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PERFORMANCE EVALUATION CRITERIA FOR PREPARATION AND MEASUREMENT OF MACRO- AND MICROFABRICATED ION-SELECTIVE ELECTRODES

(IUPAC Technical Report)

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Performance evaluation criteria for preparation and measurement of macro- and microfabricated ion-selective electrodes

(IUPAC Technical Report)

Abstract: Over the last 30 years, IUPAC published several documents with the goal of achieving standardized nomenclature and methodology for potentiometric ion-selective electrodes (ISEs). The ISE vocabulary was formulated, measurement protocols were suggested, and the selectivity coefficients were compiled. However, in light of new discoveries and experimental possibilities in the field of ISEs, some of the IUPAC recommendations have become outdated. The goal of this technical report is to direct attention to ISE practices and the striking need for updated or refined IUPAC recommendations which are consistent with the state of the art of using macro- and microfabricated planar microelectrodes. Some of these ISE practices have never been addressed by IUPAC but have gained importance with the technological and theoretical developments of recent years. In spite of its recognized importance, a generally acceptable revision of the current IUPAC recommendations is far beyond the scope of this work.

Keywords: ISEs; microfabricated; ion-selective electrodes; potentiometry; picomolar; nanomolar; IUPAC Analytical Chemistry Division.

CONTENTS

1. INTRODUCTION

In the last 30 years, IUPAC produced several documents with the purpose of achieving standardized nomenclature and methodology in the field of potentiometric ion-selective electrodes (ISEs) due to the broad interest in ISEs and their practical importance. As a part of this work, the vocabulary for the most common terms and methods has been formulated [1,2], the selectivity coefficients have been compiled [3–6], and suggestions for the definition and measurement of the dynamic response have been proposed [7]. Some of the IUPAC recommendations are widely accepted and used throughout the field for characterizing ISEs. Others remain at the level of recommendation without wide acceptance. Finally, some are considered controversial or have become outdated with new discoveries. In recent years, the detection limits and selectivity coefficients of several known ISEs have been improved by several orders of magnitude (for review, see [8]) and novel, nonclassical response principles have been introduced [9]. To achieve nano- and picomol/L detection limits, the ISEs were often used under nonequilibrium conditions in which engineered concentration gradients [10] or external current [11,12] were utilized to eliminate minor ionic fluxes across the membrane which contaminated the sample solution in contact with the sensing membrane. On the application side, planar, microfabricated ISEs gained ground both as research tools as well as detectors in commercial blood gas analyzers [13,14]. These novel experimental and theoretical findings in potentiometry justify the critical evaluation of the existing IUPAC recommendations.

Besides the IUPAC-recommended protocols [15], there are common practices in the methodology of using ISEs, which are accepted throughout the field although they have never been addressed by the IUPAC. Some of these have a well-established scientific background and are in accord with the IUPAC suggestions. Others are believed to provide practical advantages and are practiced without explanation or questioning of their origin. However, in light of the most recent findings, some of these common practices have certain negative consequences which were not recognized until recently. Consequently, some of these practices contribute to the generally accepted view that the detection limits of ISEs are in the concentration range between 10^{-5} and 10^{-7} mol/L, both for solid-state and liquid membrane electrodes, independent of the membrane matrix and its active ingredients. Some of these practices are listed in the following examples:

- Freshly prepared electrodes are generally conditioned in a relatively concentrated ($c \geq 10^{-3}$ mol/L) primary ion solution before testing. Most commonly, the analytical performance characteristics of ISEs are tested after conditioning them overnight. However, hardly any data are available on the effect of this "required" conditioning on the performance characteristics of ISEs as a function of conditioning time or composition of the conditioning solution. The drift and the standard deviation of the measured cell voltage [1], in a solution of constant composition and at constant temperature, following the first solution contact and after extensive conditioning, are generally not reported. Similarly, only a few papers are devoted to the analysis of structural and chemical changes in the phase boundary layers of the ion-selective membranes during conditioning and their correlation with the analytical signal. Actually, in light of the most recent findings, conditioning the sensing membrane in concentrated primary ion solutions cannot be recommended and may be disadvantageous with respect to the attainable detection limit.
- In most conventional (macro) ISEs, a relatively concentrated solution of the primary ion is used as inner filling solution (IFS) (10^{-2} to 10^{-3} mol/L). It was widely accepted that it is essential to have well-defined phase boundary potentials on the IFS side of the ion-selective membrane. However, as it was proved recently [16], the use of concentrated IFS is rather disadvantageous as it may contribute to primary ion leaching across the membrane into the sample solution due to salt co-extraction. This primary ion leaching is considered the major source of biased detection limits and selectivity coefficient values published throughout the literature. Based on this knowledge, the use of highly diluted IFSs with well-controlled ion activities (e.g., buffered) is recom-

mended [10]. The concentration of the IFS should be somewhat lower or should match the concentration of the most dilute analyte solution [17].

- Although the most common method for determining the selectivity coefficient of ISEs is the so-called separate solution method (SSM), in which the ISE membrane under test is exposed to solutions of one or many interfering ions, hardly any information is available on the effect of these exposures to solutions of interfering ions (time and concentration) on the electrode performance characteristics in solutions containing primary ions following the selectivity coefficient measurements (e.g., short time stability of the measured potential before and after evaluating the selectivity coefficients) [18]. Similarly, the effect of interfering substances in the sample (ionic or non-ionic) on the electrode performance is generally not explored in detail. The mechanism as to how these substances (with parameters concentration level and exposure time) affect the membrane composition and the electrode performance in most of the cases is unknown.
- Based on practical experience, it is widely accepted that the slope of the response function of ISEs remains fairly constant during extended use while the offset voltage may drift. Drifting offset voltage can indicate changes in the membrane composition as a consequence of (i) ion-exchange processes in the presence of high concentration of interfering ions in the sample, (ii) anion co-extraction in highly concentrated samples or in the presence of highly lipophilic anions, (iii) decomposition of the ionophore and/or added charged sites in the membrane, and (iv) leaching membrane ingredients. Although a drift in the offset voltage is most probably related to changes in the membrane composition, by characterizing short- and long-term stability of ISEs, an eventual drift in the potential of the reference electrode is generally not considered. By and large, no information is provided on the stability of the reference electrodes when the performance characteristics of novel ISEs are reported. In summary, instead of addressing the origin of the drift of ISEs, they are frequently calibrated to achieve the required precision and accuracy of the determinations.

In the last decade, microfabricated, planar ISEs have been progressively gaining importance [14]. The driving force behind these developments is related to the need for simple, single-use devices for measurements in a medical context, such as in emergency situations, bed-side analysis, and in the doctor's office. These devices should be manufactured cost-effectively and work without calibration (or with minimal calibration) for the analysis of minute sample volumes (up to a few microliters). In addition, there is a continuous desire for short- or long-term in vivo monitoring of blood electrolytes, which would require sterilizable, biocompatible sensor structures with excellent stability (i.e., with negligible potential drift in the time frame of the experiment). Unfortunately, there are no IUPAC recommendations for evaluation of the performance characteristics of planar microfabricated electrodes. Consequently, the protocols used to evaluate the performance characteristics of macroelectrodes are generally adapted to characterize planar microfabricated electrodes. However, this is not always appropriate in view of the differences in the design of macro- and microfabricated planar microelectrodes.

