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Ion-Selective Membrane Electrodes for Clinical Use

Urs Oesch, Daniel Ammann, and Wilhelm Simon

We review ion-selective solvent polymeric membrane electrodes for clinical use. The particular requirements that the clinical application set on the membrane are discussed in terms of selectivity, stability, lifetime, and response time. The performance of currently available electrodes is reviewed, with consideration of actual problems that arise in clinical practice.

Among the techniques of detection used in clinical chemistry, optical and electrochemical methods are especially attractive. However, except for glass electrodes for pH measurement, potentiometric electrochemical sensors (ion-selective electrodes) gained general acceptance in clinical analyzers and in clinical instrumentation, respectively, only around 1970 (for reviews see *1–5*). Here we report on the state of the art in the design of membranes for clinically relevant ion-selective electrodes (ISE's) and on possible trends in their development.

Principles of Potentiometric Ion Sensors

Although a direct solid contact of the membrane in ion-selective membrane electrodes is possible, cell assemblies of the type

external reference electrode	sample solution	membrane	internal filling solution	internal reference electrode

ion-selective electrode (1)

are ordinarily used where high EMF stability and reproducibility are demanded (*6*). According to the nature of the basic membrane material, ISE's may be classified into the following categories (see also *7*):

(*a*) Solid-state membrane electrodes based on various crystalline materials (*8*).

(*b*) Glass membrane electrodes (*9*).

(*c*) Liquid membrane electrodes based on charged sites [classical ion-exchanger or charged ion-selective complexing agent (charged ion carrier, charged ionophore)]. The membrane consists of an organic, water-immiscible liquid phase incorporating the sites (*10*).

(*d*) Neutral carrier liquid membrane electrodes, where the membrane is usually formed from an organic solution of an electrically neutral, ion-selective complexing agent (neutral ion carriers, neutral ionophores) held in an inert polymer matrix (*11*).

(*e*) Special arrangements, such as gas-sensitive electrodes and enzyme electrodes. The potentiometric detection unit is based on conventional electrodes of the types *a–d* (*12–14*).

By selectively transferring the ion to be measured (primary ion I) from the sample solution to the membrane phase, a potential difference is generated between the internal filling solution and the sample solution, which is, ideally, a linear function of the logarithm of the activity ratio of I in the two solutions contacting the membrane. This approximation (Nernst equation) suffices in many cases, especially if the membrane is highly selective for the ion I and (or) if ions other than I are present in comparatively small concentrations. The Nernst equation is valid, if the ionophores in membranes of types *c* and *d* exclusively transport the ions I across the membrane phase (ideal permselectivity for the ion I). A semi-empirical, but nonetheless successful extension of the Nernst equation for the EMF (zero current electric potential difference between the internal and external reference electrodes at constant composition of the internal filling solution) of a practical cell assembly [cell (1)] is described in the Nikolskii–Eisenman equation:

$$EMF = E_0 + E_D + s \log[a_i + \sum_{j \neq i} K_{ij}^{Pot} a_j^{z_i/z_j}] \quad (2)$$

$$s = 2.303\ RT/z_iF = 59.16\ mV/z_i\ (25\ °C) \quad (3)$$

where

E_0, a constant potential difference, depends on the reference electrodes and on the temperature (independent on sample solution)

E_D is the liquid-junction potential difference generated between reference electrolyte and sample solution (by adequate choice of reference electrolyte, often sufficiently independent of sample solution)

R is the gas constant, 8.314 $JK^{-1}\ mol^{-1}$

T is the absolute temperature, K

F is the Faraday equivalent, $9.6487 \cdot 10^4\ C\ mol^{-1}$

z_i, z_j are charge numbers of the primary ion I and the interfering ion J, respectively (in units of the proton charge)

a_i, a_j are the activity of the primary ion I and the interfering ion J, respectively, in the sample solution (mol L^{-1})

K_{ij}^{Pot} is a selectivity factor, a measure of the preference by the sensor for the interfering ion J relative to the ion I. For an ideally selective membrane electrode all K_{ij}^{Pot} would equal zero. Although the dimension of the selectivity factor is $(mol/L)^{1-z_i/z_j}$, K_{ij}^{Pot} is commonly referred to molarity concentrations (mol/L).

Typical electrode response functions are shown in Figure 1.

Department of Organic Chemistry, Swiss Federal Institute of Technology (ETH), CH-8092 Zurich, Switzerland.

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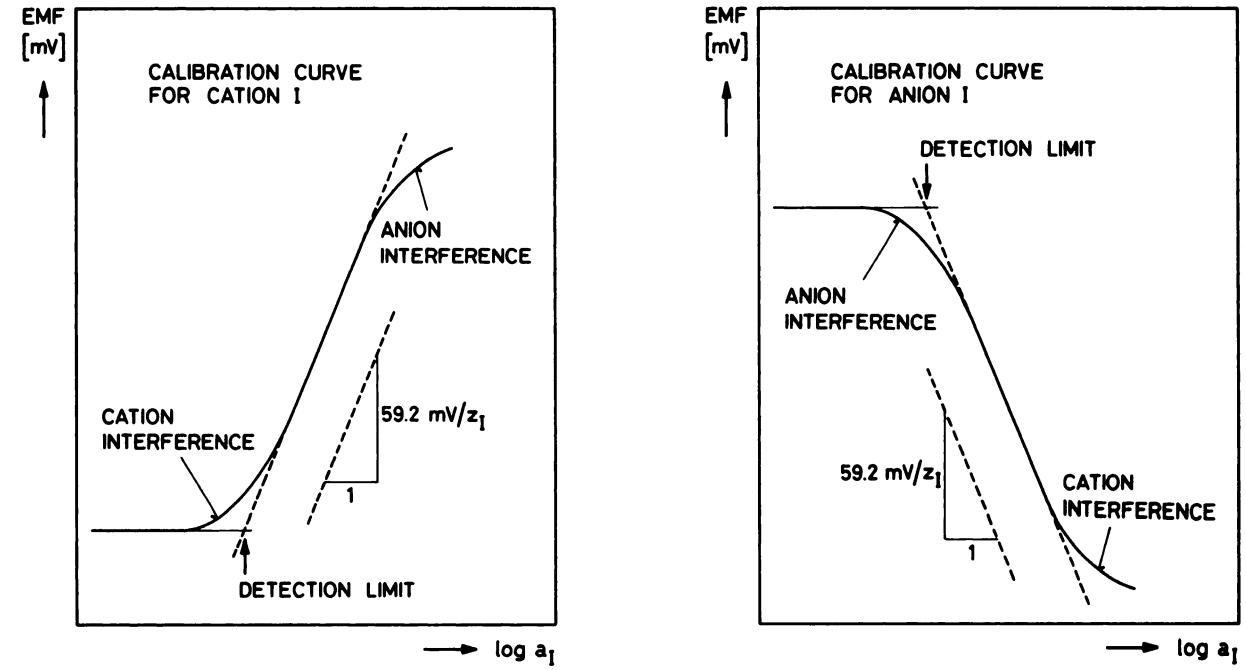

