH_3) rather than a relatively nonspecific change in pH.

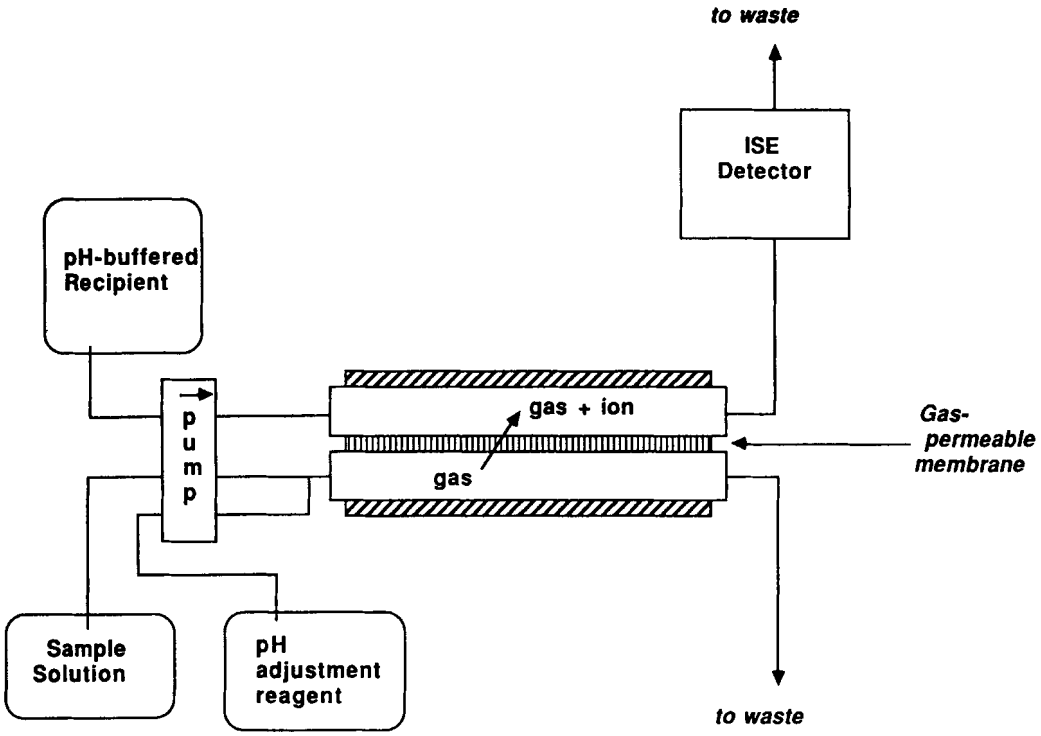

FIGURE 5. Schematic diagram of flowing gas sensor configuration. Analyte gas is generated in the sample stream at appropiate pH, passes through the gas-permeable membrane, and is collected in a pH-buffered recipient solution for quantitation by the downstream ISE detector.

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A relatively fresh approach to the design of potentiometric gas sensors involves the use of two polymer membrane ISEs in a differential measurement mode. The following galvanic cell may be used to detect dissolved carbon dioxide:[99,100]

Ag/AgCl wire in pH buffer cont'g chloride	pH-sens. polymer membrane	sample solution	pH-sens. polymer membrane	Ag/AgCl wire in $NaHCO_3$/NaCl solution

If both polymeric membranes are identical in their response to the activity of the hydrogen ions, the response of the galvanic cell is independent of sample pH. However, because CO_2 in the sample can diffuse through the proton-selective polymeric membranes, in one half-cell (that with $NaHCO_3$ as the internal solution), such diffusion results in a pH change at the inner surface of the ion-selective membrane, thereby changing the membrane potential. While gas diffusion also occurs into the second half-cell, a strongly buffered internal solution within this half-cell does not allow the diffusing CO_2 to alter the pH. Consequently, the potential difference between the two ion-selective electrodes is related only to the partial pressure of CO_2 in the sample.

