REVIEW

Quality control criteria for solid-contact, solvent polymeric membrane ion-selective electrodes

Ernö Lindner · Róbert E. Gyurcsányi

Abstract After a long history and conflicting views, solid-contact (SC) solvent polymeric membrane ion-selective electrodes (ISEs) emerged as reliable potentometric-sensing devices with unique advantages. From the large variety of proposed SCs inherently conductive polymers emerged as the materials of choice. In our view, the most attractive feature of SC ISEs is their compatibility with thin- and thick-film microfabrication technologies that can provide cheap, mass-produced sensors and sensor arrays that can be integrated with the measuring, data acquisition, and control electronics in a straightforward way. However, despite the impressive properties of certain SC electrodes and their potential advantages, they remained primarily in the research laboratories. To make the jump from the research laboratories into commercial devices, it would be essential to prove that miniaturized SC ISEs can indeed match or surpass the performance characteristics of the conventional, liquid-contact macroelectrodes. In addition, it would be important to settle on the quality control criteria and testing protocols for assessing the performance characteristics of SC electrodes. It could help in interpreting the sometimes-inconsistent experimental data. Once cheap, miniaturized, SC ISEs will mach the performance characteristics of macroscopic-size electrodes, it is expected to have an important impact in a variety of applications requiring robust, maintenance-free, or single-use ISEs, e.g., in homecare or bedside diagnostics, environmental analysis, and quality control assessment. In addition, reliable SC ISEs are expected to revitalize the field of ion-selective field effect transistors and open new possibilities in combination with nanowire-based devices.

Keywords Solid-contact ion-selective electrodes ·
Microfabrication · Quality control · Measurement protocol ·
Long-term stability

E. Lindner (✉)
Department of Biomedical Engineering,
The University of Memphis,
Memphis, TN 38152, USA
e-mail: elindner@memphis.edu

R. E. Gyurcsányi (✉)
Department of Inorganic and Analytical Chemistry,
Budapest University of Technology and Economics,
Szt. Gellért tér 4,
Budapest 1111, Hungary

R. E. Gyurcsányi
Research Group for Technical Analytical Chemistry
of the Hungarian Academy of Sciences,
Budapest University of Technology and Economics,
Szt. Gellért tér 4,
Budapest 1111, Hungary

Introduction

The superb selectivity of ion-selective electrodes (ISEs) simplified electrolyte analysis and made simple, separation-free monitoring of ionic activities in complex matrices possible [1–5]. Although clinical applications of ISEs account for more than a billion of blood electrolyte measurements per year, comprising a large share in the *in vitro* diagnostic sensor market, chemists still consider ISEs primarily as simple, routine tools for direct potentiometry and as indicator electrodes in potentiometric titrations. When ISEs are used for the analysis of environmental and pharmaceutical samples or for the determination of physico-chemical constants, the available sample volume typically does not impose restrictions on the size of the electrodes. Therefore, conventional ISEs are generally quite large with

Fig. 1 Conventional ISE designs. **a** Schematic representation of a symmetrical cell with liquid contact on both sides of the ion-selective membrane (M^+ is the primary ion, L is the ionophore, R^- is the mobile site, ML_n^+ is the ionophore ion complex). **b** Photograph and cross-sectional view of the Philips IS 561 liquid membrane electrode body (Möller Glassbläserei, Zürich Switzerland). **c** Microscopic image of the "Christmas tree" electrode array. The *inset* shows the layered construction of this microfabricated planar sensor with a hydrogel layer compartment holding the inner filling solution
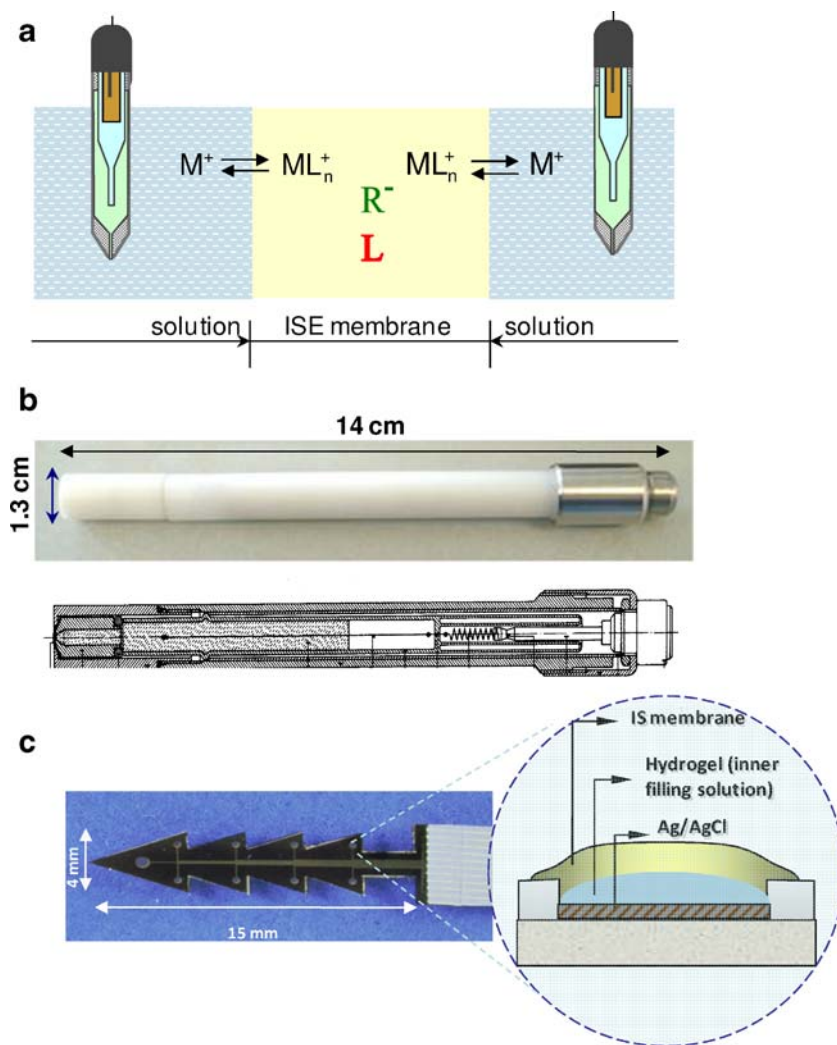
sensing surfaces between approximately 3 and 100 mm^2. In these conventional ISEs (macroelectrodes), the sensing membrane is sandwiched between two solutions, the sample and the inner filling solutions (Fig. 1a, symmetrical cells). Thus, the membrane potential is measured between two reference electrodes, most often $Ag|AgCl|Cl^-$ electrodes. Conventional macro-ISEs are robust, have excellent potential stability, long lifetime, and are hardly susceptible to electrical noises, e.g., the popular IS-561 Philips liquid membrane ISEs (Fig. 1b).

Throughout their history, ISEs adapted well to wide-ranging applications by preserving the inner filling solution-based symmetrical configuration. They complied with the requirements in industrial applications and met the requirements essential for in situ environmental analysis such as the monitoring of sedimentation processes in waters [6]. Conventional-size electrodes in combination with micro-fluidic cells were utilized for assaying minute sample volumes. ISEs were prepared also in extremely small sizes for probing single cells, to follow ionic transients in the

ischemic heart [7] and to analyze a few microliters of blood or other biological samples. They were made in the form of vibrating, self-referencing probes [8–10] for ion flux measurements in plant physiology. Multiplexed measurements could be done even under in vivo conditions by using microfabricated ISE arrays, such as the "Christmas tree" sensor array arrangement shown in Fig. 1c with sensing areas of the individual electrodes between 0.008 and 0.05 mm^2 [7]. Two-dimensional mapping of ion concentration profiles became possible through interfacing ion-selective microelectrodes with high-resolution scanning devices, which is known as scanning potentiometric microscopy [11].

In solid-contact (SC) ISEs, the sensing membrane is sandwiched between the sample solution and a SC (Fig. 2a–c, asymmetric sensor cells). Having so many successful applications with conventional ISEs utilizing symmetrical design, one may ask what the motivation behind developing and implementing SC ISEs is, especially because practically no report claims that the change from
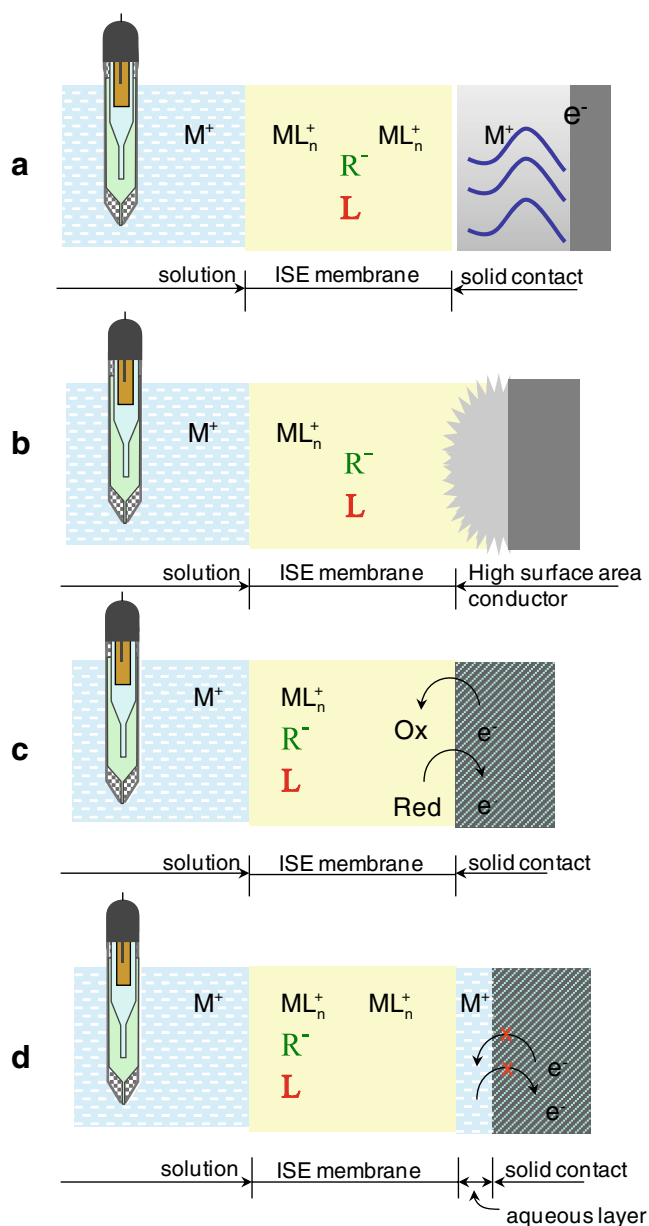
Fig. 2 Schematic representation of membrane separated potentiometric cells with: **a** conducting or redox polymer-based SC, **b** high-surface area, large-double-layer capacitance, solid electron conductor-based SCs, **c** electron-conducting metal contact to the ion-conducting sensing membrane where the membrane|metal interface is reversibly poised with a redox couple (*Ox/Red*) loaded into the membrane, **d** an unintentionally formed aqueous layer between the sensing membrane and its electron-conducting metal/SC

liquid contact to SC improves the analytical parameters of the corresponding ISEs in regular laboratory use. Indeed, in most reports on SC electrodes, matching the performance characteristics of liquid-contact electrodes is the claimed attainable goal. An obvious explanation could be that certain problems inherent to ISEs made with inner filling solutions automatically disappear with SC ISEs, e.g., the drying out of the inner filling solution (need for main-

tenance) and the limitations in applications (electrode orientation, temperature and pressure range, etc.). While maintenance-free use is a real advantage in all applications and could be essential in others, e.g., remote sensing, it is difficult to assess the significance of ISE applications that require ISEs that function at extreme pressures (deep sea ion measurements) or temperatures, especially, because besides of the liquid inner contact, other parts of an ion-selective membrane-based electrochemical cell assembly also impose limitations in the use of ISEs.

