Published in final edited form as:
Trends Analyt Chem. 2008 ; 27(7): 612–618. doi:10.1016/j.trac.2008.04.007.

Nanoscale potentiometry

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Abstract

Potentiometric sensors share unique characteristics that set them apart from other electrochemical sensors. Potentiometric nanoelectrodes have been reported and successfully used for many decades, and we review these developments. Current research chiefly focuses on nanoscale films at the outer or the inner side of the membrane, with outer layers for increasing biocompatibility, expanding the sensor response, or improving the limit of detection (LOD). Inner layers are mainly used for stabilizing the response and eliminating inner aqueous contacts or undesired nanoscale layers of water. We also discuss the ultimate detectability of ions with such sensors and the power of coupling the ultra-low LODs of ion-selective electrodes with nanoparticle labels to give attractive bioassays that can compete with state-of-the-art electrochemical detection.

Keywords

Bioanalysis; Bioassay; Ion-selective electrode; Miniaturization; Nanoelectrode; Nanolayer; Nanoparticle; Nanoscale; Potentiometry; Sensor

1. Introduction

Nanoscale potentiometry comprises a variety of topics. Potentiometric microelectrodes and sub-microelectrodes have been known for decades; they were simply not named nanoelectrodes because the term “nano” was not in vogue at that time. We discuss these devices and their modern counterparts in the first part of this review. The second part is dedicated to nanolayers that are important in all-solid-state ion-selective electrodes (ISEs) either as unwanted disturbing effects (e.g., ultrathin (10 nm) water films between solid contacts and polymeric membranes) or as monolayers or thin films generated on purpose to stabilize the potentiometric response. In this context, molecular layers on the outer surface of ISE membranes have been used to increase potential stability in the presence of proteins. Miniaturized ISEs with extremely low limits of detection (LODs) are subsequently presented. Although these ISEs are not nanoscale devices, their accessibility is a prerequisite for diverse applications based on nanoparticles, including quantum dots (QDs) as labels for the potentiometric detection of proteins or DNA, as described in the last section of this review.

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2. Ion-selective microelectrodes and nanoelectrodes

Potentiometric microelectrodes have already been in use for half-a-century, mainly for physiological studies.

Early pH [1] as well as Na^+ and K^+ selective glass microelectrodes [2] with diameters of a few tens of μm were followed by cation or anion exchanger-filled microcapillaries with diameters of <1 μm [3,4] in the 1970s (Fig. 1).

More recently, nm-scale pH electrodes have been fabricated by electrochemical deposition of a polyaniline film on conically-etched carbon fibers with tip diameters of 100–500 nm [5]. Selective ionophores have been known since the late 1960s, but their use in microelectrodes only became possible [6] after it was observed that ion exchangers (e.g., tetraphenylborate derivatives for cation-selective electrodes) must be added to the sensing membrane phases [7].

Then, within a few years, ion-selective microelectrodes based on glass micropipettes with diameters down to a few tens of nm [8] were described for a series of ions, including H^+ [9], Li^+ [6], Na^+ [10], K^+ [11], Ca^{2+} [12] and Mg^{2+} [13]. Such electrodes were at first almost exclusively used for intracellular ion-activity measurements but then several other applications emerged. An ion-exchanger-based K^+-selective microelectrode was used as a detector in open-tubular column liquid chromatography [14] with an estimated detection volume of <500 pL and an assessed amount of ca. 1 pmol of K^+. Later, 10^{-7} M I^- was determined with an anion-exchanger-based microelectrode in a similar detection volume, which corresponds to an estimated detected amount of 6 amol of I^- [15]. Microelectrodes were subsequently applied as detectors in capillary zone electrophoresis [16–20]. To avoid interferences by the high voltage, they were originally used as post-column detectors [16] but, later, on-column detection of cations [18] and anions [19,20] became possible with conically-etched capillaries [17]. A lower LOD of 5×10^{-8} M ClO_4^- was obtained [20].