An additional differential gas-sensing scheme has been introduced recently in an effort to enhance potentiometric gas sensitivity. This sensor design is illustrated in Figure 6. In a static configuration, this design employs two half-cell probes connected via a salt bridge. One half-cell (1) responds to the basic or acidic analyte gas by detecting an increase or decrease in the pH of a thin film of electrolyte (e.g., NH_4Cl for an NH_3 gas sensor) sandwiched between a pH-sensitive polymeric membrane (prepared with tridodecylamine as membrane-active species) and an outer gas-permeable membrane. The second half-cell (2) detects the analyte gas in the sample by responding to changes in the activities of the conjugate base anion or acid cation (e.g., NH_4^+ for an NH_3 gas sensor) of the analyte gas in a thin layer of buffer sandwiched between an ion-selective membrane and another outer gas-permeable film. This allows each cell, in principle, to respond similarly, but in different potential directions. Gas ammonia response slopes observed with this unique configuration for both static and flow-through configurations approach the 118 mV per decade predicted by theory. The analytical utility of this sensing

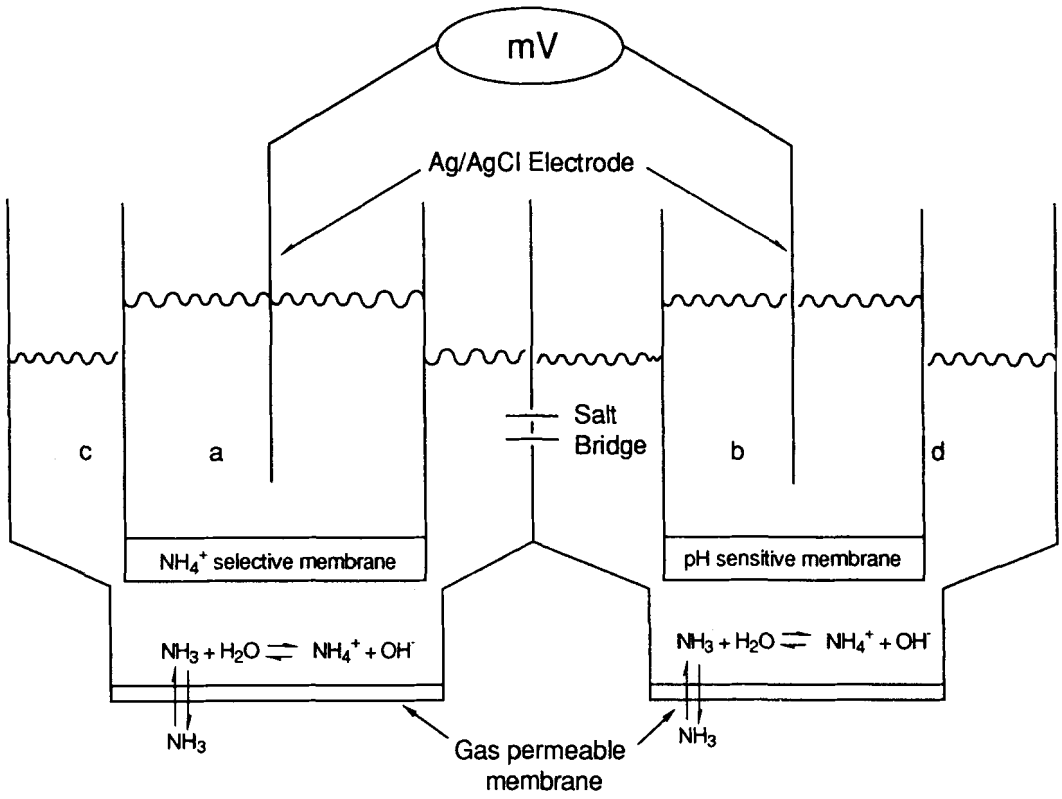

FIGURE 6. Schematic diagram of differential ammonia gas sensor fabricated with two different ion-selective membranes: (a) NH_4Cl + NaCl solution; (b) buffer + NaCl solution; (c) buffered internal solution; (d) pH-sensing internal electrolyte solution.

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arrangement has been assessed in a flow-through mode by determining accurately the ammonia-N concentrations in biorector media.[101] In principle, this differential approach could be extended to devise new, more sensitive probes for other gases, including CO_2, NO_2, and SO_2.

IV. MINIATURIZED ISEs

A. Coated Wire Electrodes

As explained earlier, the most common potentiometric cell arrangement includes two reference electrodes (internal and external) immersed in their respective solutions and separated by an ion-selective membrane. The importance of *poising* the electrode surfaces was also mentioned. A class of specially designed ISEs exists that deviates from this design. Ion-selective polymer membranes have been coated directly onto conductive surfaces, such as metal wires, for the purpose of making very small, durable solid-state electrodes. The *internal* filling solution of the conventional designs is missing from this type of electrode: the internal reference wire contacts the ISE membrane directly. This approach facilitates the construction of miniature electrodes 0.5 to 2 mm in diameter.[102]