The goal of this technical report is to direct attention to ISE practices and the striking need for updated or refined IUPAC recommendations, and to highlight critical areas which need new recommendations in accordance with the new state of the art of macro- and microfabricated planar microelectrodes. Some of these ISE practices have never been addressed by IUPAC but have gained importance with the technological and theoretical developments of recent years. Although the authors recognize the need for a new, generally acceptable revision of the current recommendations [1], this revision is far beyond the scope of the present work.

2. IONOPHORE-BASED SOLVENT POLYMERIC (LIQUID) MEMBRANE ION-SELECTIVE ELECTRODES

It was recognized quite early that the detection limit of ISEs can be extended to amount concentrations in the submicromol/L range if the primary ion concentrations were set with appropriate ion buffers [19–21]. With precipitate-based electrodes, it was also found that the limited working range of ISEs is due to primary ion contamination arising from the sensing membrane itself [22,23]. In the presence of ion buffers, this contamination is removed from the sensing surface. Under these conditions, the detection limit could be extended to much lower concentrations [24,25], and the selectivity coefficients were improved [26]. Unfortunately, in the absence of the ion buffers (i.e., during the analysis of real samples), the appealing selectivity coefficients and detection limits could not be reproduced. The knowledge that the source of the limited working range of neutral and charged carrier-based solvent polymeric membranes is related to minor ionic fluxes across the membrane came much later [10,16]. However, once the origin of the contamination (i.e., minor ionic fluxes across the membrane) had been eliminated (or minimized), spectacular improvements in the detection limit and selectivity coefficients of ISEs were achieved [27]. Ideal membranes do not contaminate their boundary layers, but real membranes, with less than perfect ion- and permselectivity, do. It is not always realized that highly selective ionophores are not enough for detection at submicromol/L concentrations in complex matrices. Recognizing the role of the other membrane components, especially charged sites and lipophilic electrolytes, has been essential in membrane optimization. Unfortunately, the optimized membrane performance may change during use as membrane components decompose or leach out from the membrane into the sample, which limits the sensor's lifetime [28–32].

2.1 Charged sites in solvent polymeric membranes

Membranes frequently contain built-in "fixed" ionic sites, e.g., negatively charged immobile $-SO_3^-$ groups in sulfonated cross-linked polystyrene membranes, or intentionally added hydrophobically trapped "mobile" ionic sites, e.g., tetraphenylborate derivatives in plasticized poly(vinyl chloride) (PVC). The response of ISEs, based on ionophore- or ion carrier-loaded solvent polymeric or liquid membranes (i.e., membranes with an incorporated lipophilic complexing agent that reversibly binds ions) strongly depends on the charge sign and amount concentration of these fixed and mobile ionic sites. The importance of fixed and mobile sites in ionophore-based solvent polymeric membranes has been advocated very early [33,34]. The presence of ionic sites is commonly used to explain the permselectivity of neutral carrier-based solvent polymeric membranes, which is a prerequisite for ISE responses. A smaller fraction of ionic sites regularly originates from impurities of membrane components, such as the membrane plasticizer or the polymer matrix (e.g., PVC) [35]. The dominating fraction of ionic sites, however, is deliberately incorporated into the membrane in order to improve its performance and characteristics. Deliberately added ionic sites are nowadays routinely used to decrease the electric resistance of the membrane [36] and the interference of lipophilic counterions related to salt co-extraction into the membrane (Donnan exclusion failure) [37]. Added sites are also utilized to control the potentiometric selectivity [38] and to shorten response time [39] of polymeric membrane electrodes.

Since the materials utilized in ion-selective membranes (e.g., polymers or plasticizers) almost always contained some ionic impurities, it was not trivial to recognize the importance of charged sites in the ion-selective membranes and very little is still known about the properties of membranes free of ionic sites. For understanding the role and to demonstrate the need for ionic sites in ion-selective membranes, the potentiometric properties of membranes free of ionic sites had to be compared to those with "regular" composition (containing only fixed ionic sites or both fixed and mobile ionic sites) [40]. To better understand the influence of ionic sites on the potentiometric responses of solvent polymeric membrane ISEs, optical second-harmonic generation (SHG) at the liquid–liquid interfaces of ionophore-free and neutral ionophore-incorporated membranes has been measured simultaneously with

cell voltage measurements in the presence and absence of ionic sites (Fig. 1) [41,42]. Changes in the primary ion concentration of the aqueous sample solutions result in Nernstian potential responses of electrodes fabricated with membranes containing fixed or mobile ionic sites. The corresponding changes in the extent of charge separation at the sample/membrane interface are manifested by changes in the concentration of oriented SHG-active species. In contrast, membranes without ionic sites showed no potential response or only transient potential response upon changes in the primary ion concentration. In site-free membranes, the concentration of SHG-active primary ions or primary ion complexes at the interface is very low and independent of the primary ion activity in the sample solution. The extent of charge separation at the membrane/solution interface of ionic site-free membranes remains constant even when the concentration of the sample solution has been altered. This confirms that ionic sites, with charge signs opposite to that of the primary ions, are a necessity for counterion-independent primary ion responses in ionophore-based potentiometric sensors [42].