Potentiometric microelectrodes also serve as detectors in scanning electrochemical microscopy [21]. After first applications of Ag [22] and Sb-microdisk electrodes [23] to assess Cl^- and H^+ activities, ionophore-based single-barreled and double-barreled microelectrodes with diameters of 1–20 μm were used to determine K^+, NH_4^+, and Zn^{2+} concentration profiles in electrochemical-model experiments, during enzyme-catalyzed reactions, corrosion processes, and electrochemical reduction of Zn^{2+} [24,25].

Astonishingly, not much effort has been invested in combining microelectrodes with miniaturized total analysis systems (μ-TAS). So far, the smallest membrane used in μ-TAS had dimensions of 20×20 μm^2 and the Ba^{2+}-selective electrode gave rather noisy, drifting signals [26].

Microelectrodes have also been used in the vibrating mode to measure ion fluxes (e.g., a Cd^{2+}-selective microelectrode, optimized in terms of its selectivity and lower LOD [27,28], was used to characterize the Cd^{2+} flux near plant roots).

Since potentiometric measurements in pL volumes are possible, and lower potentiometric LODs of $\leq 10^{-10}$ M have been achieved, we may want to ask what the limits are in term of total measurable amount. Indeed, is it possible to detect single ions by potentiometry? Interestingly, this question had already been discussed more than 20 years ago [29].

First, it must be kept in mind that the potential at the membrane or sample-phase boundary arises as a consequence of a local charge separation of cations and anions at the membrane surface. This occurs within a very thin layer of the order of 10 nm, and even for microelectrodes

having a surface of ca. 10^{-8} cm^2 involves an estimated amount of ca. 10^{-19} mol or ca. 10^4 ions [30]. The second limitation is the measuring current, which is of the order of 1 fA or 10^{-20} mol/s, even with advanced instruments. Thus, during a measurement time of only 100 s, the ion content of the sample changes by the order of 10^6 ions. Also, bulk and surface resistances of the electrode must be considered. Microelectrodes often have bulk resistances of ca. 10^{11} Ω, requiring a measuring station with an input impedance of $>10^{14}$ Ω. While this is not a basic problem today, further miniaturization might require more sophisticated instruments. Surface resistance must also be considered. It is related to the exchange current, which must be higher than the measuring current. Otherwise, depending on the direction of the current, sub-Nernstian or super-Nernstian responses might be induced at low sample concentrations. Corresponding response curves were estimated by applying the Butler-Volmer equation [29]. For a measuring current of 10 fA, it was estimated that polarization due to an insufficient exchange current occurs with a microelectrode exhibiting an area of 10^{-9} cm^2 below a sample activity of ca. 10^{-6} M (the limiting current is proportional to the membrane surface and to the square root of the sample concentration). In a volume of 1 pL, this corresponds to 10^5–10^6 ions. Based on these considerations, it is not possible to determine single ions by potentiometry. Of course, the situation is different if the total concentration of ions is large and a very low concentration of free ions is kept constant by using ion buffers. If the equilibrium is fast enough, very low concentrations of free ions may nominally be measured by potentiometry (e.g., a solid state Ag_2S ISE showed a potentiometric response to a calculated activity of 10^{-25} M Ag^+ in an ion buffer consisting of 0.1 M Na_2S and 1.0 M NaOH [31,32]).

The major disadvantage of conventional micropipette-based electrodes is the difficulty of handling them, their fragility, and their short lifetime, so, more recently, efforts have been made to develop rugged microelectrodes. Robustness was increased by using an inner solid contact (i.e. metal wire or glassy carbon sealed into a glass capillary). A conducting polymer layer was then deposited on the surface of the disk electrode, which was subsequently covered with a conventional PVC membrane [33]. Electrode diameters of about 5 μm have been achieved with this design.

In a modified procedure, the membrane was placed in a microcavity obtained by recessing the above disk electrode through chemical etching (Fig. 2) [34]. A membrane ca. 1 μm in diameter was obtained by a similar process that made use of an inner Ag/AgCl electrode in contact with a microscopic hydrogel layer covered with a polymer membrane [35]. These electrodes were used as selective probes in experiments using scanning electrochemical microscopy [34,35].