It is common to identify the inner filling solution of ISEs as the major obstacles toward miniaturization. This point of view is certainly questionable, since pulled micropipette-type ISEs, where the inner filling solution is preserved, can be fabricated with diameters down to 0.1 μm [9, 10, 12]. Unfortunately, the fabrication of pulled micropipette-type ISEs is a delicate task, and the microelectrodes are rather fragile. Consequently, their application is generally limited to experiments in cellular physiology [9, 10]. Thus, the motivation for SC ISEs could be the simple, cost-effective fabrication of robust, reliable, and maintenance-free mini-aturized sensors. From a manufacturing point of view, the most attractive feature of SC ISEs is clearly their compatibility with thin- and thick-film microfabrication technologies that can provide cheap, mass-produced sensors and sensor arrays integrated with the measuring, data acquisition, and control electronics [13, 14]. Therefore, the present discussion focuses on miniaturized SC electrodes for laboratory use and biomedical applications.

Considering the long history of SC ISEs and the projected advantages for the combination of miniaturized SC ISEs and microfabrication, it is somewhat surprising that the advantageous properties of SC ISEs are frequently demonstrated only through examples in which the electrode body and the sensing area of the studied ISEs are comparable to that of conventional macro-ISEs. Although ISEs with large membrane volume or surface area are advantageous in certain applications, e.g., in when the dissolution of the membrane ingredients limits the lifetime of the sensor, experimental results on these macro-size sensors do not necessarily prove that the same advantageous performance characteristics can be achieved with micro- or nanometer-size SC ISEs. In addition, they are not adequate for recording local concentration changes or imaging concentrations with high spatial resolution (Fig. 1c). Since this is not the only contradiction in the field, in this review, we focus on the critical aspects in SC ISE development. We are proposing essential guidelines to elude some common misconceptions rather then to provide a comprehensive overview on the various materials and methods used for preparing SC ISEs. Emphasis is given to SC ISEs with ionophore-based solvent polymeric membranes, because the ionophore-based electrodes are now in

the forefront of the interest, and the development of proper SCs for this class of ion-selective membranes seems to be the most challenging.

Solid contacts for ISEs

Selective ion exchange processes provide thermodynamically well-defined phase boundary potentials at the two membrane solution interfaces of symmetrically bathed membranes (Fig. 1a). Well-defined phase boundary potentials are the requirement for adequate long-term potential stability and reproducibility in potentiometric measurements. In ISEs with solid-state membranes, e.g., silver halide-based sensors, the compatibility between the semiconductor membrane (e.g., AgCl) and an electron-conducting metal contact (e.g., Ag) provides straightforward coupling. However, to interface an electron-conducting SC with an ion-conducting liquid ion-selective membrane is challenging (Fig. 2a–c). Inadequate SCs in combination with liquid ion-selective membranes led to unreliable sensors with drifting potentials and poor reproducibility. In addition, they were subject to interferences related to the transport of small molecules, e. g., H_2O, CO_2, O_2, etc., across the membrane. Such interferences were hardly experienced with conventional liquid-contact electrodes or with SC ISEs utilizing impenetrable solid-state membranes [15].

The history of SC ISEs can be traced back to the early 1920s [16]; however, emerging microfabrication technologies and the appearance of ion-selective field effect transistors (ISFETs) provided the largest boost for the development of miniaturized ISEs with SCs [14, 17, 18]. For details on the early approaches for designing SC electrodes, the reader is referred to the excellent review of Nikolski and Materova [19]. According to the authors, the first experiments were aiming to replace the inner solution in pH-sensitive glass electrodes with solid-state materials, such as alkali metal amalgams or glasses that due to their high Fe^{2+}/Fe^{3+} and Na^+ content exhibited both ion and electron conduction (redox activity). Precipitate-based electrodes, such as the PbS-based lead-selective electrode, were interfaced through several mixed layers of PbS, Ag_2S, and Ag, pressed in pellets [20]. Similar, reversible solid inner contact was developed for the LaF_3 single-crystal-based F^--selective electrode. In this SC fluoride-selective electrode, a LaF_3-doped AgF layer, deposited onto the backside of the sensing membrane by heat fusion, served as an ion-to-electron transducer. At the two boundaries of the silver ion-conducting AgF layer, fluoride ion exchange and the $Ag|Ag^+$ redox equilibrium provided well-defined phase boundary potentials [21].

Regarding SC ISEs with solid-state membranes, it should be emphasized that although, in principle, it is

rather simple to envision reversible SCs for these sensors, their fabrication is rather demanding especially in very small sizes. In addition, since many of the involved materials are light, temperature, and oxygen sensitive, improper control of the fabrication of the SCs can lead to large variations in the E^0 values and electrode failures. Consequently, with a few exceptions, e.g., pH-selective field effect transistors, the symmetric cell configurations with hermetically sealed inner solution retained their importance, e.g., in the conventional pH-sensitive glass electrodes.

The implementation of different SCs for liquid membrane-based ISEs commonly preceded the exploration and understanding the mechanism of the potential determining processes at the ion-selective membrane|SC interface. The advent of the coated wire electrode [22], in which a 0.018-in.-thick platinum wire was dip coated with a calcium didecylphosphate-loaded, dioctylphenylphosphonate-plasticized poly (vinyl chloride) (PVC) membrane is a relevant example. The problems with the coated wire electrodes stimulated valuable discussions and extensive research in SC ISEs, which resulted in the formulation of the essential criteria for stable potential SC ISEs [19]: (1) reversible transition from ionic (membrane) to electronic (SC) conduction, (2) ideally nonpolarizable interface with high exchange current density, which is not influenced by the input current of the measuring amplifier, and (3) SC with stable chemical composition.

In accord with these criteria, materials utilized as SCs for liquid-membrane ISEs should have both redox and ion exchange properties. The redox properties of these materials secure the phase boundary potential at the SC|metal interface, while their ion exchange properties provide ion exchange equilibrium-based stable potential at the liquid membrane|SC interface (Fig. 2a). One of the first implementation of this paradigm utilizes metallic silver (Ag)| silver tetraphenylborate (AgTPB)|cation-selective membrane configuration. In this system, the TPB^- anions present both in the cation-selective membrane (to induce permselectivity) and in the SC provide a well-defined phase boundary potential at the membrane|AgTPB interface while the Ag^+ ions at the AgTPB|Ag metal interface [23]. It is not clear why has this intuitive approach been almost completely forgotten in ion-selective potentiometry. The light sensitivity and the limited lipophilicity of the tetraphenylborate anions [24] may have discouraged further investigations. However, since this early work, new tetraphenylborate derivatives emerged, with much less light sensitivity and much better lipophilicity, which makes this approach worth revisiting.

The incorporation of a silver-selective ionophore into the membrane of sodium and ammonium ISEs can be considered as a variation of the same idea. It is claimed that the reversible redox reaction $AgL^+_{(m)} + e^- \leftrightarrow Ag_{(s)} + L_{(m)}$ (where

L is the silver-selective ionophore in the membrane phase (m) and (s) denotes the solid phase) stabilizes the potential at the interface of the membrane and its silver epoxy film-based SC [25]. The disadvantage of the incorporation of the silver ionophore into the membrane is that it can be detrimental to the overall sensor response, first and foremost to the selectivity of the membrane toward the primary ion. Another possible approach for providing thermodynamically well-defined phase boundary potentials at the membrane|SC interface is shown in Fig. 2c, where the electron-conducting SC is reversibly poised with an $Os(bpy)_3^{3+}/Os(bpy)_3^{2+}$ redox couple loaded into a Nafion-based membrane [26, 27].

From the large variety of proposed SCs (Ag|AgCl, redox-active self-assembling monolayers [28], silver epoxy [29], Prussian Blue [30], carbon-based composites [31, 32], and redox polymers [33], etc.) for liquid-membrane ISEs, inherently conductive polymers emerged as the materials of choice as ion-to-electron transducers [34–36]. The Nobel Price in Chemistry (2000) made the conductive polymers-based SC ISEs a hot topic in ISE research with excellent overviews on the different materials and technologies from the leading research groups in Finland (Abo Academi University) [34, 35, 37–39] and Poland (Warsaw University, Warsaw University of Technology) [36, 40–46]. More recently, nanostructured materials, with large double-layer capacitances, came into sight as attractive SCs. Examples of such three-dimensional ordered materials are (1) macroporous carbon [47], (2) platinized porous silica [48], and (3) single-walled carbon nanotubes (Fig. 2b) [49].

In Fig. 3, the chemical structures of thiol derivatives used for the formation of redox-active self-assembled monolayer contacts in planar ISEs [28, 40, 50, 51] and the chemical structure of monomers that are commonly utilized for the fabrication of conducting polymer-based SCs for ISEs are summarized. Conducting polymer-based SCs can be deposited with unique spatial specificity using electrochemical methods. Conducting polymer films can be grown by electropolymerization from nanometer to micrometer thicknesses. This is in sharp contrast to electrochemically deposited insulating polymers, e.g., size exclusion membranes, where the thickness of the polymer is limited to the electron-tunneling distance [52]. With the help of an electrochemical quartz crystal microbalance the growth of the film thickness can be assessed in real time [53]. The film thickness can also be estimated from the charge used during the polymerization process. In the first attempt to miniaturize liquid-membrane ISEs with conducting polymer-based SCs, Gyurcsányi et al. have deposited polypyrrole (PPy) on a 25-µm-diameter disk-shaped gold electrode [54] and cast an approximately 100-µm (7.8×10^{-5} cm^2)-diameter solvent polymeric membrane over this SC. With this fabrication protocol, the advantage of the

small-size SC (25-µm-diameter PPy-coated disk) is partially lost. The authors had overcome this limitation by using recessed microelectrodes in which an approximately 100-µm-deep recession, prepared by controlled etching, accommodated both the conducting polymer and the ion-selective membrane. Using this protocol, potassium- and calcium-selective electrodes with 4.9×10^{-6}-cm^2 sensing area (25 µm diameter) were prepared [55].