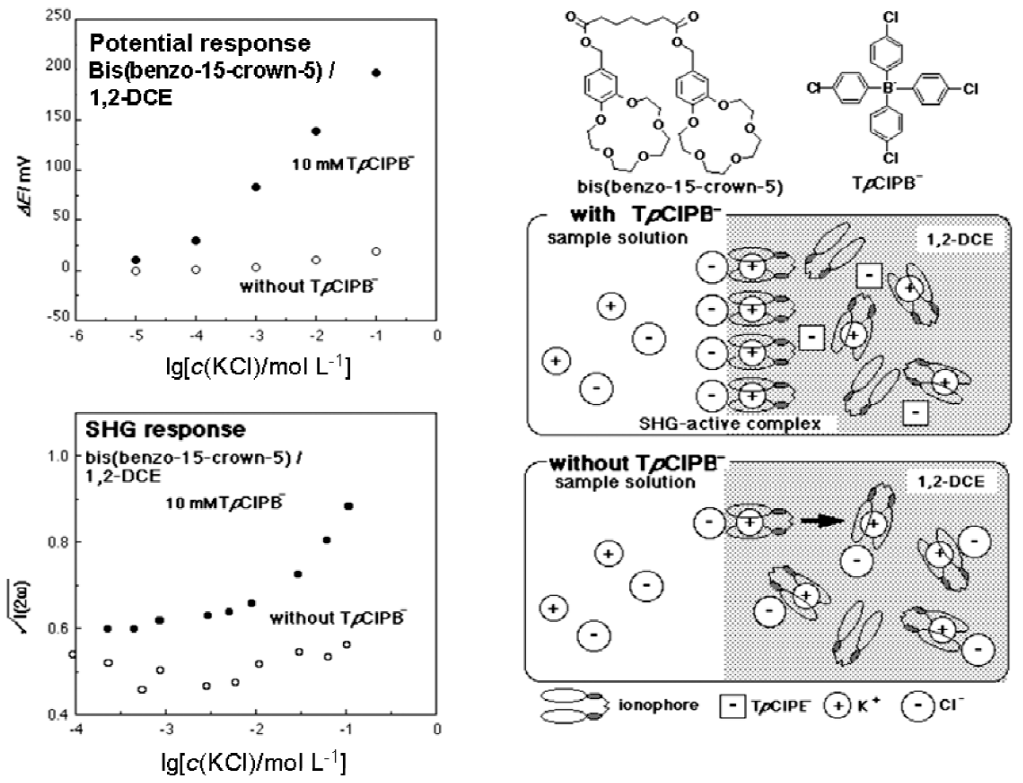

Fig. 1 Dependence of membrane potential change ΔE and the optical SHG response, $\sqrt{I(2\omega)}$, on the concentration of added lipophilic anionic sites [tetrakis (*p*-chlorophenyl) borate, T*p*ClPB] (left), and schematic representation of the corresponding surface model that shows orientation of SHG-active K^+-ionophore complex molecules across the interface in the presence of the added anionic sites in the membrane phase (right) [41,42]. *I*: intensity of SHG response, ω: angular velocity; bis(benzo-15-crown-5: bis(1,4,7,10,13-benzopentaoxacyclopentadecin-15-ylmethyl) heptanedioate, DCE: 1,2-dichloroethane.

2.2 Determination of the concentration of charged sites in solvent polymeric membranes

Precise control of the total concentration of intrinsic and added ionic sites in neutral carrier-based ISE membranes can improve many aspects of electrode performance. The charge sign of either fixed ionic

sites or mobile hydrophobic ion sites determines whether a membrane is permselective for cations or anions of the sample. The mole ratio of the ionophore to the intrinsic or added ionic sites in an ion-selective membrane can significantly influence the selectivity of ionophore-based electrodes. Consequently, full knowledge of the ionic site "inventory" in an ion-selective membrane may be essential when new, uncharacterized polymers or plasticizers are used for ISE fabrication. Accurate ionic site control, however, requires a simple method for the determination of ionic sites in solvent polymeric membranes and membrane components. Unfortunately, most of these methods are quite cumbersome and cannot be easily adapted for screening the ionic site concentration in novel membrane matrices and plasticizers [43–48]. More recently, a simple spectrophotometric method was developed for the fast and accurate determination of ionic site concentrations (covalently attached functionalized groups and/or ionic impurities) in plasticized polymeric membranes and membrane plasticizers [49]. The method is based on the determination of the degree of protonation of hydrogen ion-selective chromoionophores incorporated into these membranes or dissolved in the membrane plasticizers. In electroneutral membranes, the positively charged protonated chromoionophore concentration is equal to the total concentration of negative sites [49], i.e., the quantitative measurement of the protonated chromoionophore concentration in membranes and membrane plasticizers provides the concentration of negatively charged ions in the same matrices. The method was applied to the determination of ionic sites (both positively and negatively charged) in PVC materials (different purity grade, and bearing various functional groups), polyurethanes (aliphatic, aromatic, and polycarbonate-based), and selected plasticizers [2-nitrophenyl octyl ether and bis(2-ethylhexyl)decanedioate]. The simplicity of the method qualifies it for fast quality control or fast screening of optically transparent existing and new membrane materials. However, if dyes susceptible to photochemically initiated decomposition are used as "indicator" molecules in these experiments, it is essential to know the influence of such decomposition on the precision and accuracy of the determination of the ionic site concentrations.

2.3 Changes in the membrane composition through leaching and decomposition

In vivo measurements require small sensor sizes. Small ion sensors are delicate devices with fragile (a few μm thick) membranes which contain only a few nanograms of active components. Delamination or perforation of the membrane results in complete loss of the sensor functions. However, in addition to catastrophic failure of an ion sensor, the gradual deteroriation of its analytical performance due to the dissolution of the membrane components into the sample as function of time is also a serious concern. Since the regular and precise calibration of implanted ion sensors is not feasible, evaluation of changes in the analytical performance of permanently implanted ion sensors (i.e., the determination of factors limiting the lifetime of miniaturized ISEs) is a critical issue. The lifetime of a potentiometric sensor was defined as "the time interval between the conditioning of the membrane and the moment when at least one parameter of the functionality characteristics of the device changes detrimentally" [50]. When plasticized polymeric membrane ISEs are exposed to aqueous samples, the equilibrium partitioning of ions at the sample/membrane interface leads to a phase boundary potential. However, together with the sample ions, all membrane components (ionophore, plasticizer, salt additives) partition between the membrane and solution phases. For neutral carrier-based membrane sensors, Oesch and Simon [32] showed that the leaching of the ionophore from the membrane results in a loss of sensitivity and selectivity, thereby limiting the lifetime. The loss of selectivity is related to the change in the optimal ionophore-to-ionic site (fixed or mobile) ratio [51–53], but it is also accompanied by increased membrane resistance, worse detection limit, increased noise, and possibility of initiation of inflammatory responses in vivo [54], etc.

Besides leaching, decomposition of the ionophore and the charged-site additives can change the optimized composition of an ISE or optical sensor (optode) membrane [31,55–57]. Ratio changes in the concentration of ionophores to that of charged sites have been documented as a consequence of the decomposition of certain tetraphenylborate derivatives [56,57], which are widely used as ionic additives

in ion-selective membranes. Derivatives of phenoxazine dyes, e.g., 9-(diethylamino)-5-octadecanoylimino-5H-benzo[a]phenoxazine) (ETH 5294), serve as pH-sensitive ionophores both in potentiometric and optical sensors. The decay in the concentration of the protonated ionophore has been linked to the photochemically initiated, singlet oxygen-mediated decomposition of the ionophore [30]. The rate of decomposition depends on the wavelength of the incident light and the structure of the phenoxazine derivative. No decomposition was detected with ETH 2439 {9-dimethylamino-5-[4-(16-butyl-2,14-dioxo-3,15-dioxaeicosyl)phenylimino]benzo[a]phenoxazine}, and the rate of decomposition was negligible with ETH 5350 {9-diethylamino-5-[(2-octyldecyl)imino]benzo[a]phenoxazine}. However, the rate of decomposition of ETH 5294 was facilitated in the presence of tetraphenylborate (TPB^-), and tetrakis(4-chlorophenyl) borate ($Tp\mathrm{ClPB}^-$) anions, but no photochemically initiated decomposition of ETH 5294 was detected in the membranes loaded with tetrakis[3,5-bis(trifluoromethyl) phenyl] borate ($TFPB^-$) anion. Thus, the use of $TFPB^-$ salts is encouraged to control the ionic site concentration in ISE membranes which are more stable and more lipophilic than TPB^- or TpClPB^- [30,56,57].