In another approach, arrays of silicon-nitride micropipettes with diameters in the range 0.25–6 μm were prepared by microfabrication technology [36]. Arrays of 24 or 16 Ca^{2+} [37], K^+, and NH_4^+ micropipette electrodes were constructed for monitoring extracellular ion activities in cell cultures. Due to the rather large internal membrane reservoir, excellent lifetimes (>1 month) have been achieved [38].

3. Nanolayers in potentiometry

There have been a limited number of studies about the functionality of nanoscale membranes in potentiometry. There was some anecdotal concern that, to be permselective, ISE membranes require a minimum thickness beyond the so-called Debye length [39]. This notion appears to be rebutted by early results of the Eisenman group [40], and, more recently, by the Umezawa group, who observed similar potentiometric behavior of bulk PVC membranes and hanging lipid-bilayer membranes doped with valinomycin (Fig. 3) [41], so potentiometric sensors based on nanoscale films may indeed become a reality.

However, in most research, the ion-selective membrane is on the μm scale. Recent work by the Neshkova group introduced thin electrodeposited films of chalcogenide ISE membranes

on platinum supports for ion sensing in flow-injection analysis [42]. However, the thickness of the films was not reported in detail. As discussed in the previous section, microelectrodes usually also have thicknesses in the μm range. Clearly, nanoscale layers were most often applied for potentiometric sensors at the front or the back of the actual membrane.

3.1. Outer layers on ion-selective membranes

A recent study involving polyelectrolyte multilayers deposited on the sample side of the ISE membrane revealed that traditional potentiometric read-out is not affected by the presence of this nanoscale layer [43]. By contrast, other electrochemical-excitation experiments (e.g., electrochemical impedance spectroscopy or pulsed galvanostatic excitation) revealed a mass-transport barrier when such layers were present. In potentiometry, the underlying phase-boundary potential is dictated by an electrochemical-extraction-equilibrium process that may not always be affected by the structure of the nanoscale film. This points to potentiometric sensors being more robust than other electrochemical sensors in real-world applications.

For this reason, outer nanoscale films deposited onto ISE membranes have had important roles in improving the characteristics of these sensors indirectly, rather than attenuating the ISE response. With a perrhenate ISE, Imato and Nakamura used an outer anionic polyelectrolyte layer to improve its LOD by two orders of magnitude [44]. This was attributed to the preconcentration characteristics of the outer layer, but a reduction of undesired ion fluxes from the membrane because of the mass-transport barrier characteristics of the polyelectrolyte layer may also have played a role.

The Cha group used a thin hydrophilic coating of cellulose acetate on a hydrophobic polyurethane Cl^--ISE membrane to reduce the response to salicylate compared with uncoated membranes [45]. Here, the outer layer was used as an effective kinetic barrier against dilute interfering species, and the sensor was successfully tested in serum measurements.

The Meyerhoff and Brown groups applied thin outer hydrophilic coatings (hydrophilic polyurethane) to hydrophobic polymeric ISE membranes to immobilize enzymes (e.g., urease and adenosine deaminase) [46]. This was done with a view to microfabricating solid-state enzyme-based biosensors.

In a more direct approach, Koncki et al. covalently immobilized human IgG onto ISE membranes for H^+ and NH_4^+, forming a nanoscale assembly of IgG on the sensor surface [47]. A competitive assay involving an anti-human IgG conjugated to urease gave a potentiometric response to the ammonia released in the presence of urea.

The Bachas group used heparinized outer coatings on cellulose-acetate membranes and found that the resulting membrane selectivity and potentiometric response characteristics were not altered significantly [48]. However, the biocompatibility was markedly improved, supposedly because of the anticoagulant action of the immobilized heparin coating, and measurements were performed in undiluted serum samples.