Fig. 1. Characteristic features of electrode response functions of membrane electrodes for cations (*left*) and anions (*right*)

At low activities of I, deviations from the linear response and ultimately constant EMF values are observed, owing to a constant interference by ions J of the same charge sign (equation 2). At high activities of I, interference by species of opposite charge (not described by equation 2) may lead to a deviation from the linear electrode response. In the linear range the basic response towards the primary ion I is (equations 2 and 3): a 1-mV change in EMF corresponds to $z_i \cdot 4\%$ change in the activity of the primary ion I.

Except for the assay of Cl^- in studies of cystic fibrosis (*15*), solid-state membrane electrodes of type *a* are clinically hardly relevant. In contrast, glass membranes with selectivity for H_3O^+ and Na^+ are widely accepted in clinical analysis (*1*). Further improvements in their performance cannot be expected. In the following we will focus on the liquid-membrane electrodes of types *c* and (especially) *d*, whose fields have experienced increasingly vigorous research activities in the last two decades and where substantial improvements are continuously being made, owing to the vast possibilities offered.

Ion-Selective Components

Liquid membrane electrodes based on charged sites (type *c*) generally show permselectivity for oppositely charged ions. In the case of no selective interactions between sites and counterions (classical ion-exchangers) the selectivity of the sensor is dictated mainly by the extraction behavior of the solvating membrane medium. Therefore, the following monotonic selectivity sequence is obtained for membrane electrodes based on classical cation-exchangers (e.g., tetraphenylborate in a nitro-aromatic solvent):

$$R^+ > Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$$

and analogously for classical anion-exchangers (e.g., quaternary ammonium salts in appropriate solvents)

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

where R^+ and R^- are lipophilic organic ions.

For liquid membranes with a selective interaction between charged site (charged ion carrier) and counterion, the potentiometric selectivity depends in a rather complicated way on both the ion-extraction selectivity of the membrane solvent and the ion-binding selectivity of the charged ionophore (*16, 17*). For example, the same ligand [bis-(2-ethylhexyl) phosphate] is used in certain Ca^{2+}-selective electrodes (membrane solvent: di-*n*-octyl-phenylphosphonate) and in divalent ion sensors with comparable selectivities for Ca^{2+} and Mg^{2+} (solvent: 1-decanol) (*18*). Neutral carrier membrane electrodes (type *d*) make use of the outstanding inherent ion selectivity of certain natural and synthetic ionophores.

Solvent Polymeric Membranes

Liquid membranes have been long known as suitable organic phases in potentiometric arrangements (*19*). To hold the organic liquid in place, a glass or ceramic frit or a filter paper was soaked with it. In this way virtually any organic liquid could be used as a membrane solvent. On the other hand, it was mechanically rather poor and not easy to use in certain applications.

The introduction of poly(vinyl chloride) (PVC) as a polymeric matrix in 1967 (*20*, see also *18*) to trap the organic solvent improved much of the flexibility of the membrane in the physical sense as well as in the sense of its application. The solvent has to be a swelling agent for the polymer, which is held in place by solvation forces; thus not just any organic solvent is applicable, only plasticizers are. In most cases this is barely a restriction, because in addition to the unique mechanical advantages gained there is available a large variety of plasticizers of different chemical, physical, and electrical properties. Typically, such a solvent polymeric membrane contains about 70 g of plasticizer and only 30 g of PVC per 100 g. This membrane can still be considered as a liquid phase, because diffusion coefficients for a dissolved low-molecular-mass component (e.g., an ionophore) are on the order of 10^{-7} to 10^{-8} cm^2 s^{-1} (*21*). Only at very low plasticizer contents (<20 g/100 g), diffusion coefficients may be 10^{-11} cm^2 s^{-1} and smaller, approaching values that are found for solids. The electrical membrane resistivity is about 1 to 100 MΩ cm (depending on the membrane

composition), which is, e.g., up to four orders of magnitude smaller than that of glass membranes.

Other plasticized polymers—such as polystyrene (*22*), poly(methyl methacrylate) (*22*), poly(vinyl butyral) (*23*), polyamide (*22, 24*), and polyimide (*25*)—have been tried with modest success. More successful has been the use of elastomers such as polyurethane (*26*), poly(fluorophosphazene) (*27*), silicone rubber (*28, 29*), and certain block copolymers of poly(dimethylsiloxane) (*26, 30*). Although these polymers do not contain any solvents, the high flexibility of their polymer chain permits a sufficiently high mobility of an incorporated ionophore, which is a prerequisite for a reversible electromotive behavior. Owing to the simplicity of its use and its outstanding properties, plasticized PVC currently is by far the polymeric membrane matrix most widely used for ion-selective electrodes.

Required Characteristics of Membranes

This section will deal only with required physicochemical features of the membrane and its components that are imposed by the interaction between the membrane and the sample to yield a signal that is reliable for the variable being measured. The sample considered here will be whole blood, plasma, and serum. The same discussion could be done in a similar way for urine or other clinical samples.

For measurements of ions of clinical interest a well-balanced optimization of all the electrode characteristics relevant for the application should be discussed rather than the superiority of a single property. Such relevant properties are selectivity, useful life-time of the sensor, stability, and response time.