The outer surface of such a coated wire electrode (CWE) behaves similarly to membranes we have already described. Depending on the ion-selective agents present within the membrane, the outer boundary of the membrane developes a phase-boundary potential according to the ion activity a_i present in the sample. Electrodes can be made sensitive to ions for which there exist the usual ion-selective liquid/polymer membranes. The slope and selectivity characteristics of these membranes are usually similar to those exhibited by conventional liquid/polymer membrane-based electrode designs, and they have found use in the determination of many species.[103] However, the phase boundary potential of the inner surface is often poorly defined. In most cases, it is the presence of some redox

couple in the membrane, introduced either intentionally (e.g., ferrocene/ferricene) or incidentally, that defines the potential at the membrane/wire interface.[104] Examples of the latter include oxygen diffusing through the membrane from the sample, or simply redox impurities in the membrane materials. While measurements can be made with such CWE devices, the analyst can expect a certain amount of *drift* in the electrode potential from one set of analyses to the next, as conditions at the membrane/wire interface change. Thus, successful use of CWEs requires frequent calibrations. A variant of this configuration has been reported in which a thin polyvinyl alcohol (PVA) gel of internal KCl electrolyte is immobilized between an Ag/AgCl wire and a polymer membrane responsive to potassium ions.[105] The internal wire is thus poised and more stable readings can be achieved. Drift will occur, however, if water vapor diffuses preferentially into or out of the internal gel. This situation occurs upon changes in the osmolarity of the sample; it results not only in a change in the activity of chloride ions within the internal solution, but a physical swelling or shrinking of this layer as well.

B. ISFETs

An ISE design of major interest places an ion-selective polymer membrane in contact with the gate region of a solid-state field-effect transistor.[106–108] These devices are sometimes known as ISFETs or CHEMFETs. They operate on the principle that the outer membrane phase-boundary potential affects the voltage experienced by the FET gate; when the FET is in a current-measuring circuit, the resulting current should be indicative of the membrane potential and, ultimately, the activity of the analyte ion in the sample. The design has been used, for example, in the development of a variety of solid-state sensors (Na^+, K^+, Ca^{2+}, etc.).[109,110] These devices can boast of being very small, low-noise, self-contained, and rugged probes. The device comprises a large segment of the ordinary ISE measuring circuit, with the membrane, reference electrode, and connecting wires all being replaced within one tiny microelectronic chip. In principle, several membranes may be incorporated into one unit for multicomponent sensing.[111,112]

Drawbacks do exist, however. Most prominent among these is the same problem mentioned above in the context of the coated wire ISEs: because there may be no well-defined potential at the gate/membrane interface, these sensors are suceptible to drift caused by various species diffusing through the membrane from the sample. As the coated wire electrodes mentioned above are sensitive to redox couples at the metal surface, the surface of the FET gate (usually silicon oxide or silicon nitride) is sensitive to pH changes at the interface. Therefore, CO_2 and organic acids present in the sample can diffuse through the polymer membrane and contact the gate, altering the phase-boundary potential at the membrane/gate interface.[113,114] In addition, there are engineering difficulties in encapsulating the device such that the aqueous sample does not "short" it out. Attempts to alleviate some of these problems by altering the polymer matrix have already been mentioned in Section II.C. Other approaches have included the covalent attachment of a hydrogel [poly(hydroxyethyl) methacrylate] to the silicon oxide gate of the ISFET.[115] This hydrogel layer is subsequently swollen in a buffered electrolyte and the sensing membrane then deposited. This design seems to effectively remove interferences from acidic species (i.e., CO_2) and stabilizes the output voltage of the miniature device.

C. ISE Catheters

The simplest yet most reliable approach for preparing highly stable miniaturized ion-selective sensors is to use a configuration in which a defined volume of internal electrolyte exists behind the ion-selective membrane. This has been the strategy taken to fabricate a host of ISEs suitable for continuous *in vivo* ion measurements. Figure 7 illustrates one design introduced by Band and co-workers.[116,117] A narrow diameter, dual lumen PVC tube is plugged at the end with a porous ceramic frit. This plugged end is dipped into a polymer membrane casting solution containing the appropriate carrier. Once dried, the porous plug serves as a physical support for the ion-selective membrane. This reduces physical pulsation of the membrane upon implantation. An appropriate internal reference solution is placed within