3. 2. Inner layers at ion-selective membranes

With a view to achieving small, mass-fabricated potentiometric sensors, so-called solid-contact or all-solid state ISEs are important in modern sensor research. Cattrall and Freiser coined the term coated-wire electrode, which typically suffered from an ill-defined inner interface, hence exhibiting long-term potential instabilities, and was suitable for special applications only [49]. It was much later postulated that a nanoscale layer of water may form between the ISE membrane and the unmodified metallic support, whose electrolyte composition may change as a function of the outer bathing solution because of counter-diffusion fluxes [50]. Very

recently, De Marco, using neutron reflectometry, gave spectroscopic evidence of the existence of such a water layer, in this case having a thickness on the order of just 10 nm [51].

One approach to eliminating this undesired water layer was to introduce hydrophobic monolayers containing redox-active functionalities. Fibbioli et al. used fullerene and tetrathiafulvalene functionalities and, using potentiometric reconditioning protocols, showed convincingly that an inner water layer was absent (Fig. 4) [52]. More recently, the Malinowska group explored ferrocene-terminated thiols as more convenient inner monolayer coatings and also reported the absence of a water layer [53].

Alternatively, conducting polymers can be employed as inner layers for ISEs. These efforts were reviewed recently [54]. While some materials (e.g., polypyrrole [55] and poly(3,4-ethylenedioxythiophene) (PEDOT) [56]) electropolymerize conveniently, alternative compounds (e.g., poly(3-octylthiophene)) are often solvent cast, but they exhibit desired lipophilicity characteristics and reduced redox interference from potentially-interfering solution species [53–55].

Recently, the Bühlmann group suggested that coated-wire electrodes may be stabilized by increasing the inner surface area. Nanostructured macroporous carbon was used as unmodified solid-contact material for fabricating all solid state ISEs [57]. The much larger effective surface area at the inner membrane side was shown to render that interface essentially non-polarizable, even though no defined ion-to-electron-transduction mechanism was involved. The resulting potential drifts were of the order of a mere 11 μV/h. Similarly, both porous silicon [58] and single-walled carbon nanotubes [59] seem to make the presence of a redox couple at the membrane-solid interface unnecessary.

4. Improved limits of detection with miniaturized electrodes

After understanding the adverse effect of transmembrane ion fluxes [60,61], which may bias the primary ion concentration in the vicinity of the membrane, ISEs with lower LODs in the range 10^{-8}–10^{-11} M total-ion concentrations have been developed for more than 10 ions [62, 63]. Although, due to spherical diffusion, microelectrodes should be advantageous in this respect, so far, they have not shown such low LODs. However, miniaturized electrodes have been designed with excellent LODs [64–66]. They allow measurement of low concentrations in small sample volumes and are a prerequisite for applying nanoparticles as labels for protein and DNA analysis (see Section 5, below).

In a first approach, lipophilic monolithic capillaries (2–5 mm long, inner diameter 200 μm) were filled with the polymer-free membrane material. Due to impeded diffusion through the monoliths, the sensor responses did not depend on the composition of the inner solution, and excellent lower LODs comparable with the best optimized liquid-contact electrodes were achieved [64]. Later, similarly excellent performances were obtained with both hard PVC membranes in polypropylene-micropipette tips of the same diameter [65] and methylmethacrylate/*n*-decylmethacrylate copolymer-based miniaturized solid-contact electrodes [66].

With such ISEs, trace-level measurements in confined samples are possible (e.g., detection of 10^{-10} M Ca^{2+}, Pb^{2+} or Ag^{+} in samples of 3 μL (about one tenth of a droplet) was achieved with the respective ISEs combined with a similarly miniaturized Na^{+}- selective pseudo-reference electrode). This corresponds to the determination of 300 amol of the respective ions. At this concentration, the signal was several 100 times greater than the noise. The estimated LODs according to the 3σ rule were found for the three ions to be 2.5 amol, 25 amol, and 1 zmol, respectively [65].