The decomposition rate of phenoxazine derivatives in the membrane is also facilitated by anions in the sample solution (e.g., Br^-, I^-, and Cl^-) as they partition into the membrane by salt co-extraction. Consequently, when ETH 5294 is used for the indirect determination of the site concentration of polymeric membranes and membrane plasticizers [49], HNO_3 or H_2SO_4 has to be used to protonate the ionophore to avoid difficulties in interpreting the data due to the decomposition of the protonated form of the chromoionophore.

2.3.1 Determination of the ionophore/site concentration in ion-selective membranes

Changes in the concentration of membrane "components" due to their leaching or decomposition are particularly significant when microfabricated electrodes and microsphere optodes are exposed to large volumes of sample or used in long-term in vivo applications, because the total amounts of components are extremely small in these sensing devices [14,28,58,59]. For example, the amount of ionophore in a 5-μm thick, 50-μm diameter disc-shaped membrane cast over a planar sensor surface is only about 0.2 ng. In case of an average molecular weight of 1000 Da, the amount of ionophore is about 0.1 to 0.2 pmol. To identify electrode failures related to loss of membrane components and/or to estimate the residual lifetime of a sensor in use, the ionophore concentration in the membrane should be followed as a function of time. A simple chronoamperometric method, based on a single transient measurement, provides both the free ionophore and the ionic site concentration in a solvent polymeric membrane with about 8–10 % accuracy if the experimental conditions, such as the geometric parameters of the sensing membrane, the diffusion coefficient of the ionophore, and the mobile ionic sites in membrane matrix are known [28,29]. The diffusion coefficients of the different ionophores and ionic sites in plasticized membranes of the common membrane composition with 2:1 plasticizer-to-PVC ratio are fairly similar ($\approx 2 \times 10^{-8}$ cm^2/s [60] and $\approx 3 \times 10^{-9}$ cm^2/s [61]), which can provide an approximate value for the evaluation. However, if the required parameters for the determination of absolute concentration are not known, a relative measurement is still possible. It can provide fractional changes in the membrane composition upon exposure to different samples.

3. DETECTION LIMIT OF ION-SELECTIVE ELECTRODES

In recent years, potentiometry has been extended to trace analysis and has become an alternative technique for replacing stripping voltammetry, atomic absorption spectrometry, and even inductively coupled plasma/mass spectrometry (ICP/MS) [8]. Today, optimized ISEs can reach detection limits down to the low parts-per-trillion level in concentrations (10^{-12} mol L^{-1}) for a number of analytes. The key to these spectacular improvements has been the reduction of transmembrane ion fluxes contaminating the sample solution in the vicinity of the sensing membrane. Since ISEs have a wide dynamic range, in analytical practice several orders of magnitude difference may exist between the two sides of the sens-

ing membrane (sample and IFS). Most commonly, a relatively concentrated solution of the primary ion is used as the IFS (10^{-2}–10^{-3} mol L^{-1}). Under such conditions, minor ionic fluxes are established between the two sides due to the non-ideal ion- and permselectivity of practical sensor membranes, which generates an elevated primary ion concentration at the sample side of the sensor surface. The effect is negligible down to 1 μmol L^{-1} sample concentration, however, it plays a decisive role at the submicromol/L concentration level and may cause a large bias in determinations of the detection limits and the selectivity coefficients toward highly discriminated ions.

Using an inner solution that exactly matches the concentration of the sample solution can eliminate ion fluxes across ISE membranes. Unfortunately, matching the concentration of the sample is not a realistic alternative for analysis of sample lots with high variability in analyte concentrations. However, for the analysis of highly dilute samples, using highly diluted IFSs is advisable in which the concentration of interfering ions is also around the mean expected concentration value of the sample lot. Under such conditions, the ion-exchange processes are expected to be similar on both sides of the membrane. Creating a large flux of analyte across the membrane toward the IFS, by selecting exceedingly low primary ion activities in the IFS, is also disadvantageous. It depletes sample ions near the membrane and leads to a non-ideal, "super-Nernstian" responses.

As shown in Fig. 2, under such experimental conditions the IUPAC method [1] for determining the detection limit cannot be unambiguously used. However, the concentration of the primary ion at the point of intersection of the extrapolated lines of the Nernstian (high concentration) and nonresponsive (low concentration) segments of the curve (A) can be considered as an "attainable" detection limit under the stated experimental conditions (i.e., IFS composition, interference concentration level, etc).

Besides the attainable detection limit, the Nernstian response range can also be used to characterize an ISE. The Nernstian response range is defined as the activity range in which the electrode response is in accordance with the Nernst equation. The activity values associated with the points labeled C or D in Fig. 2 could be considered as the low activity limits of the Nernstian response range of an ISE with "super-Nernstian" response function. These activities can be determined from the intersection of the extrapolated lines of the Nernstian (high concentration) and "super-Nernstian" segments of the curve (C) or from the measured cell voltage which deviates 18/z mV from the Nernstian response curve (D). Finally, although intersection (B) is apparently in accord with the existing IUPAC recommendation [1], its practical value for evaluating the detection limit of an ISE is questionable.

The detrimental effect of concentration gradients across the membrane and the related transmembrane ion fluxes contaminating the sample solution can be minimized through membrane optimization. Any measures that decreases ion fluxes in the membrane is beneficial for reaching a lower attainable detection limit [17,62]. The possibilities of advanced membrane technology include the use of membranes with (i) immobilized ionophores, plasticizers, and ionic sites; (ii) increased polymeric content for decreased diffusion coefficients; (iii) increased membrane thickness; (iv) decreased ionic site concentration; (v) decreased effective surface; (vi) incorporated lipophilic particles; and (vii) incorporated lipophilic electrolytes. Intensively stirred or flowing solutions can remove the "residual" traces of primary ion contamination from the ISE surfaces, which could not be prevented by membrane optimization. In fact, better detection limits were achieved in vigorously stirred or flowing solutions [11,12,63]. Very low currents can also be used to compensate zero-current ion fluxes [11,12]. Although attractive, because it is easier than optimizing the IFS composition, this current polarization method has the same fundamental problem that a given current setting is optimal for only one "typical" sample solution concentration. Finally, one of the fundamental sources of transmembrane ion fluxes (i.e., leaching of primary ions from the IFS) can be completely eliminated by using solid-contact electrodes [64–70]. Consequently, solid-contact electrodes in combination with sensor miniaturization are expected to improve the detection limits by several orders of magnitude [67,69].