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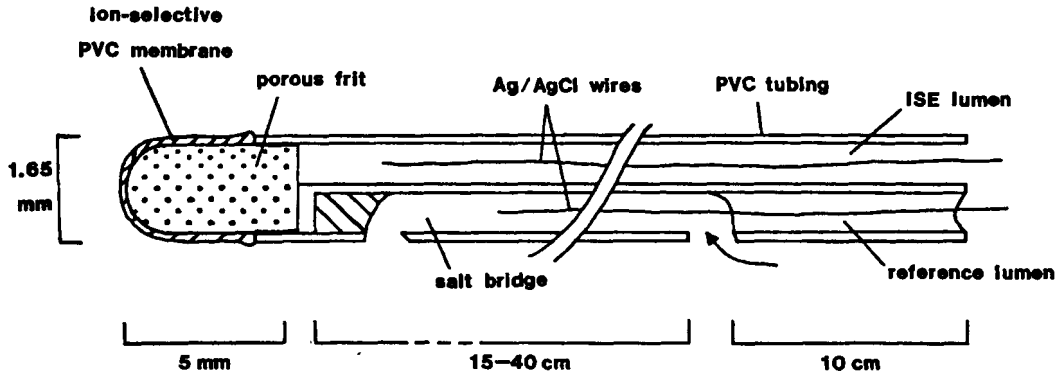

FIGURE 7. ISE catheter of Band and Treasure. (From Band, D.M.; Treasure, T. *J. Physiol.* **1977**, *266*, 12P. With permission.)

the ISE lumen. As shown, external reference electrode contact to the animal's blood is achieved by using the second lumen of the catheter as a salt bridge.

A more recent, simplified design involves the direct impregnation of the walls of narrow bore silicone or PVC tubing with the ion-selective agent.[118] This is accomplished by swelling the polymer in a suitable solvent (e.g., xylene, freon, etc.) that contains the ionophore. When removed from this solution, the solvent evaporates, leaving the walls of the tubing doped with the ionophore. Once the end of the tubing is plugged and the tube is filled with internal reference solution, the EMF developed across the wall of the tubing is proportional to the activity of analyte ion in the sample. Single-lumen catheters of this type can be readily fabricated with diameters in the range of 0.5 to 1.0 mm, and almost any of the ionophores shown in Figure 2 may be used to prepare *in vivo* sensors with varying ion selectivities.

A further attractive ISE catheter design that allows simultaneous measurements of *in vivo* ion/p_{CO_2} status is depicted in Figure 8. The sensor consists of an internal tubular polymer pH electrode that is housed inside silicone rubber tubing previously impregnated with an ion complexing agent. Ion sensing is performed by measuring the potential generated across the outer silicone-responsive membrane with respect to an external reference electrode situated in the side arm of an intravenous catheter placement unit. The CO_2 measuring scheme is developed in a manner analogous to the Severinghaus principle mentioned earlier (see Section III). Carbon dioxide diffuses through the silicone rubber tubing, changing the pH of bicarbonate electrolyte solution contained between the walls of inner and outer tubings. This pH change is monitored by the inner pH membrane electrode. Perturbation of the pH response by CO_2 diffusing through the wall of the inner tubing is prevented by a strongly buffered internal solution. Measurements with this particular arrangement was demonstrated to correlated well with discrete blood gas-electrolyte analyzer values during *in vivo* monitoring of K^+/p_{CO_2} in anesthetized, systemically heparinized dogs.[119,120] In the absence of heparin treatment, deviations from this correlation have been encountered and largely attributed to biocatalytic activity produced by thrombus formation on the surface of the sensor.[120]

Alternatively, dual-lumen silicone rubber tubing has proven useful to further simplify and miniaturize dual pH/p_{CO_2} sensors. Here, a segment of tubing (typically 1.0 mm o.d.) is impregnated with the hydrogen ionophore tridodecylamine that renders all inner and outer walls of the tubing H^+ responsive. When one lumen is filled with a strong buffer and the other with a bicarbonate/NaCl solution, the electrochemical potential between the two lumens (measured via the use of two Ag/AgCl reference wires) is proportional to the logarithm of the CO_2 partial pressure in the solution bathing the catheter. Simultaneous monitoring of sample pH and p_{CO_2} can be acomplished by contacting the sample with an external reference electrode and measuring the difference in potential between this external reference electrode and the lumen containing the strong buffer.