5. Potentiometric detection of nanoparticle labels

Biochemical assays routinely require a chemical- or electrochemical-amplification step that translates the analyte-binding event into a detectable signal even at ultra-trace-analyte concentrations. This amplification is often performed with nanoscale materials attached to a secondary bioreagent used to form a so-called sandwich complex with the analyte and primary capture probe, which is often immobilized onto a surface. With potentiometric sensors exhibiting very low intrinsic LODs and good scope for miniaturization, further chemical amplification should lead to highly attractive LODs.

One of the earliest preliminary works in this direction made use of rat-liver microsomes that were used as a biocatalytic reagent to liberate iodide from microsomal-hormone thyroxine, which was measured at sub-micromolar concentrations with an I^--selective electrode [67].

Some analogy to this approach is found in a later work by the Meyerhoff group, who utilized chemical-amplification steps involving polymeric membrane-based polyion-selective electrodes and polyion-cleaving enzymes as biochemical labels [68].

Another early pioneering approach in potentiometric bionsensors made use of liposomes loaded with marker ions. Antigens were dissolved in the lipid bilayer membranes of the liposomes, which were then destroyed by the immunoreaction the marker ions were lysed [69].

Indirect potentiometric detection of bioreactions is also possible by measuring the modulation of ion fluxes through nanopores due to such reactions [70].

To couple potentiometric sensors exhibiting ultra-trace LODs with biochemical assays containing amplification labels is quite new. Recently, a heterogeneous sandwich immunoassay was reported with gold nanoparticles as labels on a secondary antibody [71]. After completing the assay, the gold nanoparticles were chemically plated with silver, thus forming enlarged silver clusters. These were subsequently dissolved with hydrogen peroxide, which is more compatible with the final potentiometric detection step than the nitric acid used earlier for adsorptive stripping voltammetric detection [72]. The liberated silver ions were detected with a solid-contact Ag^+-selective microelectrode, yielding promising results.

Shortly afterwards, a lower LOD of <10 fmol (i.e. improved by several orders of magnitude) was achieved for IgG using cadmium-selenide nanocrystals as labels, which were directly dissolved with hydrogen peroxide in microtiter plates without further chemical plating or enhancement (Fig. 5) [73]. A Cd-ISE with liquid inner contact served as the detecting system. The improved LOD achieved with these QDs indicates that the amplification by chemical plating may go at the expense of non-specific signal originating from plating reactions occurring at sites other than the nanoparticles of interest, so it is not always beneficial. However, further optimization of the assay may improve the LOD obtained by this approach.

The use of cadmium-sulfide QDs as amplification labels was recently extended to aptamer-based potentiometric assays [74]. For this purpose, a solid-contact Cd-ISE exhibiting an nmol LOD in 200-μL microwells was developed and used to detect thrombin with aptamer-based chemistries in a way analogous to the sandwich-immunoassay principle outlined above. The LOD for thrombin was found to be ca. 5 ppb, which competes favorably with other the LODs of electrochemical assays.

Most recently, this same general principle was also applied to the detection of DNA using a surface-adsorbed capture DNA probe and a secondary DNA strand containing the cadmium-

sulfide nanocrystal label. Potentiometric read-out yielded a 10 pM (2 fmol) LOD, which competes well with comparable stripping voltammetric techniques.

6. Conclusions

Nanoscale potentiometry is a natural progression in the history of ISEs that makes use of nanoscale materials to improve the characteristics of ISEs and expand their potential uses. Understanding chemical processes at the interface is key to advancing the field, and the science of thin multilayers and nanostructured materials is starting to make a significant impact in the field of potentiometric sensors. These advances will certainly make it possible to harness the already impressive low LODs of these devices and translate them into extremely low detectable quantities that will be very useful for a number of applications. Already today, miniaturized potentiometric sensors, coupled to appropriate amplification labels such as metal and semiconductor nanoparticles, can compete with state-of-the-art electrochemical bioassays. However, without amplification, it appears theoretically impossible for potentiometric sensors to detect single ions.