Among the inherently conductive polymers PPy was used first as SC in ISEs [56]. In electropolymerized PPy, the conducting polymer chains are positively charged, and anions, incorporated into the film during polymerization, provide the electroneutrality of the film. To tune the chemical and mechanical properties of the PPy film, different anions were incorporated into the conductive polymer, e.g., BF_4^- [56], chloride, hexacyanoferrate (III) [57, 58], and poly(sodium 4-styrenesulfonate) [59], etc. The early works on PPy-based SC ISEs initiated fervent activity in the field to identify the best-conducting polymer for SCs in ISEs. Once it has been shown that the potential of poly (3,4-ethylenedioxythiophene) (PEDOT) SC-based K$^+$ electrodes are less sensitive to O_2 and CO_2 than their PPy-based counterparts [60], PEDOT came into the focal point of attention. The implementation of poly(3-octylthiophene) (POT) was the result of the search for hydrophobic conducting polymers [39, 61, 62] with enhanced adhesion properties to liquid membranes. POT films are generally solvent cast because the high charge density in electropolymerized POT films had a detrimental effect on the SC ISE performance [62].

Unfortunately, the elimination of ill-defined or blocked interfaces [63] between the sensing membrane and its SC through the selection of the most adequate conductive polymer film did not solve all the problems of SC ISEs. SC ISEs even with apparently adequate ion-to-electron conduction showed erratic responses in certain experiments. More recently, most of the inconsistencies could be traced back to the buildup of a thin water layer between the ion-selective membrane and its SC (Fig. 2d) [64]. ISEs with a thin liquid film sandwiched between the sensing membrane and its SC cannot be considered as "real" SC devices. Indeed, they behave like liquid-contact ISEs with extremely small inner solution volumes and an inadequate inner reference element. The inner reference element is considered inadequate if it does not provide a reversible phase boundary potential in its environment; for example, the potential of a Ag|AgCl inner reference element is not well defined in the absence of chloride ions. Similarly, the potential of a Pt electrode is not well defined in the absence of a redox couple in a relatively high concentration, e.g., $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$. However, even if the phase boundary potential at the inner reference element|aqueous layer interface can be considered as well defined, the

Fig. 3 Thiol derivatives used for the formation of redox-active self-assembled monolayer contacts on gold electrodes and monomers of commonly utilized conducting polymers for the fabrication of SC ISEs: **a** thiol with fullerene moiety [28], **b** thiol with ferrocene moiety [51], **c** pyrrole, **d** 3,4-ethylenedioxy-thiophene, **e** *n*-octyltiophene, **f** aniline
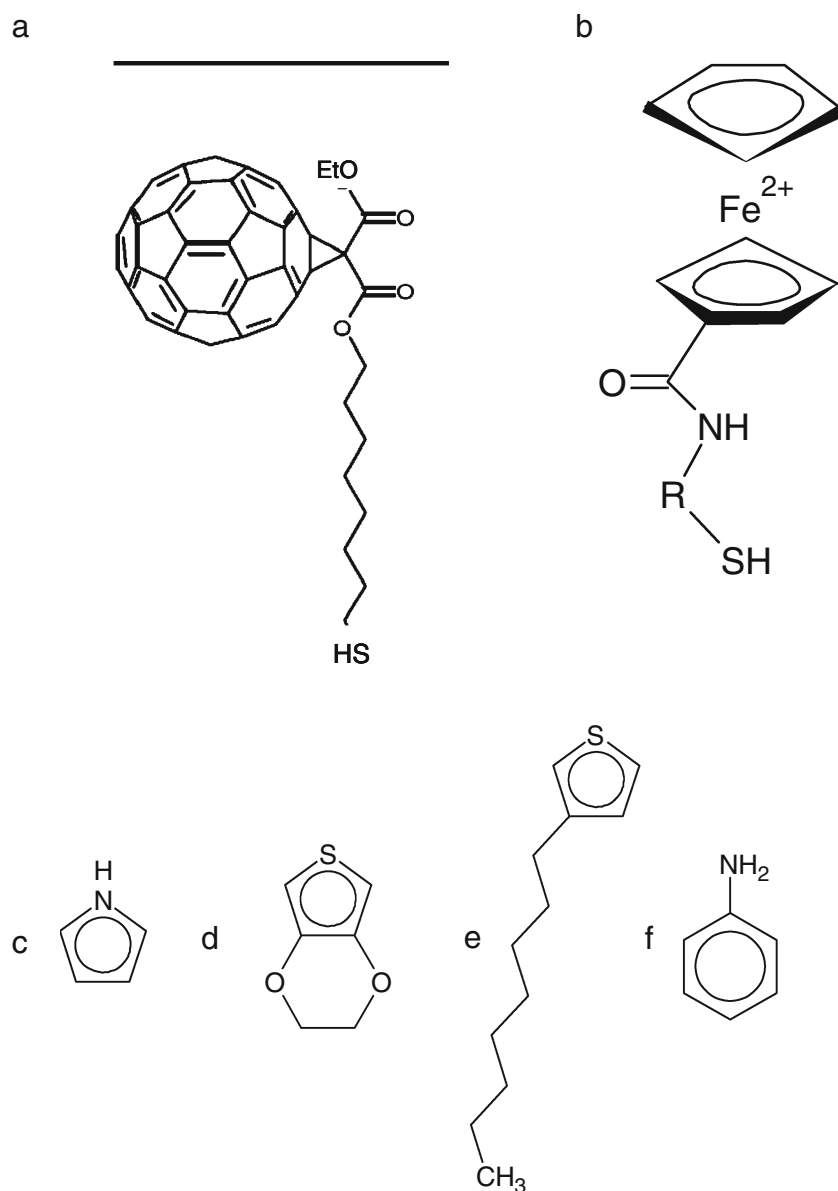
overall electrode potential may drift due to composition changes in the aqueous film sandwiched between the sensing membrane and its SC. Such concentration changes are related to the transport of water or other neutral species across the sensing membrane, e.g., CO_2, NH_3, small organic molecules, etc., into the small-volume aqueous film following changes in the composition or osmolality of the sample solution.

Recent discoveries related to the importance of minute ionic fluxes across the ion-selective membranes brought ISEs back into the spotlight because the careful elimination of these ionic fluxes allowed the extension of the detection limit of ISEs from micromolar to subnanomolar concentrations [65]. Indeed, the better understanding of ionic fluxes across ISE membranes led to the interpretation of the

drifts related to the undesirable water film formation in SC ISEs [64].

Measurement protocol for testing solid-contact ion-selective performances

In evaluations of the performance characteristics of ISEs, the practices recommended by the International Union of Pure and Applied Chemistry (IUPAC) are generally followed [66]. However, the IUPAC-recommended proto-cols are not necessarily adequate in testing microfabricated SC sensors aimed for single-use devices or for *in vivo* and closed-loop monitoring [67]. These sensors are expected to have optimal performance characteristics without con-

ditioning and provide accurate and reproducible results eventually without calibration. In addition, if possible, they should be sterilizable, biocompatible, and present excellent stability in the time frame of the measurements, e.g., up to several hours without calibration to monitor the status of patients during treatment [68]. These requirements are much more demanding than for ion sensors incorporated into blood electrolyte analyzers, in which repeated multi-point calibration protocols are common.

Consequently, the characterization of microfabricated SC electrodes should be started by recording the electrode potential of the freshly prepared or newly acquired electrodes following their first contact with an aqueous electrolyte solution. Such measurements can provide the required conditioning time for high-stability potential measurements. The acceptable potential stability should be formulated upon the requirements of the intended application. Fully conditioned potassium electrodes with PPy-based [58] or three-dimensional ordered macroporous carbon SCs [47] showed less than 30 or 17-µV/h drift, respectively. However, for the objective assessment of the potential stability of a novel SC electrode, the measurement should be made simultaneously with the potential stability measurement of conventional electrodes prepared with the same sensing membrane, but liquid inner contact and the potential stability of the reference electrode should also be considered.

Before the widespread application of inherently conductive polymers as inner contacts, the most promising results with planar microfabricated sensors were achieved when a thin layer of aqueous gel was interposed between the sensing membrane and the inner contact of the sensor [7, 69–73]. These sensors clearly should not be considered as SC electrodes; they are rather planar versions of the classical macro-ISEs with very small inner filling solution volumes. Accordingly, for the stable membrane potential, the aqueous gel was generally loaded with the chloride salt of the primary ions, and Ag|AgCl served as the inner contact [71]. In combination with a pH-sensitive membrane, a 1:1 mixture of quinone and hydroquinone (quinhydrone) was loaded into the hydrogel film, and a Pt electrode served as inner reference element. With these hydrogel-contacted electrodes, the best potential stabilities were achieved when they were manufactured with relatively large inner filling solution volumes. For planar pH sensors with 250-µm-diameter and 100-µm-deep sensor wells, potential stabilities around 70 µV/h were reported [71].

Unfortunately, as it is shown in Fig. 4a, these electrodes with Ag|AgCl|hydrogel or Pt|hydrogel inner contacts required very long equilibration times to reach the reported 70 µV/h potential stability after the first contact with the sample solution (approximately 24 h). During this equilibration process, they showed severe positive potential drift

[58, 71]. The drift is related to the water transport from the sample across the sensing membrane into the hydrogel layer to reach osmotic equilibrium. The time required for equilibration and the total voltage change during this equilibration process depends on the thickness of the
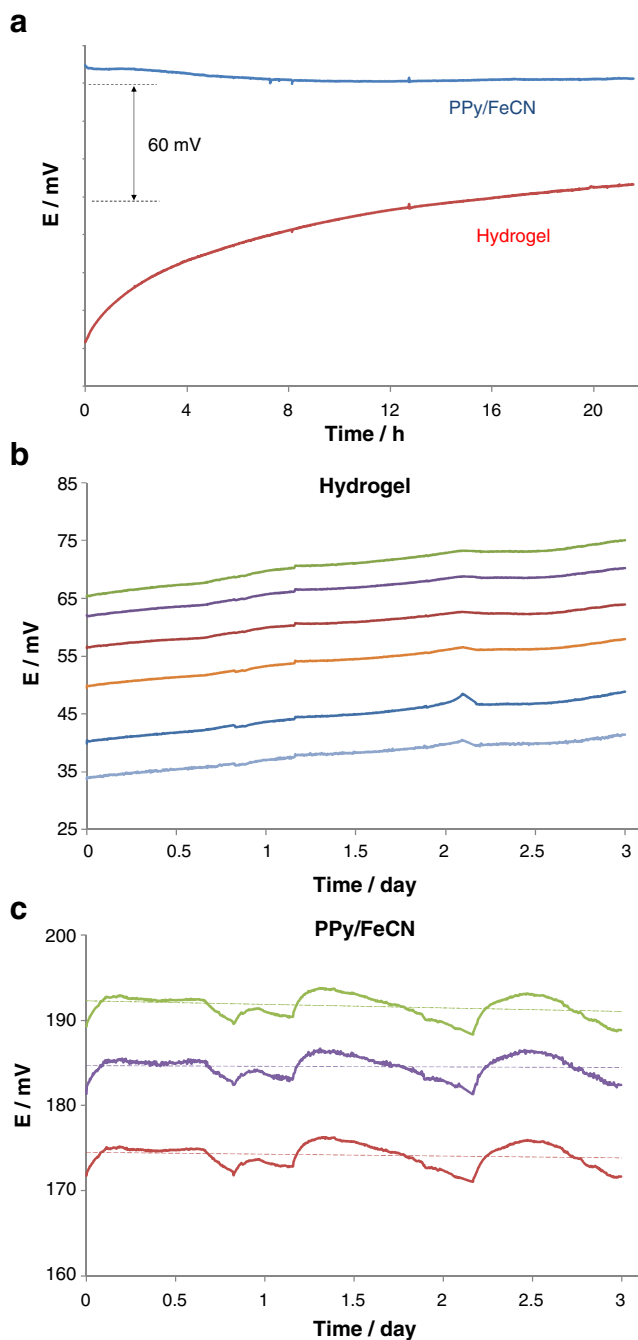