By combining several of these strategies, detection limits down to nano- and even picomol/L concentrations were reported for a variety of ISEs, and it has been demonstrated that potentiometric ISEs are adequate for monitoring metal ion concentrations in drinking water [27,71,72]. These achievements

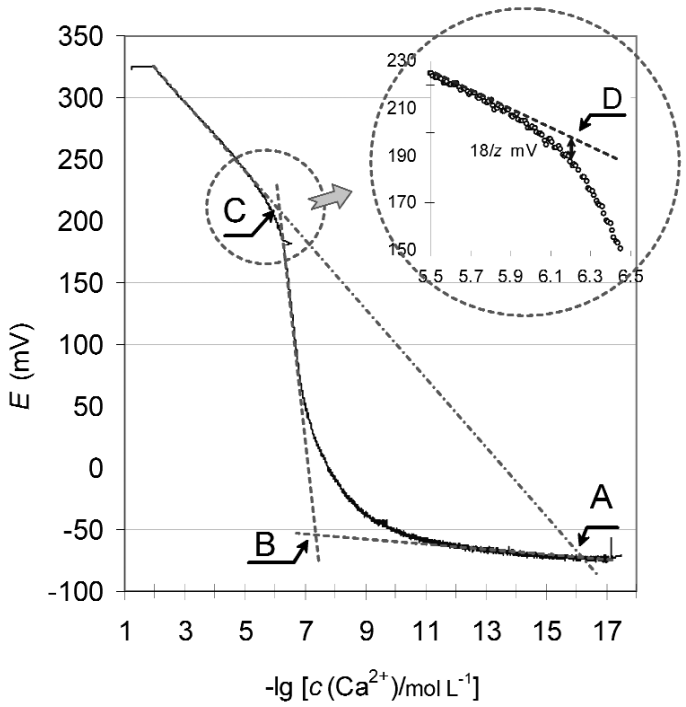

Fig. 2 Difficulties in the unambiguous determination of the detection limit of ISEs demonstrated on the calibration curve of an ETH 1001-based calcium-selective electrode. Membrane composition: 2 mg ETH 1001, 1.32 mg KTFPB, 100 mg o-NPOE, and 200 mg high-molecular-weight PVC. IFS: 10^{-3} mol L^{-1} $CaCl_2$ + 0.05 mol L^{-1} EDTA at pH = 6.55. Calculated free Ca^{2+} ion activity in the IFS: 6.3×10^{-10} (pCa = 9.2). The inset is an enlarged section of the circled area of the curve where the experimental data start to deviate from the Nernstian response. A, B, C: ion activity levels corresponding to the intersections of the extrapolated linear sections of the curve; D: ion activity level at which the measured cell voltage deviates 18/z mV from the Nernstian response curve. ETH 1001: diethyl 12,12'-[(2*R*,3*R*)-butane-2,3-diylbis(oxymethylenecarbonyl)bis(methylimino)]bisdodecanoate; KTFPB: potassium tetrakis[3,5-bis(trifluoromethyl) phenyl] borate; o-NPOE: 2-nitrophenyl octyl ether; PVC: poly(vinyl chloride).

would not be possible without the simultaneous improvement of the experimentally determined selectivity coefficients of the electrodes. In ISEs in which the bias due to primary ion fluxes has been eliminated, selectivity coefficients have been found many orders of magnitude more favorable than those originally reported [26,73–75].

4. REEVALUATION OF THE PUBLISHED SELECTIVITY COEFFICIENT DATA

The most discussed, criticized, misinterpreted, and misused terms of the 1994 IUPAC recommendations are related to the selectivity coefficients of ISEs and their determinations [1]. The selectivity coefficients are the foremost important characteristics of ISEs, informing about the ability of the sensing membrane in discriminating the primary ion against other ions of the same charge sign. Although the 1994 IUPAC recommendations stressed that the recommended procedures should only be applied (or have significant meaning) when the electrode exhibits ideal (Nernstian) response slopes for both the primary and the interfering ions, these prerequisites were not tested, or could not be fulfilled. A general equation to calculate the selectivity coefficient according to the extended Nikolskii equation is

$$\lg K_{\mathrm{I,J}}^{\mathrm{pot}} = \frac{z_{\mathrm{I}} F\left(E_{\mathrm{J}} - E_{\mathrm{I}}\right)}{RT \ln 10} + \lg\left(\frac{a_{\mathrm{I}}}{a_{\mathrm{J}}^{z/z}}\right) \tag{1}$$

where E_{I} and E_{J} are the measured membrane potentials for a solution containing only the salt of the primary ion $\mathrm{I}^{z_{\mathrm{I}}+}$ ion or the interfering ion $\mathrm{J}^{z_{\mathrm{J}}+}$ with charges of z_{I} and z_{J}, respectively. The symbols F, R, and T have their usual meaning.

The selectivity coefficient is a constant parameter for a given electrode if Nernstian response slopes are observed for both the primary and the interfering ions. This can be illustrated by inserting the Nernst equation for the primary and interfering ions (eqs. 2 and 3) into eq. 1

$$E_{\mathrm{I}} = E_{\mathrm{I}}^{\mathrm{o}} + \frac{RT \ln 10}{z_{\mathrm{I}} F} \lg a_{\mathrm{I}} \tag{2}$$

$$E_{\mathrm{J}} = E_{\mathrm{J}}^{\mathrm{o}} + \frac{RT \ln 10}{z_{\mathrm{J}} F} \lg a_{\mathrm{J}} \tag{3}$$

$$\lg K_{\mathrm{I,J}}^{\mathrm{pot}} = \frac{z_{\mathrm{I}} F}{RT \ln 10}\left(E_{\mathrm{J}}^{\mathrm{o}} - E_{\mathrm{I}}^{\mathrm{o}}\right) \tag{4}$$

Apparently, the selectivity coefficient for ion-selective sensors with Nernstian slopes for both the primary and interfering ions can be calculated from the respective E^{o} values. However, until recently, most ISEs did not show Nernstian response slopes for most of the interfering ions. Indeed, the electrode potentials in the presence of highly discriminated ions generally did not show any concentration dependence because the primary ion concentration leaching out from the membrane controlled the response, i.e., the reliable determination of the E^{o} values for the interfering ions were not possible.

Similar to the detection limit, improved selectivity coefficient data could be determined in flowing solutions. The improvements could be attributed to the removal of primary ion traces leaching from the sensing membrane from the electrode surface. Consequently, these "dynamic" selectivity coefficient values were dependent on the experimental conditions (i.e., flow rate, cell geometry, etc.) used for their determination.

Recently, it has been reported that Nernstian, or nearly Nernstian, response slopes can be observed even for highly discriminated ions if one of the following three procedures is used:

(1) Perform the measurement in the presence of a complexing agent, complexing only the primary ions but not interacting with the interfering ions [26].

(2) Use membranes for the selectivity coefficient determinations which have never been in contact with primary ions. Obviously, the lipophilic salt additives incorporated into the membrane and the IFS on the backside of the membrane must also not contain primary ions [74]. The requirement to attain Nernstian response slope for the discriminated ions is that they can fully displace the ions originally present in the membrane. Achieving complete displacement is often very difficult or hardly feasible if the membrane contains the primary ions. In addressing this difficulty the calibration procedure should be started in solutions of the most discriminated ion, if it is known, which generates the least interference. Conditioning the electrode membrane in the corresponding discriminated ion solutions before measurement is advisable.

(3) Perform the measurement under experimental conditions in which the flux of primary ions is directed toward the IFS and the composition of the IFS remains unchanged during the measurement [76]. It can be achieved by using strong chelating agents [77,78] or ion-exchangers [79] in the IFS, which set a very low primary ion concentration in the IFS and preclude the primary ion leaching toward the sample.