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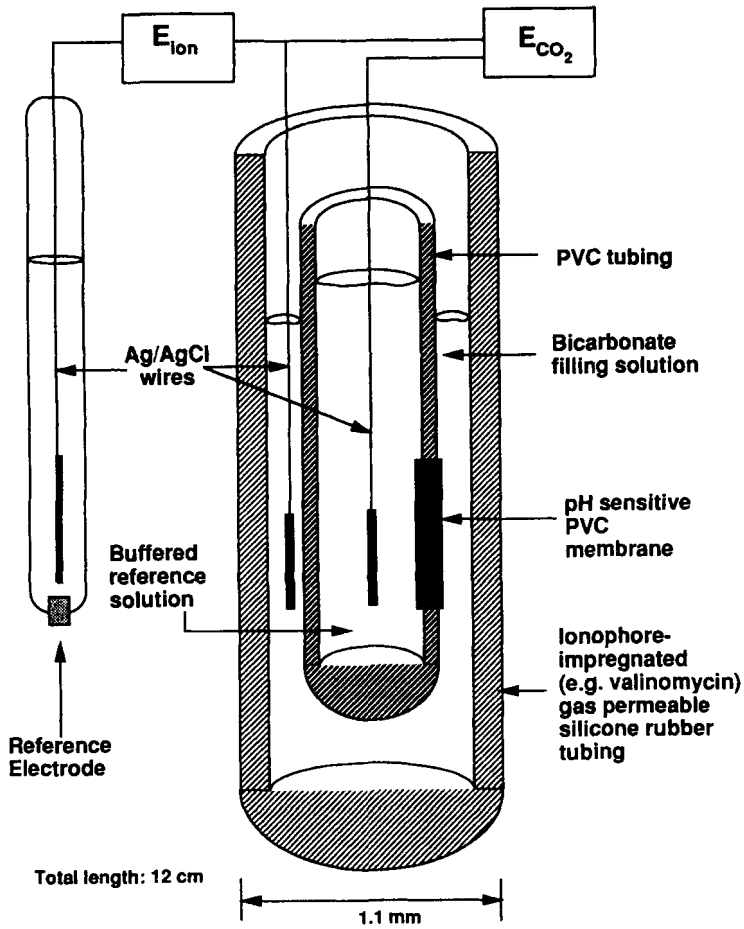

FIGURE 8. Schematic diagram of a dual potassium/carbon dioxide sensing catheter.

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V. BIOSENSORS

Our discussion up to this point has centered on the detection of charged species or gases that exist as ions when dissolved in water. While it is true that some charge separation must ultimately occur in a potentiometric measurement, membrane electrodes also can be prepared for the measurement of a wide range of uncharged, complex molecules of biological interest.[121]

A. Biocatalytic Electrodes

The gas-sensing configuration described in Section III forms a very useful basic unit for potentiometric measurements of biologically important species. An enzyme is immobilized at or near the gas probe, held in place either by covalent immobilization or physical entrapment (Figure 9). The gas sensor measures the amount of characteristic gas produced by the reaction of analyte molecule with the enzyme (in the presence of an appropriate substrate, if necessary, added to the sample). Reviews of this work have been provided by Kobos,[122] Gullbault,[123] and Rechnitz.[124]

For example, a biocatalytic urea sensor can be constructed by the immobilization of urease onto the gas-permeable surface of an ammonia gas sensor (see Figure 9). When the probe is inserted into a sample containing urea, the enzyme catalyzes its conversion to ammonia:

$$\text{Urea} + H_2O \rightleftarrows 2NH_3 + CO_2$$

The generated ammonia diffuses through the gas-permeable membrane and is sensed by the probe's internal ISE as described in the previous section. A steady-state signal is reached within minutes, as the rate of ammonia diffusion away from the sensor equals the rate of its generation from urea. Probes of this type prepared with various internal ISEs have been developed to measure creatinine,