Fig. 4 Potential–time traces recorded in 2 mM KCl solution with planar potassium selective electrodes fabricated with hexacyanoferrate (III) ion-doped PPy-based (PPy/FeCN) solid- and hydrogel-based liquid contacts without temperature control a during conditioning, b during long-term potential stability measurements with hydrogel-based liquid-contact electrodes, c during long-term stability measurements with PPy/FeCN-based SC electrodes

sensing membrane, the volume of the hydrogel layer, and the concentration difference between the hydrogel layer and the sample solution. The hydrogel layer of such electrodes commonly loses water during fabrication or storage. This lost moisture is recaptured in a diffusion-controlled process during the equilibration (conditioning) of the sensor. Throughout this rehydration process, the primary ion activity in the hydrogel layer gradually decreases, which is reflected in the positive potential drift. As it is shown in Fig. 4b, this equilibration process can be very long. The preconditioning time required for high-stability measurement can be decreased from hours to a few minutes if the planar sensors with hydrogel inner contact are stored in containers with 100% humidity [74].

The equilibration process of SC electrodes is generally much faster, and the total voltage change during the equilibration process (the overall voltage drift) is much smaller (Fig. 4a) [58]. In addition, the potential stability of microfabricated, planar ISEs with adequate SC can be much better. In Fig. 4c, the results of long-term stability measurements recorded with planar ISEs fabricated with hexacyanoferrate (III) ion-doped, PPy-based inner contacts are summarized. The measurements were performed at room temperature; consequently, the potential recordings show a fluctuation reflecting the daily fluctuations in the laboratory temperature. Due to the temperature fluctuations, the short-term potential stability of these electrodes (four hour periods during the day) varied between 34 μV/h and 1.5 mV/h (Fig. 4c). However, when the potential stabilities were calculated for the entire experiment (3 days), they turned out spectacularly better, between 9 and 17.3 μV/h (see the dotted lines fitted to the potential time traces in Fig. 4c). Similarly, impressive potential stabilities were achieved with planar sodium ion-selective electrodes and PPy/FeCN-based SCs [68] using light-protected flow cell without thermostating. The results of these experiments emphasize the necessity of proper temperature control (thermostating) and the importance of continuous potential recording. In addition, these results show how significant differences can exist between short-, middle-, and long-term potential stabilities.

If a SC electrode behaves similarly to electrodes with hydrogel inner contacts during conditioning, i.e., shows large positive drift (up to 100–150 mV) during the equilibration process and require extensive times for stable potential readings (up to 24 h), it is an indication of inadequate SC. But, despite of its important information, the time course of the measured voltage during conditioning is generally not recorded or reported in publications on SC electrodes. Consequently, the equilibration time needed for acceptable electrode potential stability is not considered as a decisive electrode characteristic. However, to move micro- and nano-fabricated SC ISEs from the research laboratories to commer-

cial devices, aimed for single-use measurements and short-term in vivo monitoring, these measurements will be unavoidable. The determination of the equilibration time should be a part of the protocols to characterize SC ISEs, and the equilibration time should be used as one of the quality control criteria in assessing the performance characteristics of planar sensors. With sensors that are not in continuous use, i. e., kept dry between measurements, the determination of the reproducibility of the equilibration process, i.e., recording the time course of the equilibration process repeatedly, could also be very informative.

Assumed vs. confirmed solid contacts: the aqueous layer test

As we discussed above, miniaturized sensor designs in which a thin hydrogel film with submicroliter volume in combination with a Ag|AgCl inner reference element is utilized on the backside of the sensing membrane has much longer equilibration time than SC electrodes and are sensitive to osmolality changes in the sample. In combination with pH-sensitive membranes, they are also sensitive to changes in the partial pressure of CO_2 in the sample. As CO_2 passes through the sensing membrane, it changes the pH in the hydrogel film, which leads to a change in the measured cell voltage. This CO_2 interference induced pH change on the backside of the sensing membrane is utilized in the differential CO_2 sensors for the measurement of CO_2 concentrations in patient blood samples [75–77].

Quite recently, it has been realized that a thin aqueous film can also form unintentionally between the sensing membrane and its SC [64]. The indicators of the formation of such an aqueous film are phenomena that were considered as disadvantages of planar electrodes with hydrogel-based inner contact: (1) slow equilibration process, (2) sensitivity to osmolality changes in the sample, (3) slow potential drift experienced upon the appearance (positive drift) and removal (negative drift) of interfering ions from the sample, and (4) sensitivity to the CO_2 partial pressure (especially in combination with pH sensitive membranes). Since the possibility for the formation of an aqueous layer between the sensing membrane and its SC was not even considered in papers published before 2000, its effect on the SC sensor responses was also not contemplated. Consequently, in the light of our understanding on the role of an unintentionally formed thin aqueous layer in SC ISEs, the critical evaluation of the conclusions of earlier papers would be desirable after revisiting the original data. It may change our stand with respect of the most adequate SCs for fabricating miniaturized electrodes in combination with plasticized solvent polymeric membranes.

In general, only the water uptake and transport through the ion-selective membrane has been considered as a source for the formation of the undesirable aqueous layer in SC electrodes. Early studies have shown evidences on water uptake and diffusion in PVC membranes [78–82]. However, the aqueous layer could have been present long before the membranes' first contact with an aqueous sample. To the best of our knowledge, there are no reports claiming fabrication protocols under dry conditions, i.e., in which specific measures were taken to exclude the possibility for the presence of water during the fabrication of SC electrodes. On the contrary, the humidity of the air is generally not controlled during electrode preparation, and the water content of the tetrahydrofuran (THF)-based ion-selective membrane cocktails is not considered as a source of water traces, although the hygroscopic properties of THF are well known. Most commonly, these membrane cocktails are used for the deposition of the ion-selective membrane over the SC by drop casting. However, in certain fabrication protocols, the use of freshly distilled THF is recommended for membrane casting. In addition, certain conductive polymer-based SCs are prepared by electrosynthesis in an aqueous solution, but generally, no specific measures are applied for the careful removal of the water residues from such electrochemically deposited films. In these electrochemically deposited conductive polymeric films, the ionic concentrations are rather high. The high ionic concentrations are from anions that compensate the positive charge of the polymeric backbone and from physically entrapped salts from the background electrolyte utilized in the polymerization process. Our study has shown that if the entrapped salts are not carefully removed from the polymeric film, the likelihood of aqueous layer formation becomes very high [83]. To remove the entrapped salts from the hexacyanoferrate (III)-doped PPy film [83] following electropolymerization, the electrodes were rinsed with excess of water and then left in a large-volume stirred deionized water for 4 h and rinsed again with water. Due to the very low ionic concentration of high-purity water, the anions incorporated by charge compensation are not removed (exchanged) from the film but only the physically entrapped salts. The PPy films were then dried at room temperature and rinsed with small amounts of water-free THF to remove residual traces of water, right before casting the membrane.

To test for the presence of an aqueous layer, Pretsch and coworkers introduced an original method [64] in which the potential drifts experienced in the presence of an undesirable aqueous layer is utilized as an indirect indicator. The protocol is very simple. It is basically the same as the separate solution method for evaluating the selectivity coefficient of an ISE for a particular ion in which the tested sensor is sequentially exposed to concentrated solutions (e.g., $c = 0.1$ mol L^{-1}) of the primary and the interfering ions. Following the exposure of the electrodes to the different solutions, the electrode potential is continuously recorded. The only important difference compared to the "common" selectivity coefficient determination is the time frame of the experiment. Depending on the thickness of the sensing membrane and the diffusion coefficients in the membrane, the electrode potential has to be recorded for extended periods of time. Experiments lasting up to 45 h were reported [40, 47].

When a membrane in equilibrium with primary ions is exposed to a solution of interfering ions on its sample side, ion exchange processes at the membrane solution interface induce changes in the membrane composition and subsequently on the other side of the membrane in the aqueous layer, if present. Since the volume of the undesirable aqueous layer is generally extremely small, even minute ionic fluxes can create large changes in its concentration. This concentration change is the source of the membrane diffusion-controlled slow drift in the measured electrode potential. The drift is positive when the primary ion solution is switched to an interfering ion solution and negative when it is switched in the opposite direction. Since the method requires ion exchange-induced fluxes across the sensing membrane, adequate selection of the interfering ion is essential in this test. Highly discriminated ions cannot be expected to induce significant ion exchange; that is, they hardly are useful in this respect. The directions of the expected concentration changes and the concomitant potential transients for a potassium-selective membrane are illustrated schematically in Fig. 5.

As Lai et al. [47] emphasized, the test is most sensitive when the aqueous layer volume is extremely small, and the results could be misinterpreted with systems in which the aqueous layer volume is relatively large. For example, in the "Christmas tree" array, shown in Fig. 1c, the sensing membrane is cast over an approximately 50-μm-thick hydrogel layer [71]. In other applications, utilizing screen-printed ISE arrays, a 100-μm-thick hydrogel layer was applied on the backside of the membrane [58]. The behavior of planar sensors with such relatively thick hydrogel layers approaches the behavior of macroelectrodes with very large inner filling solution volumes. They are indeed less sensitive to changes in the osmolality or interfering ion concentration in the sample. However, the unintentionally formed aqueous films rarely grow to such a large volume. The aqueous layer formed beneath the ion-selective membrane of coated wire electrodes is of about 100 Å [84]. When the thickness of the undesirable water layer is around this value, the ion exchange-based water layer test is expected to give unambiguous results.