To emphasize that the measured selectivity coefficients or detection limits are not corrupted by primary ion contamination due to ion-exchange or co-extraction processes, the attributes "true" [26], "unbiased" [74], and "ultimate" [76,80] are frequently added in front of the selectivity coefficients. The attribute "ultimate" intends to emphasize that experimental conditions that guarantee the least possible interference were used.

The three above-mentioned procedures provide very similar selectivity coefficient values that reflect the ion-exchange selectivity of the membrane with a thermodynamic meaning. However, the first two methods are somewhat cumbersome and thus not practical. In procedure (1), it is not always possible to find an appropriate buffer system that can be used for a variety of interfering ions. On the other hand, procedure (2) can be performed only once. After the membrane has been exposed to the most preferred primary ion, the electrode will no longer respond in a Nernstian manner to extremely discriminated ions. This method is therefore inapplicable to electrodes which have been previously used in real samples or were conditioned overnight in concentrated primary ion solutions, as is often recommended. It is also inadequate for monitoring time-dependent changes in the selectivity coefficients of practical membranes. Among the three methods, the last one (3), in which primary ions are drained toward the IFS, appears to be the most generally applicable. However, it must be recognized that the IFSs suggested in the literature for probing the practical limits of ISEs are generally prepared with a high concentration of complexing agent {e.g., 5×10^{-2} mol L^{-1} Na_2EDTA [disodium dihydrogen (ethane-1,2-diyldinitrilo)tetraacetate]} in a pH-controlled environment in which the interfering ion concentration levels may be as high as 0.12 mol L^{-1}. The disadvantage of using high concentrations of interfering ions in the IFS in experiments aimed for evaluating the "true", "unbiased", or "ultimate" selectivity coefficients is related to the induced transport of the highly discriminated ions towards the sample.

Most recently, an original method has been proposed for the determination of the ultimate span ISEs [76,80]. The span of an ISE has been defined by IUPAC as the potential difference between the upper and lower detection limits of the electrode [1]. Once the span is known, the ultimately attainable detection limit of the ISE can be calculated by using its theoretical response slope. If the concept of span measurement is extended to interfering ions, it is suitable for the determination of the ultimate selectivity coefficients. The span measurement in combination with subsequent exponential dilution provides the response slopes for both the primary and the interfering ions, which can be utilized for calculating the selectivity coefficients and to confirm the detection limit. Although this comprehensive "span method" (the simultaneous measurement of the ultimate detection limit, the selectivity coefficients in combination with the response slopes for the different ions) is very promising for characterizing ISE responses, the method must be tested for a variety of ionophores (e.g., with low ion-ionophore stability constants and in the presence of ions with large hydration energies) before it can be recommended as a general procedure. Obviously, this note is related to the attainment of the Nernstian behavior [5,81].

5. MEASUREMENT PROTOCOLS FOR OPTIMAL ION-SELECTIVE ELECTRODE PERFORMANCE

In contrast to the latest IUPAC document on the "Measurement of pH" [82], the previous IUPAC recommendations [1,2] on ISEs do not provide detailed protocols for the determination of the analytical characteristics and performance of ISEs and do not contain information on the uncertainties of the determinations. In spite of these limitations, the recommendations were acceptable because small differences in the widely accepted practices hardly modified the measured values. However, the advancement in the theory and practice of ISEs since the publication of the last IUPAC recommendations provides ample evidence that changing the commonly used protocols and practices can lead to orders of magnitude improvements in the selectivity coefficients and the detection limits of ISEs. Some of these new practices were discussed in Sections 3 and 4. In addition, the protocols that proved to be adequate for conventional macroelectrodes with large volume of IFS are not always adequate in testing microfabricated planar sensors, due to differences in their construction.

5.1 Planar microfabricated electrodes

Microfabricated potentiometric sensors are planar versions of the conventional macroelectrodes (i.e., parallel to the dramatic reduction in size), the general three-dimensional electrode structures are compressed into two-dimensional, multilayered arrangements. The first flat-form, plastic membrane, potentiometric electrodes were manufactured in the early 1970s (Eastman Kodak [83]). Microfabrication technologies have entered the field of ion sensors with the implementation of ion-selective field effect transistors [84,85]. These planar, sensor structures can be manufactured cost-effectively in large numbers by thin- and thick-film microfabrication technologies through depositing (casting, spin coating, electropolymerizing, printing, etc.) and patterning multiple membrane layers over solid surfaces. The technology for these so-called "all solid-state" electrodes projected numerous advantages in design (smaller sizes, thus smaller sample volumes), sensor handling (maintenance free), and applications (long-term in vivo monitoring) [14,86,87]. Due to their low cost and small size, microfabricated ISEs are often aimed for the measurements of a few microliters of samples (e.g., whole blood, serum, plasma, urine, or saliva) in single-use cartridges [13]. They have also been tested for short-term in vivo measurements or closed-loop monitoring of ion activities in biological samples [14,87,88].

Sensors aimed for in vivo or closed loop measurements should be sterilizable, biocompatible, and present excellent stability in the time frame of the measurements (e.g., up to several hours to monitor the status of a patient during treatment). In addition, the sensors are expected to have optimal performance characteristics without conditioning and to provide accurate and reproducible results without or with minimal calibration. If microfabrication can provide identical sensors, the calibration of a limited number of sensors from a batch (wafer) should be adequate to characterize the response function of the whole batch (wafer). These requirements are much more demanding than the requisites for ion sensors incorporated into blood electrolyte analyzers, in which repeated multipoint calibration protocols are common.

Unfortunately, dramatic miniaturization may lead to undesirable consequences. Planar sensors prepared like "coated wire" electrodes (with an ion-selective membrane cast directly over an electronically conducting metal/semiconductor surface, e.g., Pt, Au, a conductive polymer, or the gate area of a field effect transistor [89,90]), without IFS or with extremely small IFS volume, were very sensitive to transmembrane fluxes (e.g., H_2O, CO_2, O_2, etc). They required longer equilibration (conditioning) time [67,88] and were characterized by drifting potentials and modest repeatibility. The majority of the problems experienced with these "all solid-state" microelectrodes were related to the mismatch ("blocked" interfaces) between ion-conducting (IS membrane) and electronically conducting phases (substrate electrode) in which the charge carriers cannot pass from one phase into the other [86].

To form reversible interfaces and to adapt the classical inner reference electrode to the planar ISEs, the IFSs of large volume were replaced by small-volume chambers filled with layers of hydrogel integrated into the planar structures. Although the incorporation of "liquid reservoirs" into a sensor structure using thin film microfabrication technologies is difficult, planar potassium and hydrogen ISEs fabricated [14,88] with these hydrogel layers provided potential stabilities in the order of 0.1 mV/h and were used successfully for in vivo monitoring of ion concentration changes [91]. Unfortunately, the benefits of these hydrogel film-based sensors quickly faded away with decreasing sensor sizes (hydrogel volumes). Long-term drifts were experienced in solutions with high concentration of interfering ions and when the concentrations of osmotically active species were fluctuating in the sample solution [92].