Selectivity

Clinical samples exhibit well-defined physiological normal concentration ranges, so clear-cut requirements can be imposed towards the selectivity over other ions having the same kind of charge. Using the Nikolskii–Eisenman formalism (equation 2) and assuming representative physiological concentration ranges (Tables 1 and 2) the required selectivity factors can be calculated with equation 4.

$$K_{ij,max}^{Pot} = \frac{a_{i,min}}{(a_{j,max})^{z_i/z_j}} \cdot \frac{p_{ij}}{100} \qquad (4)$$

with

$K_{ij,max}^{Pot}$ being the highest tolerable value of the selectivity factor

$a_{i,min}$ being the lowest expected activity of the measuring ion I^{z_i}

$a_{j,max}$ being the highest expected activity of the interfering ion J^{z_j}

and

p_{ij} being the highest tolerable error in the activity a_i due to interferences of a_j (in percent)

If this calculated value for the required selectivity is actually achieved by a membrane, it means that the error made in the activity of a_i due to the interference of a_j is less than that set value p_{ij} even in the worst case. The required log K_{ij}^{Pot}-values given in Tables 1 and 2 are calculated for $p_{ij} = 1$, i.e., for a maximally tolerable error of 1%.

All efforts in the design of new ionophores take into consideration these demands imposed by the required selectivity. Since the ionophore does not solely determine the selectivity of the membrane, a well-selected choice of the membrane solvent and membrane additives may improve the selectivity to reach the required value. And such membrane systems might then be proposed for the clinical application although the membrane solvent and/or the membrane additives might be the failing factors in other properties like stability or lifetime (*21*).

Lifetime

Beside mechanical defects, electrical leakage pathways, severe surface contaminations, and membrane poisoning, the lifetime of solvent polymeric membrane electrodes is to a large extent dictated by the loss of membrane components (ion carrier, plasticizer, additives) into the sample solution (*21*). Such a loss affects membrane characteristics such as selectivity and membrane resistance. Through adequate membrane technology the required concentrations of membrane components may be maintained in the membrane phase for at least a desired time period. The lipophilic character of the sample (whole blood, plasma, serum) favors a substantial and fast extraction of the membrane components. Therefore, to minimize this the lipophilicity, P [defined as the partition coefficient between octane-1-ol and water (*32*)] of the membrane components should be extreme-

Table 1. Required Stabilities and Selectivities for Cation-Selective Electrodes for Whole Blood, Plasma, and Serum Applications

Cation I	95% normal concentration range, mmol/L	Required EMF stability,[a] mV	Required selectivity factor[b] log K_{ij}^{Pot} for interfering cation J						
			H^+	Li^+	Li^{+c}	Na^+	K^+	Mg^{2+}	Ca^{2+}
H^+	$4.3 \cdot 10^{-5}$ to $5.6 \cdot 10^{-5}$	0.35	0	−4.4	−6.5	−8.5	−7.0	−7.7	−7.7
Li^{+d}	<0.01	—	0.1	0	—	−6.2	−4.7	−5.3	−5.4
Li^{+c}	0.7–1.5	0.97	2.1	—	0	−4.3	−2.8	−3.5	−3.6
Na^+	135–150	0.12	4.4	2.1	−0.1	0	−0.6	−1.2	−1.3
K^+	3.5–5.0	0.46	2.8	0.5	−1.7	−3.6	0	−2.8	−2.9
Mg^{2+e}	0.45–0.8	0.37	8.9	4.4	0.1	−3.9	−0.9	0	−2.4
Ca^{2+e}	1.0–1.2	0.12	9.3	4.8	0.4	−3.6	−0.6	−1.9	0

[a] Required standard deviation of the EMF for a fivefold subdivision of the given concentration range with a 95% confidence limit.

[b] Required potentiometric selectivity factor for a maximal interference of 1% by other cations (worst case).

[c] Therapeutic level.

[d] Due to the undefined lower limit of the physiological concentration range, the required selectivity factors have been calculated for 0.01 mmol/L lithium concentration.

[e] Ionized cation.

Table 2. Required Stabilities and Selectivities for Anion-Selective Electrodes for Whole Blood, Plasma, and Serum Applications

Anion I	95% normal concentration range, mmol/L	Required EMF stability,[a] mV	Required selectivity factor[b] log K_{IJ}^{Pot} for interfering anion J								
			OH^-	Cl^-	Br^-	HCO_3^-	SCN^-[c]	$H_2PO_4^-$	HPO_4^{2-}	SO_4^{2-}	CO_3^{2-}
OH^-	$2.6 \cdot 10^{-4}$ to $4.1 \cdot 10^{-4}$	0.58	0	−7.6	−4.8	−7.0	−3.8 (−4.8)	−4.7	−6.8	−6.8	−4.9
Cl^-	95–110	0.19	3.4	0	0.7	−1.4	1.7 (0.8)	0.9	−1.2	−1.3	0.6
Br^-	0.009–0.17	3.8	−0.6	−6.1	0	−5.4	−2.3 (−3.2)	−3.2	−5.2	−5.3	−3.4
HCO_3^-	21.3–26.5	0.28	2.7	−2.7	0.1	0	1.1 (0.1)	0.2	−1.9	−1.9	0.0
SCN^-[c]	0.007–0.017(0.15)	1.1	−0.8	−6.2	−3.4	−5.6	0	−3.3	−5.4	−5.4	−3.5
$H_2PO_4^-$	0.034–0.14	1.8	−0.1	−5.6	−2.8	−4.9	−1.8 (−2.7)	0	−4.7	−4.7	−2.9
HPO_4^{2-}	0.26–0.89	0.8	6.6	−4.2	1.4	−2.9	3.4 (1.5)	1.6	0	−2.6	−1.8
SO_4^{2-}	0.3–1.0	0.77	6.7	−4.1	1.5	−2.8	3.5 (1.6)	1.7	−2.5	0	−1.7
CO_3^{2-}	0.10–0.17	0.34	6.2	−4.6	1.0	−3.4	3.1 (1.1)	1.2	−3.0	−3.0	0

[a] Required standard deviation of the EMF for a fivefold subdivision of the given concentration range with a 95% confidence limit.
[b] Required potentiometric selectivity factor for a maximal interference of 1% by other anions (worst case).
[c] Values for non-smokers, for smokers in parentheses.

ly high. The correlation between lipophilicity P_{TLC} of a component [lipophilicity P as determined by thin-layer chromatography (*33*)] and its partition coefficient K in the membrane/sample system can be assessed by equations of the type (*34*):

$$\log K = 0.48 + 0.33 \log P_{TLC} \quad (5)$$

Equation 5 holds for a dioctyl sebacate/PVC membrane and for blood as sample.