Acknowledgements

The authors would like to acknowledge the National Institutes of Health (GM59716 and EB002189) for supporting their electrochemical-sensor research. We thank D. Wegmann, G. Mori, and R.E. Gyurcsányi for careful reading of the manuscript, and R.E. Gyurcsányi for providing Fig. 2.

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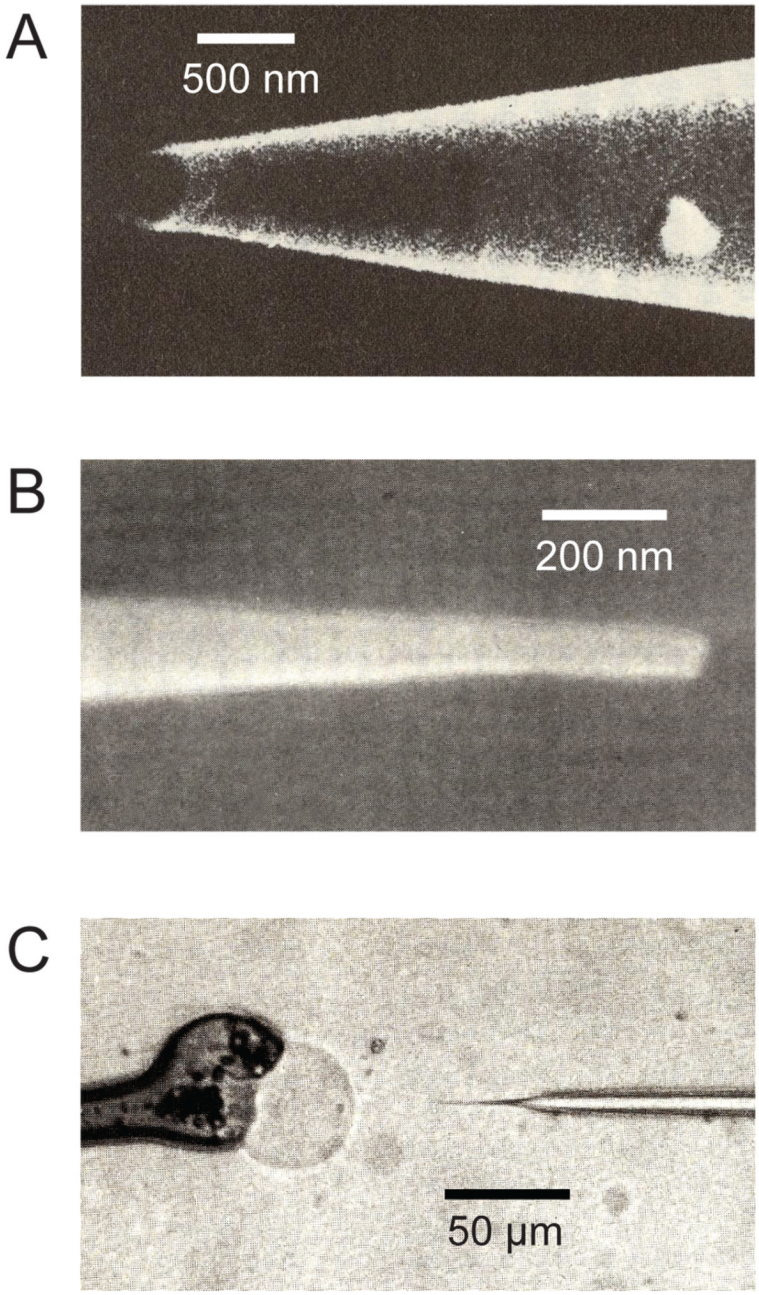

Figure 1.
Early examples of nanoscale potentiometric sensors based on micropipettes filled with ion-selective membrane materials (adapted from A) [75], B) [75], C) [76]).