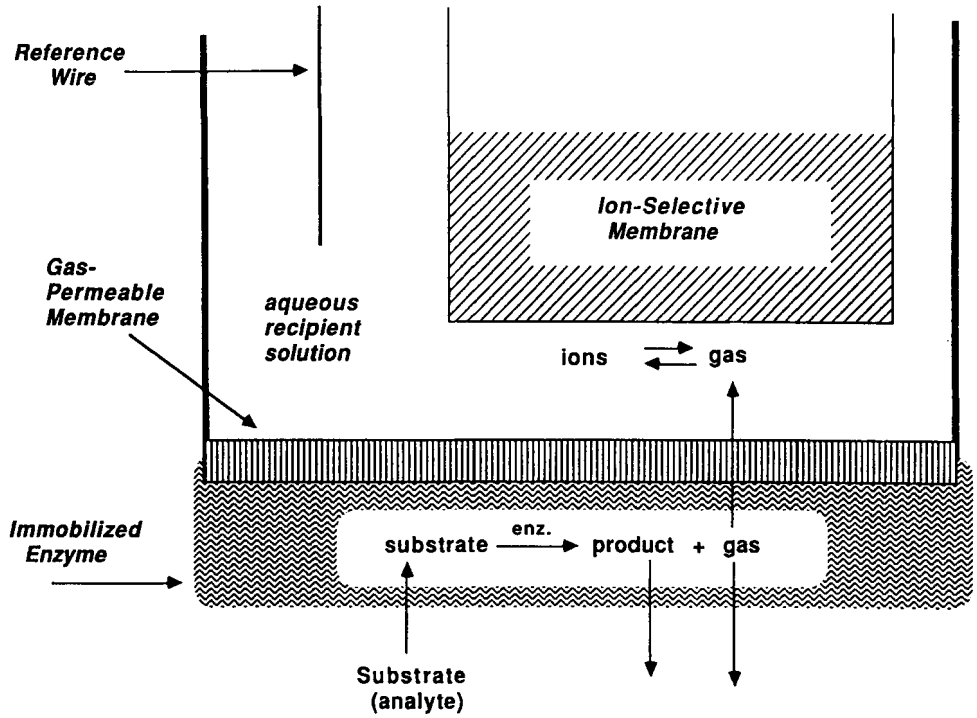

FIGURE 9. Sensing region of enzyme probe. Analyte diffuses from sample solution into the immobilized enzyme layer and reacts to generate gas, which is detected by the gas sensor.

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glutamine, histidine, phenylalanine, amygdalin, adenosine, adenosine monophosphate, tyrosine, acetylcholine, and other biological molecules. Sensor selectivity is dictated by the innate selectivity of the inner ISE or gas detector, as well as the specificity of the biocatalyst immobilized at the surface of the probe.

Pure, stable enzymes are not available for all molecules of interest. However, the scope of this technique can be expanded by the use of *in situ* enzymes: in this scheme, intact cells[125–127] or tissue sections[128,129] are immobilized on the surface of the probe. Several advantages exist in this approach:

1. Low cost (enzyme extraction and purification are unnecessary).
2. *In situ* enzymes tend to exhibit greater activity and any necessary co-factors are naturally present.
3. The enhanced stability of *in situ* enzymes extends the lifetime of these probes.
4. *In situ* multistep pathways may be exploited for the performance of analyses that require such sequences.

Response times of these bacterial and tissue electrodes can be long (up to 10 min) due to the complex transport phenomena inherent in their operation. Nevertheless, their stability makes them attractive for clinical analysis, and probes of this type have been reported for many important species, including glutamine, arginine, tyrosine, adenosine, glutamate, nitrilotriacetic acid, nitrate, guanine, and serine. Development of these probes is hindered by the fact that, in a complex matrix such as a tissue section, there are likely to exist many different bioactive species. The selectivity of such a device can thus be less than optimal.[130] One exception to this problem is the glutamine sensor of Arnold and Rechnitz, which uses immobilized porcine kidney cells with an ammonia gas sensor; this electrode system has been used to determine glutamine directly in cerebral spinal fluid.[131]

The selectivity problems encountered with both enzyme and cell-based biosensors are not limited to the intrinsic selectivity of the immobilized biocatalytic reaction. Indeed, a major analytical interference also can come from the inherent response of the basic ion or gas transducer to endogenous gas and ion species present in the sample. A particularly useful approach to overcome these interfering errors has been the inclusion of ion-exchange membranes allowing permeation of analyte

substrates while restricting the passage of interfering species. This concept has been demonstrated in an FIA system for the potentiometric measurements of urea in blood serum and L-glutamine in hybridoma reactor media.[132] Similarly, a somewhat analogous FIA scheme utilizing an in-line tubular ion exchanger arrangement (i.e., Nafion tubing) has been devised to pretreat samples before measurement of the substrates via a flow-through enzyme reactor/electrode detection system.[133] Additional enzyme layers that can degrade endogenous interferent species (e.g., ammonia) before they can reach the underlying biocatalytic electrode have also proven useful to enhance the practical analtyical utility of certain enzyme electrodes.[134]