Theoretical models and calculations can be used to quantify the loss into the sample on the basis of such variables as the lipophilicity, the geometry of the membrane arrangement, and hemodynamic conditions. For a typical commercial flow analyzer in continuous use in contact with serum or whole blood it can be estimated that lipophilicities P_{TLC} have to be at least $2.5 \cdot 10^8$ for the ionophores and $6 \cdot 10^{12}$ for plasticizers to guarantee a lifetime of at least one month (*35*). Values obtained by such a calculation represent a worst-case situation, because there is continuous use only in few applications such as *in situ* monitoring in intensive-care units. In conventional commercial analyzers the stream of samples is usually interrupted after each sample by calibration and (or) washing solution, which do not require high values for P_{TLC}. Moreover, they allow recovery from the eventual depletion of components in the vicinity of the membrane surface by a resupply from the interior of the membrane. The lifetime will obviously be extended in such cases.

Stability of Measured Signal

Among the relevant properties discussed in ion sensor technology, stability has only been treated rarely. Indeed, there is no quantitative description of the stability defined. On the other hand, the requirements for the stability of clinical electrolyte determinations are easily recognized. They have to allow a pin-pointing of the concentration within the physiological normal range with a certain precision (*36*). Since the demands on the precision may differ between clinical laboratories or between the kind of application, it is difficult to recommend here any certain requirement. Nevertheless, for the following discussion, and particularly for relative comparisons between sensors for different ions, we demand here a fivefold subdivision of the physiological normal concentration range with 95% confidence limit. The required standard deviations in the EMF signal will then be as given in Tables 1 and 2. Aside from all the further stability discussions concerning the membrane of the ion-selective electrode, the whole electrical circuitry and the design of the reference electrode have first of all to cope with these stability requirements (*4*). Experimental stability criteria to be discussed for a certain application may be:

- shift in the standard potential (E_0 shift)
- drift
- residual standard deviation
- reproducibility

There are essentially the following two kinds of applications to be considered separately, because different criteria may be regarded as important and descriptive for them.

For batch determinations, where calibrations can be performed during the run, the reproducibility of the EMF difference between sample and calibration solution may be the relevant stability criterion. A long-term drift in the EMF of the electrode assembly is negligible, because the sample/calibration intervals are usually only on the order of about a minute or less.

For continuous in vivo applications, where intermittent calibrations can no longer be performed, the stability will be expressed by the drift and the residual standard deviation.

Inherent to both types of applications is the frequent observation with such membrane electrode systems that an E_0 shift occurs after a first contact with a protein-containing solution. E_0 shifts up to 2 mV have been observed for a K^+-selective valinomycin-based bis(2-ethylhexyl)adipate plasticized membrane (*37*) and a Ca^{2+}-selective ETH 1001-based *o*-nitrophenyl octyl ether plasticized membrane (*38*). It is expected that E_0 shifts occur owing to changes in the interfacial condition. A deposition of proteins, even only of monolayers, will change the physical nature of the interface. Therefore a change in the standard potential E_0 of this interface has to be anticipated. Since the membrane/internal reference electrolyte interface is not influenced by such an occurrence, an overall E_0 shift will be observed. A change in the physical nature of the sample-side interface may also occur as a result of the extraction of marginally lipophilic membrane components by the protein-containing sample. The rather moderate mobilities of the membrane components may not equalize sufficiently rapidly this depletion on the sample-side interface. The slow generation of even slight interfacial imbalances has to result in interfacial E_0 differ-

ences, which will then be observed as slow drifts. In this case, highly lipophilic membrane plasticizers are recommended. Whether and if, and to what extent, the plasticizer and its lipophilicity are causing the deposition of protein on the membrane surface has yet to be ascertained. The use of a protective dialysis membrane to avoid such a deposition will solve this problem, but it is controversial whether or not the interposed dialysis membrane generates additional potential differences.

Response Time

The required response time of the membrane electrode cell assembly has to be compatible with the analysis time, which is on the order of ~30 s for commercial analyzers. There are many different processes that contribute to the dynamic response behavior (*5*). Since some of these processes (e.g., diffusion of sample ions through the stagnant layer adhering to the membrane surface) are not intrinsic to the membrane itself, it is difficult properly to compare reported response times as characteristics of membrane systems. The mathematical expression for the time constant of the response of carrier-based sensors, however, indicates that the intrinsic response time of the membrane may be shortened by adequate membrane technology (*39*).

Available Membrane Electrodes

H^+-selective Electrodes

The extraordinary merit of pH glass membrane electrodes in clinical practice is undisputed. Cell assemblies with pH glass membranes exhibit outstanding electromotive behavior. Selectivity, stability, and lifetime impose no significant limitations. But certain drawbacks of glass membranes become evident if some specific features of glass membranes are compared with those of solvent polymeric membranes: the mounting of glass membranes into strongly miniaturized electrode arrangements (e.g., flow-through cells) is technically not simple and the relatively high electrical membrane resistances are often a problem. In addition, glass membranes are more likely to be disturbed by certain components from biological samples [e.g., deposition of proteins (*40*), which calls for special rinsing treatments at regular intervals]. For certain special applications handling-safety is a further serious problem (e.g., catheter electrodes). In principle, all these glass-specific difficulties can be circumvented by the use of H^+-selective solvent polymeric membranes.

A liquid membrane based on the neutral H^+ carrier tridodecylamine is especially suited for clinical use. The sensors exhibit an accuracy and reproducibility (standard deviation of single measurements: 0.001 to 0.003 pH unit) for blood-pH measurements similar to that of glass electrodes, the residual standard deviation of a correlation of pH values obtained with a solvent polymeric membrane and a glass membrane being 0.01 pH unit (*41*). Although the H^+ selectivities of sensors with tridodecylamine are impressively high [selectivity factors on the order of 10^{-10} (*42*)], the dynamic pH range of the electrode response nevertheless is inferior as compared with glass electrodes. It was, however, shown that the dynamic ranges of Nernstian H^+ response are directly related to the acidity constant of the active H^+ exchange site of the neutral carrier (*43*). By exploiting these molecular features, neutral carriers for solvent polymeric membranes with a H^+ response in selected pH ranges could be realized—e.g., between pH 0.5 and 7 for gastroenterological applications (*44*).

The replacement of glass membranes by pH-sensitive solvent polymeric membranes for routine measurements with clinical analyzers is unlikely. However, for more special applications (e.g., catheter electrodes for gastric pH measurement) non-glass systems are advantageous.