The diffusion coefficients in typical plasticized PVC membranes are on the order of 10^{-8} cm^2/s [85–88]. The
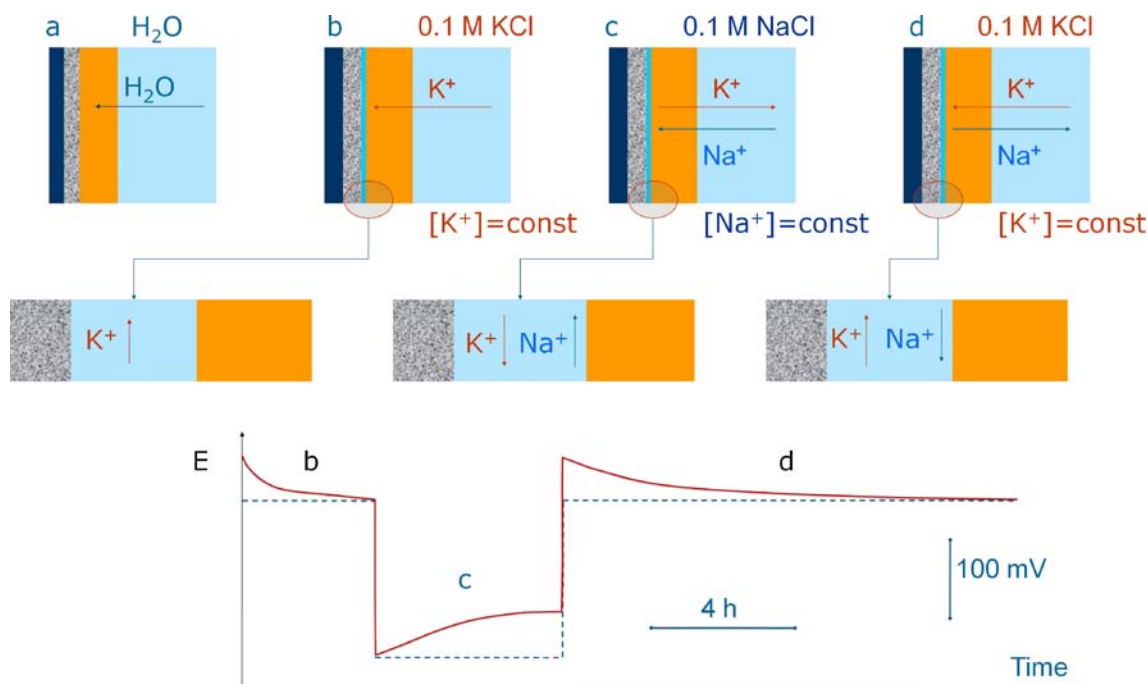
Fig. 5 Schematic illustration of fluxes, concentration changes, and the recorded potential transients in the aqueous layer test with a SC potassium-selective electrode. In the potential vs. time plot, the *dashed trace* indicates the expected potential response of an ideal SC electrode, while the *continuous trace* of an electrode with an undesirable aqueous layer between the ion-selective membrane and its SC: **a** the formation of an aqueous layer due to water transport, **b** K^+ ion concentration increases in the aqueous layer during condition-ing the membrane in a primary (K^+) ion solution, resulting in a negative potential drift, **c** replacing the K^+ ion solution to Na^+ (interfering) ion solution reverses the flux of K^+ ions through the membrane and the direction of the potential drift, as the K^+ ion concentration decreases and the Na^+ ion concentration increases in the aqueous layer, **d** placing the electrode back into the K^+ ion solution results in the readjustment of K^+ concentration in the aqueous layer, which is indicated through a drift in the negative direction

time frames of the drifts recorded with plasticized PVC membranes in these water layer tests are on the order of hours. Consequently, the feasibility of the ion exchange-based water layer test becomes questionable in membranes with much smaller diffusion coefficients, e.g., polyacrylate based membranes in which the diffusion coefficients are between 10^{-11} and 10^{-12} cm^2/s [89]. In membranes with such small diffusion coefficients, the same equilibration process would be roughly 1,000 longer; that is, the drifts become undetectable beside the noise.

The aqueous water layer test for SC ISEs with membranes in which the diffusion coefficients of the ionophore and the ionophore ion complexes are very small can be performed by recording the potential drifts upon permeation of CO_2 across the membrane into the aqueous film [40]. The test uses the same principle that has been utilized in differential CO_2 sensors [75–77]. Upon the permeation of CO_2, the pH of an unbuffered aqueous solution layer changes. This pH change on the backside of a pH-sensitive membrane generates an asymptotic drift in the recorded potential. The feasibility of the test has been shown for a coated wire electrode in combination with a pH-sensitive polyacrylate membrane [40]. Upon purging a solution with nitrogen and CO_2 intermit-tently, the potential of the coated wire electrode fluctuated

reflecting the changes in the CO_2 partial pressure while the potential of an electrode with a hydrophobic, ferrocene-based self-assembled monolayer inner contact remained constant. Although the method is aimed for SC sensors with pH-sensitive membranes, it has been shown to work also to trace the presence of a water layer formed between a sodium ion-selective membrane and its gold electrode SC [40].

In summary, "the aqueous layer test" has fundamental importance in deciding whether the tested electrodes are indeed genuine SC electrodes or not, i.e. liquid-contact ones. Consequently, to rule out the presence of an aqueous film between the sensing membrane and its SC is essential for the unambiguous interpretation of the responses of genuine SC ISEs. The emphasis is on the importance of considering the role of an aqueous film in the overall ISE responses and not on a particular test. Particular tests may fail under different experimental conditions, but the importance of an undesirable water film in the sensor response remains.

Long-term stability of solid-contact ISEs

In applications in which the small size of SC electrodes offers unique advantages, e.g., *in vivo* or closed loop

monitoring of ionic activities, the long-term stability and reproducibility of the measured potentials are essential. Consequently, in evaluating the performance characteristics of SC microelectrodes, besides tracing the time dependence of the measured potential during the equilibration process, the potential stability of the SC sensor in a constant environment (controlled concentrations, temperature, O_2, CO_2, and light intensity levels, etc.) has to be tested for extended periods of times. The time frame of these tests should reflect the intended application of the SC sensor. However, the reproducibility of the measured potentials following short-term changes in the environment is even more important. Depending on the aimed application, testing the sensors reproducibility following changes in the concentration of the primary ion, in the concentration of possible interfering ions, in the ionic strength and the osmolality of the bathing solution, in the temperature, and in the O_2, CO_2, and light intensity levels may all be important. The magnitude of these artificially created interferences should match the expected changes in the intended application. Inadequate reproducibility of the measured potentials or unacceptably long transients is these tests can be an indication of inadequate SC. However, one must realize that the overall potential stability of a few-micrometers-size ISE is not solely controlled by the potential stability of the SC|sensing membrane interface. With decreasing sensor sizes, the contact surface provided for insulating the two sides of the sensing membrane decreases quadratically. In addition, upon extended exposure to aqueous solution, the adhesion strength between the membrane and its substrate gradually weakens [71]. This can lead to the delamination of the membrane and the complete deterioration of the sensor response or to characteristic drifts in the sensor response due to the formation of resistive shunts between the two sides of the membrane. Similar drifts can be linked to the formation of miniature pinholes across the few- micrometers thin sensing membranes of planar SC ISEs. Pinholes can form for example when the ion-selective membrane is cast over a nonperfectly smooth internal contact, e.g., a poly(hydroxyethyl methacrylate) or a conductive polymer surface. If the seal between the two sides of the membrane is compromised, related to nonperfect membrane adhesion, or due to the formation of small pinholes, the sensors still may have close to theoretical short-term responses. However, following the fast change in their phase boundary potential, upon changes in the concentration of the sample, they commonly show a slow potential drift opposite in sign.

Besides the mechanical failures, the gradual deterioration of the sensors responses due to dissolution of the membrane components into the sample is also a concern. For neutral-carrier-based membrane sensors, Oesch and Simon [90] showed that leaching of the ionophore from the membrane leads to loss of sensitivity and selectivity, which is accompanied by increased membrane resistance, worsening detection limit [91–93], increased noise, and possibility of initiation of inflammatory responses *in vivo* [94], etc. The probability of the dissolution-related deterioration of the sensor responses increases with decreasing sensor sizes because the relative concentration changes can be significant in small-size sensors. Besides leaching, the decomposition of the ionophore and the charged site additives can change the optimized composition of an ISE membrane [24, 95–97] as has been documented by imaging the concentration profiles in pH-sensitive membranes using spectroelectrochemical microscopy [96, 98]. However, changes in the optimized composition of the sensing membrane could also be related to the distribution of materials between the membrane and its SC. The membrane ingredients can partition into the conductive polymer-based SC, and the ingredients or impurities in the SC can partition into the sensing membrane [46]. For example, the partitioning and permeation of the highly lipophilic solvent cast POT polymer (or shorter oligomers present in the polymer) into the ion-selective membrane can change the selectivity of the ion-selective membrane [99]. Consequently, besides evaluating the drift during the long-term stability studies, assessing the changes in other performance characteristics of the sensor through extended periods of time, e.g., response sensitivity (slope of the calibration curve), standard potential, selectivity coefficients, detection limit, response time, etc., are also important.

In summary, it has to be emphasized that the interpretation of the results of long-term potential stability studies with small-size SC electrodes can be very complex. However, the possible sources of the potential instabilities can be sorted out or narrowed down. Drifts related to the formation of an aqueous layer can be traced by the aqueous layer test while drifts associated with changes in the membrane composition, i.e., decrease in the concentrations of the free ionophore and/or the added lipophilic salt, can be tracked by chronoamperometric methods [87, 88]. Changes in the sensing membrane composition related to the dissolution of membrane ingredients into the sample are expected to influence also the selectivity coefficients, the detection limit, and the resistance of the ISE [90]. However, changes in the composition of the SC of the sensor, due to its chemical instability, should not influence these sensor characteristics but is expected to affect the long-term stability and the reproducibility of the potential measurements. If the changes in the sensing membrane and its SC composition are related to the partitioning of components between these two phases, these changes start immediately upon the application of the sensing membrane over its SC and proceed even if the sensor is stored dry. Consequently, besides of other consequences, these processes will also

influence the shelf lifetime of SC ISEs. Changes in the most important sensor characteristics (calibration slope, standard potential, selectivity coefficients, etc.) in repeated tests, by keeping the sensors dry between the tests, can be an indication of interaction between the sensing membrane and its SC.

Adequate SCs with high potential stability are claimed of having a nonpolarizable interface with high exchange current density that is not influenced by the input current of the measuring amplifier. However, the input current of commonly used amplifiers could become an important factor with micro- and nanometer-size electrodes. The polarizing current densities from the input current of the amplifier for micro- or nanometer-size electrodes can be 10^6 or 10^{12} times larger than for millimeter-size macro-electrodes, respectively. The submicrometer-size micro-electrodes used in electrophysiology experiments indeed require specific measuring amplifiers. In addition, due to the extremely large resistances of those micro-ISEs, the measurements are commonly performed in Faraday cages. Although the resistances of submicrometer size planar microelectrodes with solid internal contact are expected to be much smaller than those of pulled pipette-type micro-electrodes, there are limits how small these electrodes can be in practical applications. Once the SC sensors indeed will be manufactured with micrometer or submicrometer dimensions, evaluating the sensitivity of these electrodes to small current polarization will become unavoidable.