B. Immunosensors

Beyond the use of biocatalytic reagents, it is also possible to couple the high specificity of immunochemical reactions to membrane electrode detection systems for the design of new bioanalytical probes. Over the past decade, considerable effort has been launched in this direction. While varying approaches have been taken, none have yet led to the development of self-contained, direct probes that respond in a selective and truly reversible manner to antigens or antibodies in sample solutions. Indeed, in many instances, membrane electrodes have been used as alternate detectors to perform indirect immunotests that could otherwise be monitored by classic spectrophotometric methods. For example, several workers have employed various ion- and gas-selective electrodes to monitor the enzymatic activities associated with both modern homogeneous[135] and heterogeneous[136,137] enzyme-linked competitive binding methodologies. Others have utilized ion exchanger-based polymeric membrane electrodes to monitor organic ions released from ion-loaded vesicles used in conjunction with immunocomplement fixation-type tests.[138,139] For these types of systems, the major advantage offered by the use of electrodes is the fact that samples need not be diluted to as large an extent (to remove spectral and turbidity interferences) and, thus, real sample detection capabilities can be improved.

Direct sensing immunoelectrodes have also been suggested. Janata et al.[140] devised a CHEMFET-type immunosensor by immobilizing antibodies or protein antigens in polymeric films cast on the gates of metal oxide field effect transistors (MOSFETs). Selective potentiometric response to corresponding antigens or antibodies in the sample solution was achieved. This response was originally attributed to a change in the surface charge at the membrane-covered gate resulting from the antigen/antibody binding reaction. In subsequent studies,[141,142] it was demonstrated that the observed potentiometric response was not due to the actual charge on the antibody or antigen, but rather on changes in the ability of small ions (e.g., Na^+, K^+, etc.) present in the test solution to partition into the organic membrane phase (see Section II.C). Solsky and Rechnitz[143] improved on this general concept by coupling low molecular weight antigens (haptens) to neutral carrier-type crown compounds selective for K^+, and then impregnating these carrier/hapten conjugates into PVC membrane electrodes. This approach is schematically illustrated in Figure 10. In the presence of anti-hapten antibodies, changes in membrane potential are observed presumably because the conjugate's ability to complex K^+ and/or Na^+ is altered by the antibody binding process (to change the phase-boundary potential). This concept is termed "potentiometric ionophore-mediated immunoassay" (PIMIA), and it has now been demonstrated for several antibody/hapten systems, including dinitrophenol,[144] prostaglandins,[145] and digoxin.[146] Additional studies have shown that such antibody-selective electrodes also can be used to detect the concentration of hapten in the test solution by a competitive binding principle: that is, the more hapten present, the fewer antibodies will bind to the carrier/hapten conjugate in the membrane, and the smaller the potentiometric response observed.

It should be noted that the PIMIA concept is not truly a direct sensing scheme. Indeed, the activities of the indicator ions (K^+, Na^+) must be constant in the test solution in order to obtain reliable analytical results. Thus, ultimate implementation of this approach will require dilution of unknown samples with an indicator ion reagent. In addition, reversibility of the antibody binding reaction is slow. Therefore, to reuse these sensors, the antibody must be washed off the membrane with a solution that promotes dissociation of the antibody-antigen reaction (e.g., buffer at low pH). Such reversibility problems may ultimately be overcome by utilizing modern monoclonal antibodies with preselected thermodynamic and kinetic properties. Moreover, if such selected antibodies can be maintained at the surface of the

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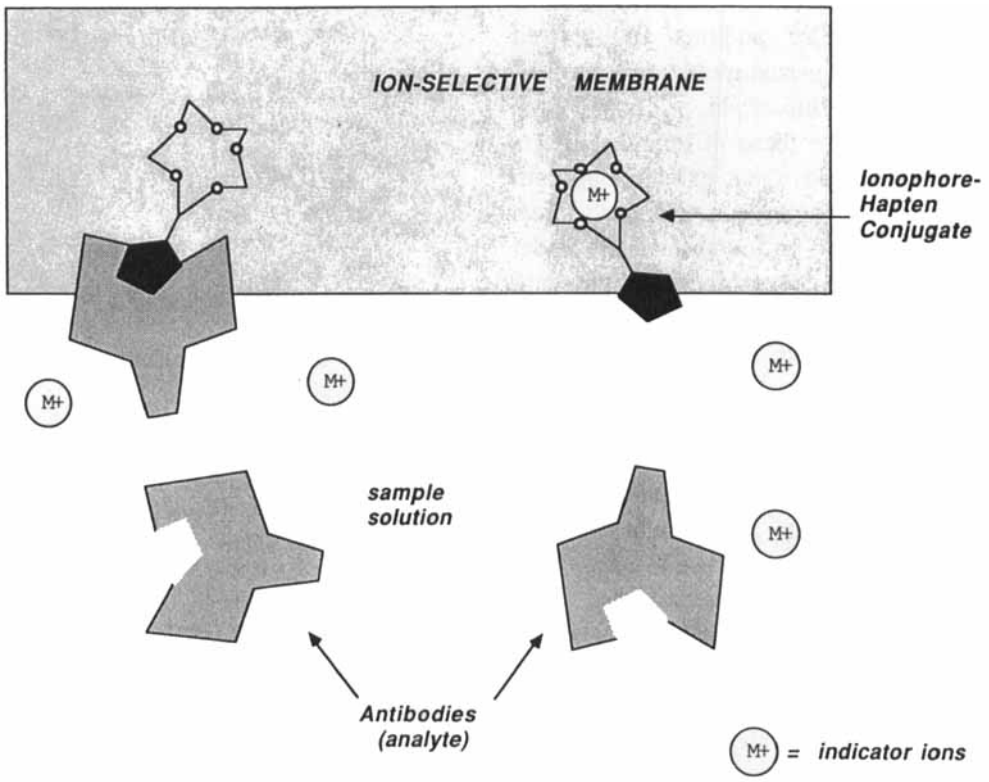