Li^+-selective Electrodes

Potentiometric Li^+ measurements have not yet been introduced into clinical practice. Indeed, direct potentiometric determination of physiological Li^+ concentrations in blood are impossible because of insufficient selectivities of the currently available Li^+ electrodes. However, there is great interest in monitoring clinically increased Li^+ concentrations in the millimolar range during the therapy of manic–depressive psychosis (*45*). Therefore, the design of ionophores with Li^+/Na^+ selectivities that meet the requirements for the assay of therapeutic concentrations is pushed with vehemence. Although none of the carriers proposed so far exhibits ideal selectivity (Table 1), two of them yield useful membranes [ETH 1810 (*46*); dodecyl methyl-14-crown-4 (*47*)]. Membranes based on ETH 1810 show a somewhat higher Li^+/Na^+ selectivity.

Because the therapeutic Li^+ range is relatively broad (0.7–1.5 mmol/L, corresponding to an EMF range of 19.1 mV), a standard deviation of about 1.0 mV in the measured EMF (Table 1) allows a fivefold subdivision of the range (95% confidence limit). If Li^+ sensors based on ETH 1810 are calibrated in the presence of a physiological Na^+ background, EMF uncertainties on the order of 1 mV are found. Despite the optimal calibration, the error is mainly due to variations in the Na^+ concentrations in the sample (*46*).

The ratio of the concentrations of Li^+ and Na^+ in blood seems to be an important factor for the control of lithium treatment of patients, because a patient with low Na^+ concentration in his blood seems to need less Li^+ to produce the same therapeutic effect than does a patient with a high Na^+ concentration. Thus, the concentration-ratio mode, where the conventional reference electrode is replaced by a Na^+-selective electrode, appears as an interesting approach with currently available Li^+ electrodes. Using such cell assemblies, the selectivity problem is reduced to a nonlinearity in the calibration (EMF vs ratio between the concentrations of Li^+ and Na^+).

Na^+-selective Electrodes

Na^+ glass membranes have found a widespread application and are routinely used in clinical analyzers. The same difficulties encountered with pH glass membranes (see above) are found for Na^+ glass membranes.

Various neutral carrier-based solvent polymeric membranes are now available. Electrodes containing the carriers ETH 157 (*48, 49*), ETH 227 (*50, 51*), ETH 2120 (*52*), a bis(12-crown-4) compound (*53*), and a hemispherand (*54*) almost satisfy the required selectivities for an application to blood samples. Membranes with ETH 227 are slightly too Li^+ unselective for Li^+ in therapeutic concentrations [required selectivity factor (Table 1): 0.8; found: 3], and membranes based on ETH 157 show a small K^+ interference (required selectivity factor: 0.25; found: 0.4). The lipophilicities of the Na^+ carrier ETH 157 and the bis(12-crown-4) compound are far beyond the value for a comfortable long-term application in blood (*35*), whereas membranes with ETH 2120 seem to meet all the requirements.

The potentiometry of Na^+ in serum has been extensively

discussed for membranes with ETH 227 (*51*). Direct potentiometric Na^+ measurements show a positive bias of about 5% when compared with indirect flame-photometric results (residual standard deviation of the correlation: 1.1 mmol/L). This is in agreement with corresponding correlations obtained with glass membrane electrodes (*55–66*). The observed deviations in Na^+ results obtained by direct potentiometry and indirect flame photometry have led to intense discussions (see below).

K^+-selective Electrodes

During the last two decades, all the K^+-selective membrane electrodes in clinical analyzers have relied on valinomycin. Most K^+ assays are performed by using plasticized poly(vinyl chloride) membranes. The only drawback has been observed during measurements in undiluted urine (*67*), where PVC membranes suffer from some anion interferences (see Figure 1). The problem could be eliminated by the incorporation of valinomycin into silicone rubber membranes, which therefore can be considered to be a membrane that is universally applicable to body-fluid samples (*68*).

It is clearly documented that valinomycin-based PVC as well as silicone rubber membrane electrodes exhibit almost ideal characteristics for use in clinical samples. Nevertheless, many efforts have been made to replace valinomycin by synthetic K^+ carriers (*69–71*). By using bis(crown) ethers, K^+ selectivities approaching those of valinomycin-based sensors have been achieved. So far, the lipophilicity of the synthetic representatives has been quite poor, but improvements are in prospect (*35*).

Mg^{2+}-selective Electrodes

Today, there is no membrane electrode available for Mg^{2+} measurements in blood. A synthetic cyclodecapeptide yields membranes with adequate rejection of H^+, Li^+, and K^+, but insufficient Mg^{2+}/Na^+ selectivity (selectivity factor found: $5 \cdot 10^{-3}$; required: $1.3 \cdot 10^{-4}$) and insufficient Mg^{2+}/Ca^{2+} selectivity (found: 0.8; required: $4 \cdot 10^{-3}$) (*72*). Furthermore, the lipophilicity of the peptides must be drastically increased for use in blood samples, and the membranes have to be optimized for satisfactory EMF stabilities in blood samples. Nevertheless, applying chemometric methods (e.g., by simultaneously measuring Na^+ and Ca^{2+}), such membrane electrodes might already be a realistic tool for certain Mg^{2+} assays in clinical analysis.

Ca^{2+}-selective Electrodes

Solvent polymeric membranes based on an organophosphate (*73*), the acyclic carrier ETH 1001 (*74*), and a bis(crown) compound (*75*) satisfy the selectivity requirements for analysis for Ca^{2+} in blood. Membranes containing ETH 1001 are most widely used, and almost each clinical analyzer for potentiometric Ca^{2+} assays relies on this type of membrane (*76*). Today, both the total calcium (*77*) and the activity or concentration of the ionized Ca^{2+} fraction (*74*) can be determined potentiometrically.

Measurements of total calcium require membranes with high Ca^{2+}/H^+ selectivity, because the bound calcium is displaced from its complexes through acidification of the serum sample (pH about 3.5). Neutral carrier-based electrodes, which exhibit a H^+ rejection larger by five orders of magnitude as compared with membranes with organophosphates, are unique in this respect (*77*). Excellent correlations between potentiometry and atomic absorption spectrometry have been obtained: residual standard deviation in total calcium concentration was ± 0.04 mmol/L (*77*).