The response to current polarization is also expected to provide valuable insight on the properties of the membrane| SC interface. Understanding the effect of external current on the response of SC potentiometric electrodes is also important because recently several methods were introduced in which ISEs are used under nonzero current conditions [87, 100–105]. These methods utilize the external current for the improvement of the detection limit of ISEs [101, 106], to increase the sensitivity of potentio-metric measurements [103], and for providing reproducible measurement conditions in certain applications [105]. With the advent of these novel potentiometric applications, the theory of chronopotentiometry of the ionophore-based membrane has been developed [107, 108]. It has been claimed that the potential stability of SC ISEs can be determined in a simple chronopotentiometric experiment without performing long-term stability studies [37]. When SC ISEs are polarized with a few nanoamperes of direct current, which is orders of magnitude higher than the input current of the high input impedance voltmeters, a potential jump (iR_b) is followed by a slow drift (where i is the applied current and R_b is the bulk membrane resistance). As shown in Fig. 6, the magnitude of this potential drift (dE/dt) is very different in SC ISEs with adequate or inadequate ion-to-electron transduction. In SC ISEs, the drift

recorded in such chronopotentiometric experiments can be related to the low-frequency capacitance (C_L) of the ion-selective membrane|SC interface and the applied current (i): $dE/dt = i/C_L$.

Light sensitivity

The light sensitivity of the potentiometric indicator and reference electrodes is related to the photovoltaic properties of certain materials, e.g. silver halides, and conductive polymers, which can convert the energy of light into electrical energy. The conductive polymers utilized in SC ISEs serve also as photovoltaic materials in solar cells. These conducting polymers are in fact semiconductors with a band gap of 1.5–3 eV between the valence and the conduction bands [109]. Activation of free electrons by photoexcitation or chemical doping can increase the electrical conductivity of these materials by several orders of magnitude [110]. Therefore, the light sensitivity can be a serious issue with SC ISEs, which should be checked for novel conducting polymer-based SC ISEs.

In conventional potentiometric cells with macro-electrodes, the electrode body generally protects these light-sensitive materials from direct exposure to the ambient light. Consequently, these devices are generally hardly influenced by changes in the ambient light intensity, although the potential of a silver|silver halide electrode can change up to about 10 mV due to photovoltaic effects [111]. However, in microfabricated planar sensors with a solid internal contact, the light-sensitive internal contact, e.g., a conductive polymer, is almost directly exposed to light intensity changes in the environment. The few-micrometers-thick, light-transparent sensing membrane
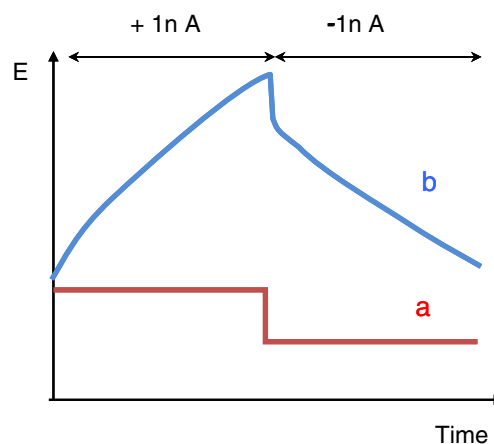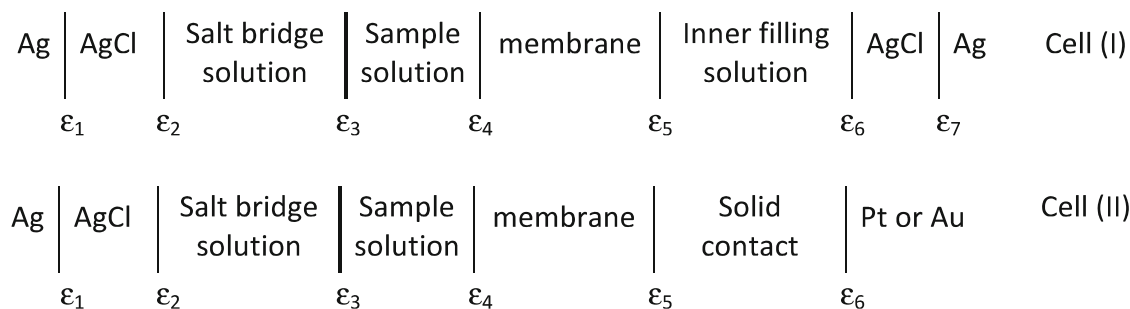

Fig. 6 Typical potential–time transients following the galvanostatic polarization of SC ISEs with positive and then negative currents: **a** with conductive polymer based ion-to-electron transduction layer, **b** with coated wire electrode [37]

over the light-sensitive SC does not act as an effective shield.

The light sensitivity of screen-printed planar potassium-selective electrodes with hexacyanoferrate (III)-doped polypyrole SCs were negligible [58], but POT-based

temperature gradient in the salt bridge, connecting the heated and nonheated half-cells, are limiting the attainable accuracy of the determinations. The temperature coefficients determined according to these protocols are termed as isothermal or nonisothermal.

Ag	AgCl	Salt bridge solution	Sample solution	membrane	Inner filling solution	AgCl	Ag	Cell (I)
	ε_1	ε_2	ε_3	ε_4	ε_5	ε_6	ε_7	

Ag	AgCl	Salt bridge solution	Sample solution	membrane	Solid contact	Pt or Au	Cell (II)
	ε_1	ε_2	ε_3	ε_4	ε_5	ε_6	

calcium-selective electrodes showed a light-dependent drift of approximately 2 mV/h [99]. In similar experiments, potassium-selective electrodes with Ag|AgCl|hydrogel inner contact showed almost 8 mV potential change upon the exposure to ambient light following measurements in the dark [58]. However, the protection of electrodes from light during measurements can lead to flawless systems even with electrodes showing large photovoltaic effects. The incorporation of the Ag|AgCl|hydrogel inner contact potassium electrodes in the flow-through system protected from ambient light eliminated all the disadvantageous light sensitivity of these electrodes [58, 68].

Temperature dependence

The total potential difference measured between the two terminal electrodes in a potentiometric cell is composed from a considerable number of local potential differences. In the electrochemical notation of the cells I and II, these local potential differences are marked as ε_1 through ε_7 or ε_1 through ε_6, respectively. Each of these potential differences has its own temperature dependence. These temperature dependences can be determined in isothermal and non-isothermal galvanic cells [112]. In isothermal cells, a $Pt|H_2$ hydrogen electrode is used as a reference electrode, and both the indicator and the reference electrodes are heated or cooled to the same temperature. The standard potential of the hydrogen electrode is set to zero at all temperatures by convention [111]. In nonisothermal cells, the potential of a heated/cooled electrode is measured against an identical electrode, which is kept in a constant temperature environment. In the nonisothermal experiment, the temperature coefficient of the diffusion potentials and the large

Due to the complexity of the measurement and interpretation of the temperature dependence of the measured potentials in potentiometric cells, the calibration and the measurement should be performed at the same temperature if possible. Thermostating small-size, microfabricated sensors, in combination with small-sample volumes, is much easier than with macroelectrodes with large inner filling solution and sample volumes. Consequently, with small-size SC sensors, which can easily be integrated into flow-through manifolds with minute sample volumes, the problems related to the temperature dependences of the measured potentials can be minimized.

Low-detection limit solid-contact electrodes

Since the attainable detection limits of ISEs are commonly determined by minute ionic fluxes from the inner filling solution into the sample, SC ISEs without inner filling solution [45, 62, 83, 113] were expected to have better detection limits than their counterparts fabricated with inner filling solution [65, 101, 106, 114, 115]. Unfortunately, these expectations in general are not fulfilled, and SC electrodes have very similar detection limits as conventional liquid-contact ISEs. This contradiction is most probably due to contaminations originating from the membrane itself that can bias the results. Atomic absorption measurements of solutions in contact with a valinomycin-based potassium-selective membranes showed always traces of potassium ions irrespective of the composition of the inner filling solution of the ISE, i.e., whether it contained KCl or not [116].

The most effective method to decrease the detection limit in conventional ISEs is the establishment of a

concentration gradient across the membrane that opposes the primary ion leaching from the membrane [65]. Since this important mean to control ionic fluxes across the membrane is unavailable in SC electrodes, novel approaches had to be implemented to obtain SC electrodes with submicromolar detection limits. One possibility is the incorporation of a complexing agent into the SC, instead of the inner filling solution. Konopka et al. achieved nanomolar detection limit with SC calcium-selective electrodes by loading 1,2-dihydroxybenzene-3,5-disulfonic disodium salt (Tiron), a complexing agent for Ca^{2+}, into electropolymerized PPy films [42]. In the same study, the importance of a proper membrane conditioning (low primary ion and relatively high interfering ion) was also emphasized as an unavoidable requirement to obtain nanomolar detection limits. In fact, the effect of conditioning has been so dramatic on the attainable detection limit that the relative importance of conditioning compared to the incorporation of Tiron into the SC cannot be unambiguously determined. Since the ion exchange capacity of the Tiron-loaded SC film is limited, i.e., even minute calcium ion flux through the membrane could lead to its saturation, proper conditioning of these electrodes is indispensable in analyzing solutions with submicromolar concentrations.

The application of an external current to control the ionic fluxes across the membrane [43] and using high-viscosity sensing membranes [62] over the SC are also feasible avenues toward SC ISEs with superb detection limits. Nanomolar detection limits were achieved with SC lead-selective electrodes based on hexacyanoferrate (III)-loaded PPy SC [83]. Even better, a subnanomolar detection limit was obtained by using poly(methyl methacrylate)/poly (decyl methacrylate) (MMA/DMA) copolymer based ion-selective membranes over drop cast poly(n-octyl)thiophene SC [62]. In MMA/DMA membranes, the diffusion coefficient of the active components is roughly three orders of magnitude lower than in conventional PVC membranes. Consequently, once again, the improvements in the detection limits cannot be unambiguously traced to the superior properties of the SC or the sensing membrane. However, the insensitivity of the POT SC sensors to oxygen is a clear advantage.

The advantages of low-diffusivity sensing membranes, such as MMA/DMA, has been also shown for Ag^+, Pb^{2+}, Ca^{2+}, K^+, and I^- ion-selective SC electrodes [117]. Although, in this study, the conductive polymer-based SC was not loaded with a complexing agent, 2×10^{-9}, 10^{-7}, and 10^{-8} M detection limits were reported for the SC silver-, potassium-, and iodide-selective electrodes, respectively. However, to achieve these impressive detection limits, elaborate conditioning protocols had to be followed. First, the membranes are conditioned in 1 mM primary ion solution, to replace the counterions of the added mobile anionic sites ([3,5-bis(trifluoromethyl)phenyl]borate) with primary ions. The conditioning in 1 mM primary ion solution is followed by conditioning the membrane in dilute (1 nM) primary ion solution for 2 days.

In summary, adequate SCs in combination with low-diffusivity membranes can provide ISEs with superb detection limits following proper conditioning. Indeed, these SC electrodes are perceived as means to elude the lengthily optimization of the inner filling solution necessary to achieve submicromolar detection limits with conventional liquid-contact electrodes. However, the attainable detection limits with optimized inner filling solution-based electrodes are still superior. But, the SC electrodes are more adequate for measurements in very small sample volumes, i.e., in immunoassays utilizing ISEs as detectors [118].