To guarantee high potential stability at the interface of the membrane and its solid-contact, films with both ionic and electronic transduction are layered between the ion conducting membrane and the electronically conducting metal [93]. Inherently conducting polymers (CPs), such as polypyrrole (PPy) [64,66,68,94], polyaniline [95,96], poly(3-octylthiophene) [97], and poly(3,4-ethylenedioxythiophene) [98,99] and redox-active self-assembled monolayers [100] have been tested. Although, ISEs without

IFS have inherent advantages (e.g., to eliminate one major source of transmembrane ion fluxes), it is still challenging to fabricate solid-contact planar microelectrodes, which meet the stability and reproducibility requirements of blood electrolyte analysis. The potential stability of CP-based planar sensors is often determined by spontaneous changes in the composition of the CP film after preparation. An additional source of potential instabilities is the spontaneous formation of a water film between the CP film and the IS membrane [101]. If this happens, the solid-contact electrodes behave similarly to planar electrodes fabricated with IFS of extremely small volume (hydrogel layer), i.e., the sensor becomes sensitive to transmembrane transport of water, CO_2 or O_2 [92].

5.1.1 Solid or liquid contact: A simple test protocol

Microfabricated planar ISEs are often intended for biomedical applications in which long equilibration times are unacceptable. Thus, the equilibration time, the time interval in which the sensor achieves its optimal performance, is a very important parameter. Planar sensors with hydrogel inner contact often show a severe positive potential drift following their first contact with an aqueous solution. The hydrogel layer of such electrodes commonly loses water during fabrication or storage. This lost moisture is recaptured during the equilibration (conditioning) process as water is transported across the membrane by diffusion until the osmotic equilibrium between the two sides of the membrane is reached. The rehydration of the hydrogel gradually decreases the primary ion activity in the hydrogel layer (inner solution of planar sensors), which is the source of a positive potential drift (Fig. 3). Storage of the "ready-to-use" planar sensors with hydrogel inner contact in containers with 100 % humidity decreased the preconditioning time from hours to a few minutes [102]. In spite of its importance, the equilibration time needed by a microfabricated, planar ISE for optimal performance characteristics is generally not reported as an essential electrode characteristic.

The equilibration time for solid-contact electrodes is expected to be much shorter as compared to electrodes with hydrogel inner contact. In agreement with these expectations, as shown in Fig. 3, high

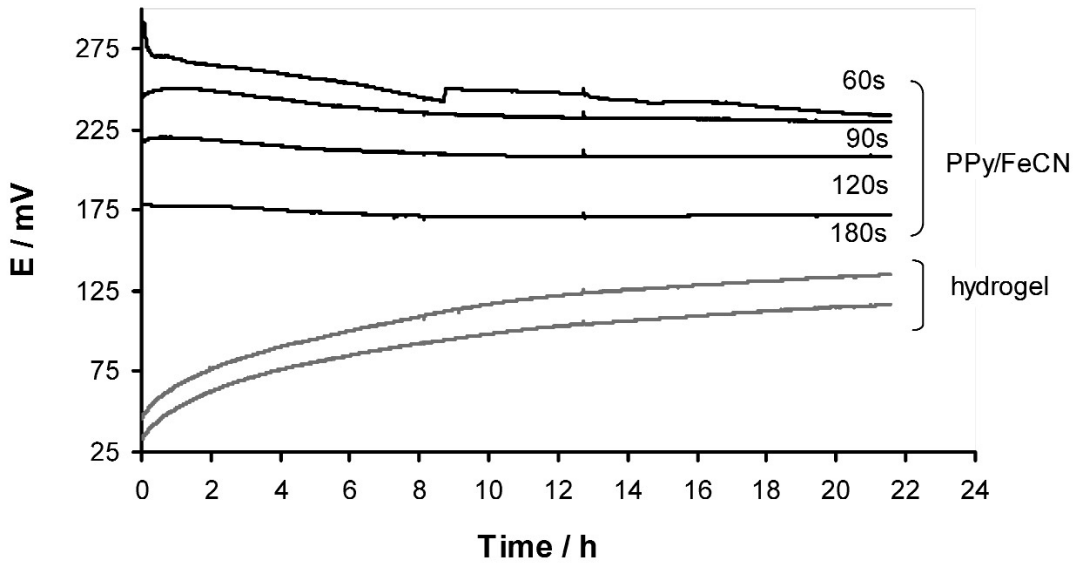

Fig. 3 Short-term stability of freshly prepared (unconditioned) planar, potassium-selective electrodes in a 5 mmol L^{-1} K^+ solution of pH 7.4 with 140 mmol L^{-1} Na^+, 1 mmol L^{-1} Ca^{2+}, and 119 mmol L^{-1} Cl^-. The planar potassium selective electrodes were prepared with potassium hexacyanoferrate (II/III) doped polypyrrole (PPy/FeCN) and hydrogel inner contact. The numbers on the potential time transients of the PPy/FeCN contacted electrodes indicate the time of the electropolymerization (which is proportional to the thickness) of the polypyrrole film.

potential stability (≤0.2 mV/h drift) has been achieved more quickly with microfabricated planar sensors using an appropriate solid contact [67]. But, apparently the equilibration time can be influenced by the characteristics of the solid-contact film. Consequently, in reporting on novel planar sensors, it is recommended that the electrode potential be continuously monitored during the first equilibration time. With cation-selective planar electrodes, a slow, asymptotic, positive drift during the conditioning period indicates the water uptake of the inner layer on the back of the sensing membrane (i.e., the formation of a thin aqueous layer between the inner reference element and the polymeric membrane). Unfortunately, this can happen with any "solid-contact" device. If a thin aqueous layer is formed between the "solid-contact" and the polymeric membrane, all or most of the claimed advantages of a "solid-contact" or "all solid-state" [64] design are lost and the sensor should not be considered to be a "solid-contact" device.

Since formation of a thin aqueous layer behind the sensing membrane could be the main source of potential instabilities, testing all new planar ISEs for the presence of such aqueous layers is considered essential and recommended [101]. It is known that adhesion strength between the sensing membrane and its solid support deteriorates gradually over time after the membrane has been exposed to aqueous solutions. The adhesion strength of a fully hydrated membrane is only a fraction of that which can be determined in dry state [88]. Accordingly, the test providing information on aqueous layer formation between the sensing membrane and its solid support should be repeated periodically in the time frame of the expected lifetime of the planar sensor.

The test is very simple. The protocol is basically the same as that used for evaluating the selectivity coefficient of an ISE for a particular ion with the SSM. The planar sensor is sequentially exposed to a concentrated solution of the primary ion (e.g., $c = 0.1$ mol L^{-1}), the interfering ion, and again the primary ion. Following each exposure, the electrode potential is continuously recorded. The only important difference compared to a "common" selectivity coefficient determination is the time frame of the experiment. Depending on the thickness of the sensing membrane, the electrode potential has to be recorded for 6 to 24 h.