For many decades, measurement of total calcium has been a conveniently accessible and well-established clinical tool. In contrast, the assay of ionized Ca^{2+} imposes several difficulties, such as standardization of activity measurements and the dependence of the ionized Ca^{2+} fraction on the pH and protein content of the blood sample. However, the dominating physiological role of Ca^{2+} as the biologically active species and the superiority of Ca^{2+} activity over total calcium data in patient care (*76, 78, 79*) have been repeatedly stressed. During analysis of ionized Ca^{2+} in undiluted blood samples, a further advantage of neutral carrier membranes over organophosphate-based membranes becomes apparent. Membranes with organophosphates must be covered with protective dialysis membranes (cellophane) in order to avoid changes in the standard potential E_0 after the first contact with serum (*79, 80*); however, a disadvantage of this procedure is the slightly prolonged response time of the sensors (*80*). On the other hand, unprotected membranes containing ETH 1001 in a PVC membrane plasticized with a highly lipophilic, nonpolar tetraester (*81*) show insignificant E_0 shifts and EMF stabilities better than 0.13 mV during as long as 6 h of continuous contact with whole blood, corresponding to about 1% change of the initial activity. Thus, in vivo monitoring over such periods of time without any intermediate calibration is feasible (*81*).

To control the influence of pH, a new generation of clinical Ca^{2+} analyzers allows simultaneous measurement of both the Ca^{2+} concentration and the pH of the blood sample. This enables the evaluation of both the actual Ca^{2+} activity and the Ca^{2+} activity corrected to a reference pH value of 7.4. Both quantities are of clinical interest (*82*): if persistently abnormal pH values exist, the actual Ca^{2+} is relevant, whereas in the case of short-term pH changes a standardized Ca^{2+} is more significant.

Cl^--selective Electrodes

At present, solvent polymeric membranes based on classical ion-exchangers are exclusively used in clinical potentiometric Cl^- assays. Although components and compositions of such membranes have been optimized extensively (*16, 83*), interference and stability problems remained. The sensors typically exhibit a Cl^-/HCO_3^- selectivity of less than 10 (*16*) [required value: 25 (Table 2)] and, because lipophilic anions are unavoidably preferred over Cl^- in such membranes, careful consideration has to be given to the interference by SCN^-, NO_3^-, Br^-, and salicylate. For example, the required Cl^-/SCN^- selectivity, which amounts to 0.02, is hardly achieved by currently used classical anion-exchanger membranes.

Therefore, there is great interest for novel, improved anion-selective membranes. The first neutral (*84*) and charged anion carriers (*85*) have been realized only recently. So far, no membrane electrode based on these compounds has met the requirements for clinical applicability.

HCO_3^-/CO_3^{2-}-selective Electrodes

No HCO_3^--selective ionophore has been described so far that would be applicable as a component in a solvent polymeric-membrane electrode of clinical relevance. On the basis of classical ion-exchanger sites there have been claims for both HCO_3^-- (*86*) and CO_3^{2-}-selective (*87*) liquid-membrane electrodes. So far, the two similar membrane systems have only rarely found a clinical application. For a recent application of such a membrane electrode in the assay of

CO_2 see refs. *88, 89*. Another type of HCO_3^--selective electrodes, based on gas-permeable, H^+-selective polymeric membranes, has been proposed (*90, 91*). When electrodes of this kind are used, attention has to be given to interferences of neutral, acidic components in the samples such as acetic acid, lactic acid, and salicylic acid rather than inorganic anions (*92, 93*). Recent results in our laboratory indicate that thin-membrane technologies, in conjunction with appropriate membrane materials, shorten the response time of such a sensor assembly down to a level which can meet the requirements for a relevant clinical application (see also *94*).

Problems in Clinical Practice

In the above sections, the usefulness of potentiometric electrolyte determinations in clinical analysis is discussed exclusively with respect to the properties of the membrane materials. Certainly, many other parameters additionally influence the results:

- mode of assay (e.g., direct or indirect)
- calibration
- nature of the sample (whole blood, plasma, serum)
- sample collection
- sample treatment
- sample storage
- type of liquid-junction (liquid-junction potentials, suspension effects by erythrocytes)
- measuring instrument

All these subjects are intensively discussed in the literature. Some important aspects are briefly summarized in the following sections (see also *3*).

Direct vs Indirect Measurements

Direct measurements (i.e., those made on undiluted blood samples) with ion-selective electrode cell assemblies that have been calibrated with activity standards offer the unique possibility to assay the physiologically most relevant fraction of an ion in blood (ion activity). Consequently, any measurement with ISE's should most logically be performed in the direct mode and be evaluated in terms of ion activities.

This approach is fully exploited in the case of pH measurements in blood. Carefully designed activity standards are available and the physician must interpret the activity data.

The superiority of information on ionized Ca^{2+} over that on total calcium concentration as a clinical parameter of high diagnostic value is now generally recognized. Direct potentiometric procedures have made this parameter accessible. Usually, free Ca^{2+} concentrations and not Ca^{2+} activities are preferentially reported (*76*).

Unfortunately, the situation for Na^+ and K^+ determinations is much more complicated. The classical flame-photometric approach yields information on their total concentrations. These quantities now are well established for diagnostic purposes, although data on Na^+ and K^+ activities definitely is more substantial. The usual substrate concentration ranges are likely to be retained, because confusion in interpretation of the data by physicians is suspected. Therefore, it has been suggested to convert the direct potentiometrically-obtained Na^+ and K^+ activities into total substrate concentrations by multiplying the results by an appropriate factor (*95*). Obviously, such a factor is valid only for an assumed activity coefficient and a typical concentration of plasma water (*96*). It is to be hoped that this not fully satisfactory procedure represents only an intermediate situation. Some time after the definite introduction of Na^+ and K^+ electrodes into clinical practice, exclusive measurements of Na^+ and K^+ activities should follow.