Interpretation of the performance characteristics of solid-contact ISEs: common misconceptions

As reviewers of papers on novel SC electrodes, we often face disturbing comments related to the interpretation of some of the experimental results. These comments commonly intend to emphasize the advantageous qualities of the advocated sensor systems without unambiguous experimental proof. Consequently, in contrast to the intentions of the authors, they point to the lack of certain data or misconceptions in understanding all the underlying phenomena. Selected examples of such misconceptions in combination with the counter arguments are summarized in Table 1.

Since the data available in publications on SC sensors are often incomplete or represent significantly different experimental conditions, the comparison of the different SC sensors based on the critical and comprehensive assessment of their performance characteristics cannot be made unambiguously. To explain what we see problematic, data summarized in Tables 1 to 3 in the review paper of Michalska [36] are used. In these tables, the author compiled the performance characteristics of potassium, chloride, and calcium electrodes with different SCs. In Table 1 of the said paper, slopes of the calibration curves recorded with the different potassium electrodes range between 56.2±0.2 and 58.8±0.8 mV per decade. The selectivity coefficients of these potassium electrodes toward sodium ions range between 1.6×10^{-4} and 6.3×10^{-5}. Similarly, spread data are reported in Tables 2 and 3 of the same paper on calcium- and chloride-selective electrodes. What is commonly missing from papers on SC electrodes is the interpretation of the differences between data reported in the study on a novel SC electrode and data available in the literature or measured simultaneously with

Table 1 Common misconceptions and counter arguments related to the interpretation of experimental data with SC ISEs

Misconceptions	Counter arguments
Close to theoretical (Nernstian) response slopes and selectivity coefficients similar to the published values prove the quality of the tested SC	According to the phase boundary model [119] and advanced nonequilibrium models [38], the SC on the backside of an ion-selective membrane should not have any influence on the selectivity coefficients of the electrode or on the slope of the calibration curve recorded with the electrode. Consequently, a Nernstian slope and adequate selectivity coefficients do not have positive predictive value. However, if the slope of the calibration curve recorded with a SC electrode differs from the Nernstian slope or the selectivity coefficients are worse compared to values reported for the same membrane with liquid inner contact, it can indicate an inadequate SC
Reproducible response slopes in repeated calibrations indicate an adequate SC. SC electrodes with reproducible response slopes are adequate for practical applications	The quality of a SC is better assessed from the reproducibility of the standard potentials (E^0) than from the reproducibility of the calibration slopes. Drifting E^0 values can indicate an inadequate SC. According to the phase boundary model [119] and advanced nonequilibrium models [38], the response slopes should not be influenced by the quality of the SC if the change in standard potential is negligible in the time frame of the calibration process. If the change in the E^0 is slow and the SC ISE is frequently calibrated, it can be adequate in a variety of practical applications
Adequate long-term potential stability with a SC ISE is a proof for adequate SC	Any system can reach equilibrium after a long period of time in a constant environment. The time required to achieve a drift (mV/h) below a threshold value following a change in the environment and the reproducibility of the measured potential in the same environment following repeated changes are the essential characteristics that should be used to evaluate the quality of a SC ISE
Long-term potential stability studies provide more valuable information on the quality of SC ISEs than short- and mid-term stability measurements	The opposite is true in practical applications. Short response time is essential in monitoring fast concentration changes. In a medical context, e.g., in emergency applications, in devices aimed for the doctor's office, etc., the first few minutes and hours are the most critical. With single-use sensors, short turnaround time is essential; that is, we are interested to have a potential reading as fast as possible with electrodes removed from packaging or storage and brought in contact with the sample
Larger drifts registered during the aqueous layer test indicate enhanced water accumulation at the membrane|SC interface	The exact opposite is true. Ion concentrations change less and slower in larger volume aqueous films than in very small volume films. Thus, the appearance of minute amounts of water at the membrane interface is causing the largest drifts during the test
SCs that are showing sensitivity to any of the environmental conditions (light, temperature, oxygen, or CO_2) are useless	Certainly, it is desirable to have inner contacts that are unaffected by changes in the environment of a SC ISE. However, it is relatively easy avoid interferences originating from light intensity and temperature changes during measurements. The sensitivity of the sensor signal to CO_2 and O_2 should be evaluated with respect of their expected fluctuations in the sample
SC ISEs have better detection limits compared to ISEs with liquid inner contact because ionic fluxes from the inner filling solution are essentially eliminated	In the practice of ISEs, this advantage generally cannot be realized. The detection limits of SC and liquid-contact electrodes in most applications are very similar because besides of ionic fluxes from the inner solution, ionic contaminations from the sensing membrane itself can also bias the detection limit. To approach the best detection limits, the utilization of sensing membranes in which the diffusion coefficients are very small and extensive conditioning of the membranes in dilute solutions appear to be the best strategy with both SC and liquid-contact ISEs

liquid-contact electrodes utilizing the same membrane or other SC electrodes fabricated with the same membrane. Consequently, even from carefully compiled data, as in Tables 1 through 3 in reference [36], it is almost impossible to draw conclusions on the quality of the different SCs or on the source of the experimentally recorded differences. It is not clear whether the recorded differences in the performance characteristics, e.g., slopes, detection limits, dynamic ranges, selectivity coefficients, potential stabilities, etc., of the tested SC electrodes are statistically

significant or not. In addition, even with statistically significant differences, it is not clear whether it is indeed related to differences in the quality of the SC, the conditioning protocol, or the ion-selective membrane itself. When statistically relevant differences indeed exist in the recorded performance characteristics, the source of such differences should be explored. According to the phase boundary model [119] and advanced nonequilibrium models [38], an adequate SC on the backside of an ion-selective membrane should not have any influence on the on the selectivity coefficients of the electrode or on the slope of the calibration curve recorded with the electrode. Consequently, if such differences are recorded they may indicate inadequate SCs. Tracking the time dependence of the measured potentials in the different solutions utilized for the determination of the response slope and selectivity coefficients could narrow down the possible sources of the differences. Drifting potentials could point to changes at the SC|liquid membrane interface when the membrane ingredients can partition into the SC or the ingredients or impurities in the SC can partition into the sensing membrane [46]. Similarly, drifting potentials could point to chemical instabilities in the SC or to interferences to certain materials that can easily penetrate the ion-selective membrane, e.g., O_2, CO_2, etc.

Toward a new generation of solid-contact electrodes

In the last decades, the advantages of SC ISEs have been demonstrated in a variety of applications. As the original metal electrode contacts were gradually replaced with new SC materials, with better potential stability and less sensitivity to interferences, they became real competitors of the conventional macroelectrodes. Among the novel SC materials, inherently conductive polymers, with mixed electron and ion conduction, received the most attention; however, superb detection limits and potential stabilities were also reported for electrodes using other materials for SCs. What still appears missing is a consensus on the best-available SC for ionophore loaded ion-selective membranes, which eventually could be used as a "gold standard" for comparison in testing new SC sensors or could be used as a general platform, as we use the Philips electrode body for studying novel ISE membranes. The lack of a generally applicable SC platform may be the reason that despite of the impressive properties of certain SC electrodes, they did not make an important breakthrough in commercial devices and remained primarily in the research laboratories. Although there are a number of commercial devices that utilize planar sensors with ionophore-loaded sensing membranes, e.g., ISTAT single-use cartridges for the analysis of blood electrolytes or the

Horiba Cardy single-ion analyzers for the analysis of small sample volumes, the actual design (layer structure) of these devices are not known. A generally applicable solid internal contact platform is missing because certain processes determining the potential on the membrane|SC interface are still not clearly understood. Due to the lack of our understanding on these processes, it generally cannot be predicted whether a SC with superb properties in certain applications would provide the same characteristics in different applications e.g., with different sensing membranes cast with a different ionophore, plasticizer, etc., or upon the application of external current.

If it is proved that a certain SC electrode has the same performance characteristics as conventional ISEs, it would be important to show that the same performance characteristics can be achieved when the sensor size is drastically reduced. A back-to-back comparison of macro- and micro-SC electrodes could serve this purpose. If statistically significant differences are found, to trace the sources of such differences would be the same importance.

Without such detailed analysis, it is difficult to imagine that the SC ISEs will replace the conventional ISEs in the near feature. On the other hand, once cheap, miniaturized, SC ISEs will match the performance characteristics of macroscopic-size electrodes, they could have an important impact in a variety of applications requiring robust, maintenance-free, or single-use sensors, e.g., in homecare or bedside diagnostics, environmental analysis, and quality control checking. In addition, reliable SC-based ISEs are expected to revitalize the field of ISFETs and open new possibilities in combination with nanowire-based devices [120].

References

1. Bakker E, Bühlmann P, Pretsch E (1997) Chem Rev 97:3083
2. Bühlmann P, Pretsch E, Bakker E (1998) Chem Rev 98:1593
3. Umezawa Y, Buhlman P, Umezawa K, Hamada N (2002) Pure Appl Chem 74:995
4. Umezawa Y, Bühlmann P, Umezawa K, Tohda K, Amemiya S (2000) Pure Appl Chem 72:1851
5. Umezawa Y, Umezawa K, Bühlmann P, Hamada N, Aoki H, Nakanishi J, Sato M, Xiao KP, Nishimura Y (2002) Pure Appl Chem 74:923
6. Maerki M, Muller B, Wehrli B (2006) Limnol Oceanogr 51:1342
7. Cosofret VV, Erdősy M, Johnson TA, Buck RP, Ash RB, Neuman MR (1995) Anal Chem 67:1647
8. Kuhtreiber WM, Jaffe LF (1990) J Cell Biol 110:1565
9. Smith PJS, Sanger RS, Messerli MA (2006) In: Michael AC (ed) Principles, development and applications of self-referencing

electrochemical microelectrodes to the determination of fluxes at cell membranes. CRC, Boca Raton, FL

10. Messerli MA, Robinson KR, Smith PJS (2006) In: Volkov AG (ed) Electrochemical sensor applications to study of molecular physiology and analyte flux in plants. Springer, New York

11. Horrocks BR, Mirkin MV, Pierce DT, Bard AJ, Nagy G, Tóth K (1993) Anal Chem 65:1213

12. Ammann D (1986) Ion-selective microelectrodes. Springer, Berlin

13. Lindner E, Buck RP (2000) Anal Chem 72:336A

14. Joo S, Brown RB (2008) Chem Rev 108:638

15. Fogt EJ, Untereker DF, Norenberg MS, Meyerhoff ME (1985) Anal Chem 57:1995

16. Portnoy HD (1967) In: Eisenman G (ed) The construction of glass electrodes. Marcel Dekker, New York

17. McKinley BA (2008) Chem Rev 108:826

18. Janata J, Huber RJ (1979) Ion-Sel Electrode Rev 1:31

19. Nikolskii BP, Materova EA (1985) Ion-Sel Electrode Rev 7:3

20. Vlasov YG, Ermolenko YE, Iskhakova OA (1979) J Anal Chem 34:1175

21. Fjeldly TA, Nagy K (1980) J Electrochem Soc 127:1299

22. Cattrall RW, Freiser H (1971) Anal Chem 43:1905

23. Simon W, Pretsch E, Ammann D, Morf WE, Guggi M, Bissig R, Kessler M (1975) Pure Appl Chem 44:613

24. Rosatzin T, Bakker E, Suzuki K, Simon W (1993) Anal Chim Acta 280:197

25. Liu D, Meruva RK, Brown RB, Meyerhoff ME (1996) Anal Chim Acta 321:173

26. Naegeli R, Redepenning J, Anson FC (1986) J Phys Chem B 90:6227

27. Buck RP, Lindner E (1998) Acc Chem Res 31:257

28. Fibbioli M, Bandyopadhyay K, Liu SG, Echegoyen L, Enger O, Diederich F, Bühlmann P, Pretsch E (2000) Chem Commun 5:339