As shown in Fig. 4, planar sensors with solid (upper curve) or liquid (hydrogel layer) contact behave completely differently in this test [101]. Replacement of the primary ion I^+ by a less preferred interfering ion J^+ provides a large positive drift after the expected instantaneous drop in the measured potential with planar sensors fabricated with a liquid contact. The magnitude of the instantaneous drop in potential depends on the selectivity coefficient of the membrane. Similar transients are experienced with "solid-contact" sensors in which an undesirable water film formed between the solid internal contact and the sensing membrane. However, no such behavior is experienced with "real" solid-contact electrodes. The transient response of liquid contact planar sensors (i.e., the apparent loss of ion selectivity of the sensing membrane) can be unambiguously interpreted by the phase boundary potential model [101]. The transient response arises from minute transport of interfering ions into the aqueous film behind the membrane and to the related significant change in its composition. The subsequent replacement of the interfering ion (J^+) by the primary ion (I^+) generates a similar potential transient of opposite sign. However, the drift toward negative potentials is much smaller.

Efforts to provide high-stability "real" solid-contact or "all solid-state" electrodes (without aqueous layer formation) were successful when redox-active, highly lipophilic self-assembling monolayers [103] or overoxidized polypyrrole films [66,69] were layered between potassium- or lead-selective membranes and gold or platinum inner contacts. However, with polypyrrole inner contacts, the quantitative removal of salts from the polypyrrole film by extensive washing was essential for success. Even small traces of water-soluble salt in an inherently conducting film can contribute to aqueous film formation as it drives water across the membrane to meet osmotic equilibrium on the two sides of the membrane.

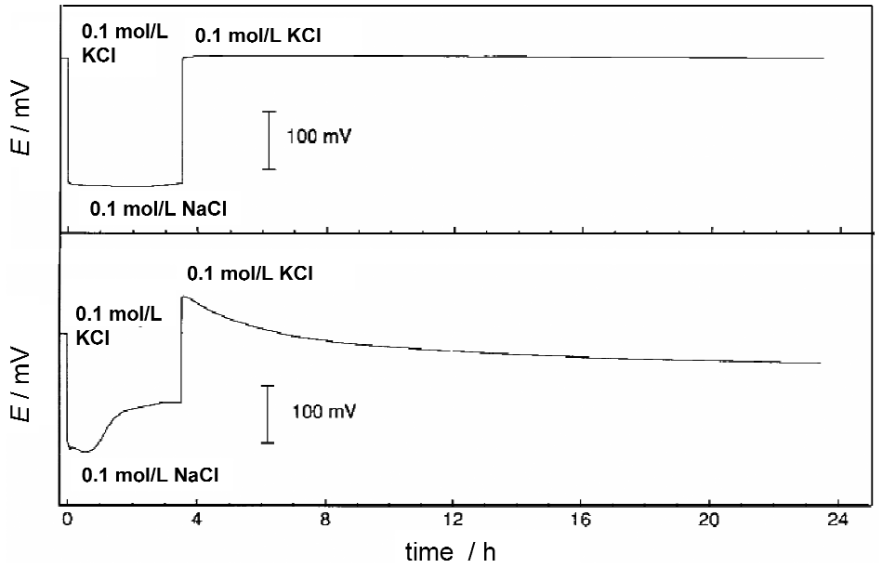

Fig. 4 Response of a solid-contacted K^+-selective electrode with (top) and without (bottom) an aqueous film between the sensing membrane and its solid contact. At time $t = 0$, the conditioning solution (0.1 mol L^{-1} KCl) was exchanged for 0.1 mol L^{-1} NaCl. At $t = 3.5$ h, the sample was replaced by the conditioning solution [Reproduced with permission from M. Fibbioli, W. E. Morf, M. Badertscher, N. F. de Rooij, E. Pretsch. *Electroanalysis* **12**, 1286 (2000)].

6. ANALYTICAL CALIBRATION CURVES (CALIBRATION PLOTS) FOR pH AND OTHER ION-SELECTIVE ELECTRODES

In IUPAC's *Compendium of Analytical Nomenclature* [15] (Section 10.3.3.2), the relation between a measured quantity x and the concentration c is called the *analytical function*. A graphical plot of the analytical function, whatever the coordinate axes used, is called the *analytical curve*. The measured quantity expressed as function of c [i.e., $x = g(c)$] is termed as analytical calibration function while the graphs corresponding to these functions are called analytical calibration curves. In Section 8.3.2.1, the analytical calibration curves of an ISE are denoted as *calibration plots* and the recommended procedure for presenting an ISE calibration plot is given as: "For uniformity, it is recommended that the cell emf is ascribed to the ordinate (vertical axis) with more positive potentials at the top of the graph and that pa_A (-log activity of the species measured, A) or pc_A (-log amount concentration of the measured species measured, A) is ascribed on the abscissa (horizontal axis) with increasing activity to the right". However, in Chapter 8.4. of the recently accepted IUPAC recommendation on the "Measurement of pH" [82], the calibration plot of a hydrogen ion-responsive electrode is plotted with decreasing H^+ ion activity (increasing pH) to the right. To have different recommendations for the H^+ ion-selective and all other ISEs cannot be justified although both recommendations are based on widely accepted practices. To have unified presentation of the calibration plots of all ISEs, it is recommended that the pa_A or pc_A values be plotted on the horizontal axis with increasing pa_A or pc_A values to the right.

7. SYMBOLS AND ACRONYMS

7.1 List of symbols

a_I and a_J	activity of the primary ion I and interfering ion J, respectively
E^o, E^o_I, and E^o_J	constant potential terms in the Nernst equation

E_I and E_J	electrode potential in solution of the a primary ion I or interfering ion solution J
ΔE	potential difference
F	Faraday constant
I	intensity of SHG response
$K^{pot}_{I,J}$	potentiometric selectivity coefficient
R	universal gas constant
T	thermodynamic temperature
z_I and z_J	charge of the primary ion I and interfering ion J
ω	angular velocity

7.2 List of acronyms

DCE	1,2-dichloroethane
ETH 1001	diethyl *N*,*N*'-[(4*R*,5*R*)-4,5-dimethyl-1,8-dioxo-3,6-dioxaoctamethylene]-bis(12-methylamino-dodecanoate)
ETH 2439	9-dimethylamino-5-[4-(16-butyl-2,14-dioxo-3,15-dioxaeicosyl)phenylimino-[a]phenoxazine
ETH 5294	9-(diethylamino)-5-octadecanoylimino-5H-benzo[a]phenoxazine)
ETH 5350	9-(diethylamino)-5-[(2-octyldecyl)imino]benzo[a]phenoxazine
FeCN	potassium hexacyanoferrate II/III
ICP/MS	inductively coupled plasma/mass spectrometry
ISE	ion-selective electrode
IUPAC	International Union of Pure and Applied Chemistry
KTFPB	potassium tetrakis[3,5-bis(trifluoromethyl) phenyl] borate
Na_2EDTA	disodium ethylenediaminetetraacetate
PPy	polypyrrole
o-NPOE	2-nitrophenyl octyl ether
PVC	poly(vinyl chloride)
SHG	second harmonic generation
SSM	separate solution method
TPB^-	tetraphenylborate anion
$TpClPB^-$	tetrakis(4-chlorophenyl) borate anion
$TFPB^-$	tetrakis[3,5-bis(trifluoromethyl) phenyl] borate anion

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