Influence of Protein and Lipid Volume

The controversy between direct (undiluted blood, plasma or serum sample) and indirect (diluted sample) measurements is partly linked to effects ascribable to the presence of remarkable amounts of proteins and lipids in blood. Deviations between results by these two approaches can be explained to a large extent by the volume fraction occupied by these macromolecules. A theoretically predicted positive bias of 6.7% for direct potentiometry (concentration in free plasma water) vs indirect assays (concentration in the total sample volume) is generally accepted for normal situations. Thus, corrections for protein and lipid content can be made. However, after such corrections have been made, a negative bias of about 1% remains. This fact was observed and has been discussed by several authors (*56–60, 63, 97–101*). Besides the correction for protein and lipid volume, the binding of Na^+ to bicarbonate (*60, 99, 102*), to proteins (*100, 103*), to unknown substances (*58, 98*), water binding on proteins (*60*), effects at the liquid-junction (*101, 104–106*), and calibration and (or) evaluation procedures (*56, 60, 96, 101, 104, 106*) have been proposed as further factors to be considered. Calculations involving the Debye–Hückel, Henderson, and Nikolskii formalisms and calibration with a 140 mmol/L Na^+ solution make it obvious that, after correction for protein and lipid volume, the negative bias cannot be attributed to uncertainties in the calibration and evaluation procedure. For a hypothetical sample deviating in the EMF value from this calibration solution by 1 mV, one would obtain a bias of <0.5% in the Na^+ concentration. This holds even if one uses, ill-advisedly, a reference electrolyte such as 140 mmol/L NaCl, or neglects corrections for changes in the liquid-junction potential or ionic strength, or both (*51*).

Calibration

Except for H^+ (*107*), there are unfortunately no generally recommended and accepted calibration procedures for membrane electrode cell assemblies of clinical relevance. Deviations in the calibration solutions of various clinical analyzers considerably contribute to differences in the results and consequently in the reference ranges. Many reports on the evaluation and comparison of clinical analyzers (*96, 108–111*) show that inter-instrumental deviations may be considerable. Thus, standardization of the calibrations would crucially contribute to comparisons of data within various laboratories. Recommendations for calibrations of electrode cell assemblies are in preparation by an IFCC committee.

Liquid-Junction

Changes in the liquid-junction potential are minimized by the use of highly concentrated equitransferent reference electrolytes (*4*). However, even the use of saturated KCl as the bridge solution will not completely eliminate contributions of liquid-junction potentials to the measured EMF when aqueous calibration solutions and blood samples are exchanged. The bias (which usually is tolerable) is further minimized if the calibration solutions show an electrolyte composition resembling as closely as possible the ionic composition of the blood sample.

If whole blood is taken as the sample, additional potentials arise due to the presence of erythrocytes. The extent of the bias depends on the salt bridge and on the hematocrit of

the blood sample. There is no clear theoretical explanation of the effect of erythrocyte suspensions at the liquid junction. It was found that the influence of erythrocytes on the liquid-junction is minimal if a 4 mol/L sodium formate reference electrolyte is used (*112*). This unusual bridge electrolyte solution should be used with great care for direct monitoring of ion activities in whole blood only because an accidental compensation of different contributions to the cell EMF may cause this apparent minimal liquid-junction effect. In general, use of highly concentrated KCl electrolytes is more advantageous.

Sampling, Storage

In the preparation of anticoagulated whole-blood samples, effects due to delay of centrifugation (e.g., loss of CO_2, osmotic movement of water across erythrocytes, K^+ release from erythrocytes), the anaerobic handling of blood samples, and the storage of such samples are, especially for the direct analysis of H^+ and Ca^{2+}, of great importance (*113–115*).

Sensor Designs

The versatility of the application of ion-selective polymeric membranes in potentiometric sensing systems is illustrated in Figures 2–5.

Flow-Through Electrodes

The membrane, which is not larger than about 1 mm^2, is molded in the wall of the sample channel (Figure 2). Several electrodes are put in series, thereby allowing the system to be operated in a continuous-flow or a discrete mode, for which only about 100 μL of sample is necessary. Because the membrane area is so small, the electrode has a rather high electrical resistance (~100 MΩ), which has to be dealt with. Most commercial electrolyte analyzers make use of this type or of similar arrangements (e.g., AVL 980, Boehringer ISE 2020, NOVA series, Corning 902). In some other analyzers (e.g., Radiometer KNA1 and ICA1) a conventional but miniaturized electrode, which can be introduced into the sample channel, is used. With the electrode assembly shown in Figure 2, extreme EMF stabilities could be achieved in the continuous-flow mode. For example, for a valinomycin-based potassium electrode an E_0 shift of 0.11 mV after 6 h of continuous contact with blood, a drift of 0.01 mV/h, a reproducibility of EMF differences between an aqueous solution and a serum sample of ± 0.1 mV (standard deviation, n = 5), and a long-term EMF stability in an aqueous solution of ± 0.03 mV (standard deviation, n = 100, 5 h) have been observed (*81*).

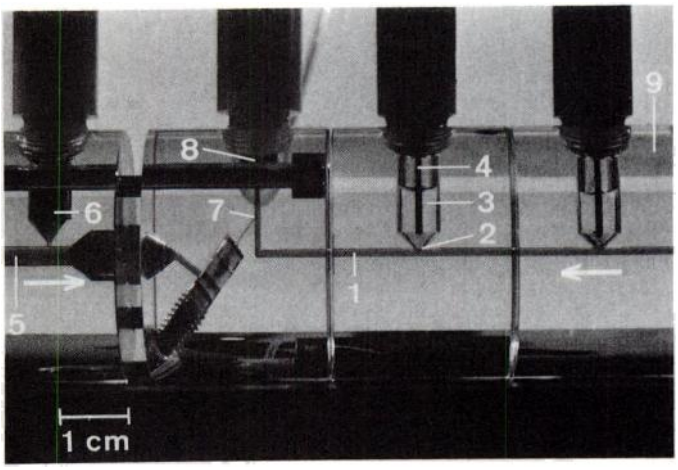

Fig. 2. Flow-through electrode system

(*1*) sample channel, (*2*) ion-selective membrane, (*3*) internal electrolyte, (*4*) internal reference element (Ag/AgCl), (*5*) reference electrolyte, (*6*) reference element (Ag/AgCl), (*7*) free flowing liquid-junction, (*8*) common electrode (Pt), (*9*) poly(methylmethacrylate) electrode module. *Arrows* indicate direction of flow

Catheter Electrodes

Solvent polymeric membranes are especially suited for miniaturized conventional electrodes, because they can be molded in any desired shape and size. Figure 3 shows a tip of a catheter-type polymeric membrane electrode. The membrane has a thickness of about 25 μm and is permanently sealed to the polymeric catheter tubing. The small outer diameter makes them suitable for continuous intraluminal and intravascular ion monitoring (*40, 44, 116*).