FIGURE 10. PIMIA (potentiometric ionophore-mediated immunoassay) scheme: antibodies in solution bind the hapten-ionophore conjugates in the membrane, changing the ionophore's ability to complex M^+. The electrode's signal for M^+ is thus dependent on the concentration of antibody in the sample.

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ISEs via a semipermeable membrane, the entire arrangement may be useful for continuous monitoring applications. This concept was demonstrated by Bush and Rechnitz, who designed a continuous sensor for 2,4-dinitrophenol.[147]

VI. FUTURE PROSPECTS

If advances over the past 3 decades are any indication, in the coming years the development of new membrane-based potentiometric devices will continue to play an important role in reducing the complexity and cost associated with direct chemical sensing. Research over the next decade will likely focus on filling voids in existing membrane electrode technology. For example, while we now have highly selective liquid/polymer membrane-type sensors for important cations, there are relatively few analogous electrodes suitable for direct determination of selected anions, particularly biomedically important bicarbonate and environmentally important sulfate and phosphate. Clearly, ionophore or charged carrier structures must exist, or could be designed using modern molecular modeling, which selectively bind these anions. If so, such species could be isolated or synthesized for use in the development of anion-selective membranes. Similarly, more attention should be directed toward discovering solid-state materials that act as ion exchangers/conductors, in much the same manner that glass membranes respond so rapidly and selectively to hydrogen ions.

Another area likely to receive considerable attention will be that of miniaturized ion-, gas-, and bio-selective sensors. Small probes containing multiple sensing sites would be extremely useful as detectors for the simultaneous measurement of several species (e.g., blood electrolytes), and the ability to mass produce such multiple sensors on small silicon chips will certainly remain an active avenue of research and development. Such chips could be employed as disposable single-use devices or as multiparameter reusable detectors in modern FIA systems. One biomedical company (I-Stat) has in fact recently introduced a silicon chip-based disposable potentiometric device for measurement of blood

electrolytes (Na^+, K^+, Ca^{2+}, and urea) in undiluted whole blood.[148] Miniaturized multiparameter sensors for *in vivo* monitoring of pH, p_{CO_2}, and electrolytes also will be the focus of renewed efforts aimed at devising sensors that exhibit long-term calibration stability. At the same time, chemical approaches will need to be found that will render such in-dwelling probes more biocompatible without interfering with the membrane chemistry that is required to make accurate electrochemical measurements.

Advances in these and other areas, particularly the development of useful potentiometric biosensors, will require truly interdisciplinary research efforts. For example, biochemists and protein chemists studying the mechanism of anion transport across cell membranes have isolated proteins that selectively bind these anions. Once the structures of the binding sites of these proteins are fully elucidated, synthetic chemists could then attempt to synthesize smaller model compounds with similar binding properties for use in the design of new anion sensors. In an analogous fashion, significant advances in the design of immunosensors will be realized only when approaches are devised to enhance the reversibility of the antibody-antigen interactions. Judicious use of modern monoclonal antibody technology may provide one means of solving this reversibility problem by enabling the preselection of antibody molecules that have the required affinity and kinetic properties. Biocompatibility of implantable membrane sensors may be improved with innovative coatings (including anti-clot enzymes) or new, more blood-compatible polymer matrices. Thus, the advent of many new and exciting membrane electrodes appears imminent, awaiting the efforts of analytical chemists, electrochemists, immunologists, biochemists, material scientists, and other imaginative parties working in concert toward this end.

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