29. Machado AASC (1994) Analyst 119:2263

30. Gabrielli C, Hemery P, Liatsi P, Masure M, Perrot H (2005) J Electrochem Soc 152:H219

31. Gallardo J, Alegret S, Munoz R, De-Roman M, Leija L, Hernandez PR, del Valle M (2003) Anal Bioanal Chem 377:248

32. Veltsistas PG, Prodromidis MI, Efstathiou CE (2004) Anal Chim Acta 502:15

33. Hauser PC, Chiang DWL, Wright GA (1995) Anal Chim Acta 302:241

34. Bobacka J (2006) Electroanalysis 18:7

35. Bobacka J, Lindfors T, Lewenstam A, Ivaska A (2004) Am Lab 36:13

36. Michalska A (2006) Anal Bioanal Chem 384:391

37. Bobacka J (1999) Anal Chem 71:4932

38. Bobacka J, Ivaska A, Lewenstam A (2008) Chem Rev 108:329

39. Bobacka J, McCarrick M, Lewenstam A, Ivaska A (1994) Analyst 119:1985

40. Grygolowicz-Pawlak E, Plachecka K, Brzozka Z, Malinowska E (2007) Sens Actuators B Chem 123:480

41. Konopka A, Sokalski T, Lewenstam A, Maj-Zurawska M (2006) Electroanalysis 18:2232

42. Konopka A, Sokalski T, Michalska A, Lewenstam A, Maj-Zurawska M (2004) Anal Chem 76:6410

43. Michalska A, Dumanska J, Maksymiuk K (2003) Anal Chem 75:4964

44. Michalska A, Konopka A, Maj-Zurawska M (2003) Anal Chem 75:141

45. Michalska A, Maksymiuk K (2004) Talanta 63:109

46. Michalska A, Ocypa M, Maksymiuk K (2006) Anal Bioanal Chem 385:203

47. Lai CZ, Fierke MA, Stein A, Bühlmann P (2007) Anal Chem 79:4621

48. Zhu ZQ, Zhang J, Zhu JZ, Lu W, Zi J (2007) IEEE Sens J 7:38

49. Crespo GA, Macho S, Rius FX (2008) Anal Chem 80:1316

50. Fibbioli M, Bandyopadhyay K, Liu SG, Echegoyen L, Enger O, Diederich F, Gingery D, Bühlmann P, Persson H, Suter UW, Pretsch E (2002) Chem Mater 14:1721

51. Grygolowicz-Pawlak E, Wygladacz K, Sek S, Bilewicz R, Brzozka Z, Malinowska E (2005) Sens Actuators B Chem 111:310

52. Gyurcsányi RE, Cristalli A, Nagy G, Nagy L, Corder C, Pendley BD, Ufer S, Nagle HT, Neuman MR, Lindner E (2001) Fresenius J Anal Chem 369:286

53. Syritski V, Gyurcsányi RE, Opik A, Tóth K (2005) Synth Met 152:133

54. Gyurcsányi RE, Nyback AS, Tóth K, Nagy G, Ivaska A (1998) Analyst 123:1339

55. Sundfors F, Bereczki R, Bobacka J, Tóth K, Ivaska A, Gyurcsányi RE (2006) Electroanalysis 18:1372

56. Cadogan A, Gao ZQ, Lewenstam A, Ivaska A, Diamond D (1992) Anal Chem 64:2496

57. Michalska A, Hulanicki A, Lewenstam A (1997) Microchem J 57:59

58. Gyurcsányi RE, Rangisetty N, Clifton S, Pendley BD, Lindner E (2004) Talanta 63:89

59. Momma T, Yamamoto M, Komaba S, Osaka T (1996) J Electroanal Chem 407:91

60. Vazquez M, Bobacka J, Ivaska A, Lewenstam A (2002) Sens Actuators B Chem 82:7

61. Paciorek R, van der Wal PD, de Rooij NE, Maj-Zurawska M (2003) Electroanalysis 15:1314

62. Sutter J, Radu A, Peper S, Bakker E, Pretsch E (2004) Anal Chem 523:53

63. Buck RP (1976) Anal Chem 48:R23

64. Fibbioli M, Morf WE, Badertscher M, de Rooij NF, Pretsch E (2000) Electroanalysis 12:1286

65. Sokalski T, Ceresa A, Zwickl T, Pretsch E (1997) J Am Chem Soc 119:11347

66. Buck RP, Lindner E (1994) Pure Appl Chem 66:2527

67. Lindner E, Umezawa Y (2008) Pure Appl Chem 80:85

68. Phillips F, Kaczor K, Gandhi N, Pendley BD, Danish RK, Neuman MR, Tóth B, Horváth V, Lindner E (2007) Talanta 74:255

69. Keplinger F, Glatz R, Jachimovitz A, Urban G, Kohl F, Olcaytug F, Progaska OJ (1990) Sens Actuators B Chem 1:272

70. Lindner E, Cosofret VV, Ufer S, Johnson TA, Ash RB, Nagle HT, Neuman MR, Buck RP (1993) Fresenius J Anal Chem 346:584

71. Lindner E, Cosofret VV, Ufer S, Kusy RP, Buck RP, Ash RB, Nagle HT (1993) J Chem Soc Faraday Trans 89:361

72. Schindler JG, Schindler MM (1989) Fresenius J Anal Chem 335:553

73. Sudholter EJR, van der Wal PD, Skowronska-Ptasinka M, van den Berg A, Bergveld P, Reinhoudt DN (1990) Anal Chim Acta 230:59

74. Cosofret VV, Erdosy M, Lindner E, Johnson TA, Buck RP, Kao WJ, Neuman MR, Anderson JM (1994) Anal Lett 27:3039

75. Yim HS, Cha GS, Meyerhoff ME (1990) Anal Chim Acta 237:115

76. Lindner E, Buck RP (1999) Fresenius J Anal Chem 364:22

77. Meyerhoff ME (1990) Clin Chem 36:1567

78. Li X, Petrovich S, Harrison DJ (1990) Sens Actuators B Chem B1:275

79. Li Z, Li X, Petrovic S, Harrison DJ (1996) Anal Chem 68:1717

80. Li Z, Li X, Petrovich S, Harrison DJ (1993) Anal Method Instrum 1:30

81. Li Z, Li X, Rothmaier M, Harrison DJ (1996) Anal Chem 68:1726
82. Zwickl T, Schneider B, Lindner E, Sokalski T, Schaller U, Pretsch E (1998) Anal Sci 14:57
83. Sutter J, Lindner E, Gyurcsanyi R, Pretsch E (2004) Anal Bioanal Chem 380:7
84. DeMarco R, Veder JP, Clarke G, Nelson A, Prince K, Pretsch E, Bakker E (2008) Phys Chem Chem Phys 10:73
85. Bodor S, Zook JM, Lindner E, Tóth K, Gyurcsányi RE (2008) Analyst 133:635–642
86. Moczár I, Gyurcsányi RE, Huszthy P, Jágerszki G, Toth K, Lindner E (2006) Electroanalysis 18:1396
87. Pendley BD, Gyurcsányi RE, Buck RP, Lindner E (2001) Anal Chem 73:4599
88. Pendley BD, Lindner E (1999) Anal Chem 71:3673
89. Heng LY, Tóth K, Hall EAH (2004) Talanta 63:73
90. Oesch U, Simon W (1980) Anal Chem 52:692
91. Bakker E, Xu A, Pretsch E (1994) Anal Chim Acta 295:253
92. Eugster R, Gehrig PM, Morf WE, Spichiger UE, Simon W (1991) Anal Chem 63:2285
93. Meier PC, Morf WE, Läubli M, Simon W (1984) Anal Chim Acta 156:1
94. Lindner E, Cosofret VV, Ufer S, Buck RP, Kao WJ, Neuman MR, Anderson JM (1994) J Biomed Mater Res 28:591
95. Bakker E, Lerchi M, Rosatzin T, Rusterholz B, Simon W (1993) Anal Chim Acta 278:211
96. Langmaier J, Lindner E (2005) Anal Chim Acta 543:156
97. Telting-Diaz M, Bakker E (2001) Anal Chem 73:5582
98. Gyurcsányi RE, Lindner E (2005) Anal Chem 77:2132
99. Sutter J, Pretsch E (2006) Electroanalysis 18:19
100. Bedlechowicz I, Sokalski T, Lewenstam A, Maj-zurawska M (2005) Sens Actuators B Chem 108:836
101. Lindner E, Gyurcsányi RE, Buck RP (1999) Electroanalysis 11:695
102. Makarychev-Mikhailov S, Shvarev A, Bakker E (2004) J Am Chem Soc 126:10548
103. Makarychev-Mikhailov S, Shvarev A, Bakker E (2006) Anal Chem 78:2744
104. Shvarev A, Bakker E (2003) Anal Chem 75:4541
105. Shvarev A, Bakker E (2003) J Am Chem Soc 125:11192
106. Pergel É, Gyurcsányi RE, Tóth K, Lindner E (2001) Anal Chem 73:4249
107. Zook JM, Buck RP, Gyurcsányi RE, Lindner E (2008) Electroanalysis 20:259
108. Zook JM, Buck RP, Langmaier J, Lindner E (2008) J Phys Chem B 112:2008
109. Wallace GG, Dastoor PC, Officer DL, Too CO (2000) Chem Innov 30:14
110. Potje-Kamloth K (2008) Chem Rev 108:367
111. Ives DJG, Janz GJ (1961) Reference electrodes, theory and practice. Academic, New York
112. Lindner E, Tóth K, Pungor E (1973) Ion-Sel Electrodes 1:205
113. Bakker E, Rubinova N, Chumbimuni-Torres K (2007) Sens Actuators B Chem 121:135
114. Vigassy T, Gyurcsányi RE, Pretsch E (2003) Electroanalysis 15:1270
115. Vigassy T, Gyurcsányi RE, Pretsch E (2003) Electroanalysis 15:375
116. Bühlmann P, Yajima S, Tohda K, Umezawa K, Nishizawa S, Umezawa Y (1995) Electroanalysis 7:811
117. Chumbimuni-Torres KY, Rubinova N, Radu A, Kubota LT, Bakker E (2006) Anal Chem 78:1318
118. Chumbimuni-Torres KY, Dai Z, Rubinova N, Xiang Y, Pretsch E, Wang J, Bakker E (2006) J Am Chem Soc 128:13676
119. Bakker E, Buhlmann P, Pretsch E (2004) Talanta 63:3
120. Patolsky F, Zheng GF, Lieber CM (2006) Anal Chem 78:4260