Ion-Selective Field Effect Transistors

The high impedance signal from the high resistive miniaturized electrodes is a problem that can be circumvented by *in situ* signal amplification. Ion-selective field effect transistors (ISFETs) make use of this. The ion-selective membrane is directly contacted with the FET. Although such devices based on standard integrated-circuit fabrication technologies have been known since 1975 (*117*), the major problems seem to be inherently related to the electrochemically ill-defined interface between membrane and gate insulator, which leads to long-term instabilities (*118*). The small area of the gate (~0.01 mm^2, see Figure 4) is intriguing, because it would allow the arrangement of several ion sensors on a very small site. But deposition, separation, and encapsulation of the different membranes for a reliable multi-function sensor imposes problems that have yet to be overcome. The use of ISFETs in clinical applications has been demonstrated for in-dwelling single-function devices (*119*) and for quadruple-function flow-through devices (*120*).

Potentiometric Analysis Slides

Ion-selective solvent polymeric membranes are used in this approach in combination with dry reagent films to form a disposable electrolyte analysis device for the discrete single-parameter ion measurement (Figure 5) (*121*). The sample (10 μL) is placed with a reference sample on the slide (Kodak Ektachem Clinical Chemistry Slide), which constitutes a whole electrode assembly, and is inserted into a voltmeter for the EMF reading (Kodak Ektachem Electrolyte Analyzer). The continuous fabrication technique for the

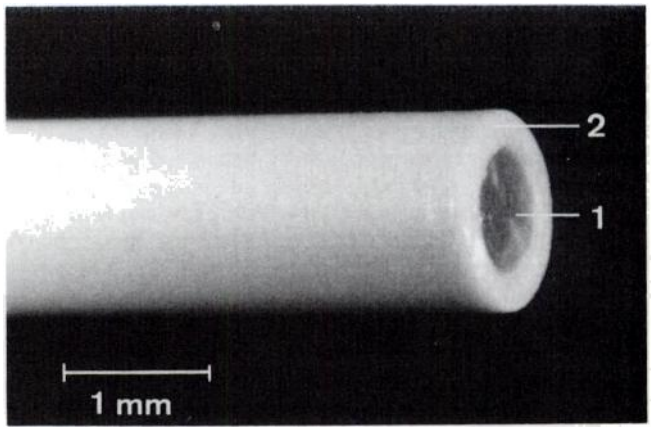

Fig. 3. All-polymeric catheter electrode

(*1*) ion-selective membrane, (*2*) poly(vinyl chloride) catheter tubing

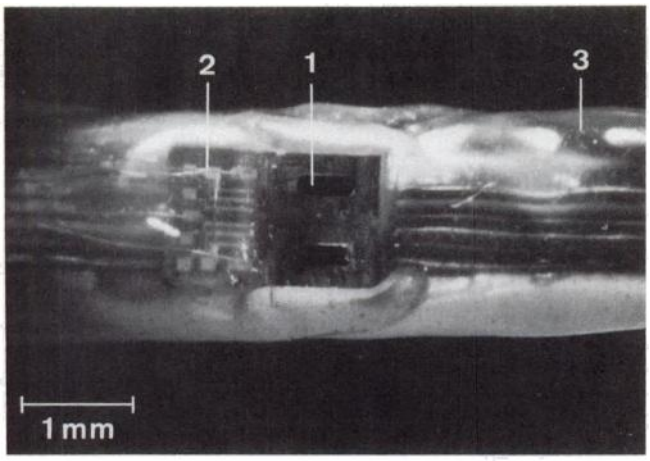

Fig. 4. Encapsulated ion-selective field effect transistor

(*1*) opening in the encapsulation filled with ion-selective membrane, covering the gate (20 × 400 μm), (*2*) pads and wires for electrical connections, (*3*) epoxy encapsulant

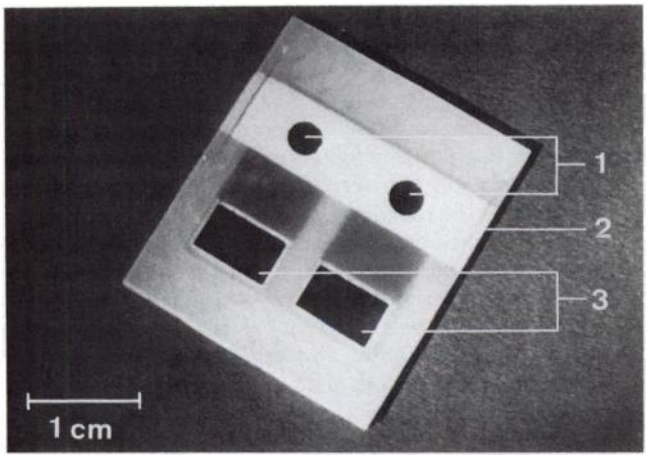

Fig. 5. Electrolyte analysis slide (Kodak Ektachem Clinical Chemistry Slide)

(*1*) sample and reference pad covered with ion-selective membrane, (*2*) paper bridge, (*3*) pads for electrical connections

dry reagent slides is highly reproducible and therefore yields highly reproducible test readings.

Outlook

For all physiologically important alkali and alkaline earth cations—except Li^+ and Mg^{2+}—there are cation-selective solvent polymeric membranes available for clinical sensors which meet all the requirements discussed in Table 1. Although clinically relevant Li^+-electrodes exist, higher Li^+/Na^+ selectivities would be welcome. Substantial efforts will be necessary to realize fully optimized electrodes for Mg^{2+} and for all the inorganic anions, especially Cl^-, sulfate, and phosphate. We hope that efforts in the field of membrane technology will soon allow the production of sensors of such high signal stability as to allow long-term on-line measurements *in situ* as well as conventional bench-top measurements with only occasional calibration.

The ISFET-technology—which was claimed to be ideal for miniaturized, disposable multiparameter sensors of low cost (*122*)—has not yet reached the stage of fulfilling the requirements outlined above. New technologies in encapsulation and in the deposition and structuring of membrane materials on gate insulators will be necessary to make polymeric membrane-based ISFETs competitive with conventional ISEs for clinical application. Although polymeric membrane-based ISFETs were introduced in 1975 (*117*), so far not even single-parameter devices have as yet made it to a commercial product.

In contrast, we assume that improvements in the planar membrane technology will help to promote devices based on the so-called dry reagent chemistry (*121*).

It is our opinion that electrochemical ion-sensors based on polymeric membranes improved by an adequate membrane technology will be widely used in clinical chemistry for at least the next two decades. On the other hand, sensors based on electrochemical transduction will be strongly challenged in the long run by optochemical transducing devices [optrodes (*123*)].

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