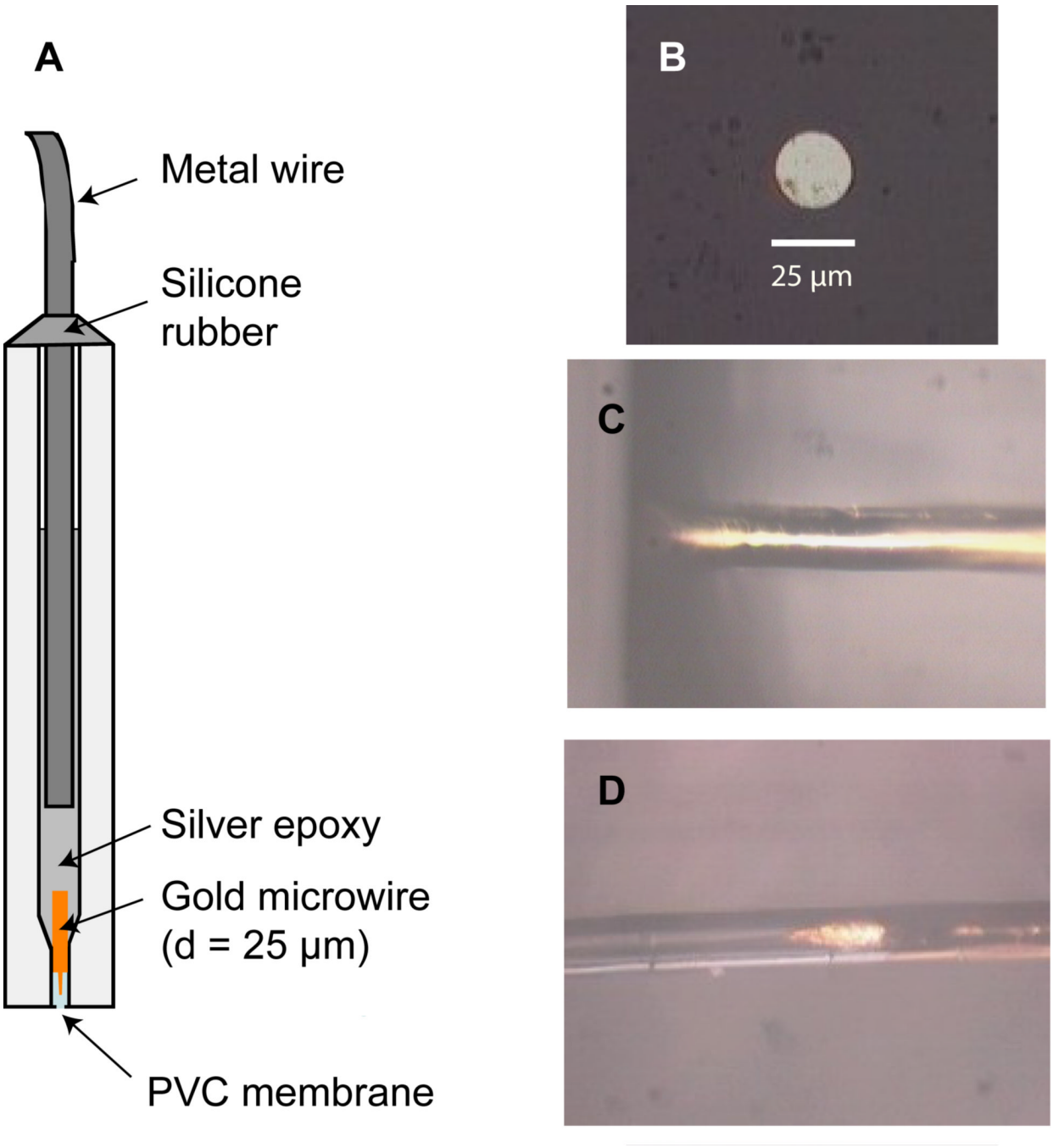

Figure 2.
All-solid state microelectrodes based on a recessed gold microelectrode covered with a conducting polymer PEDOT and an ion-selective liquid membrane [34]: A) electrode assembly; B) bottom view; C) side view of the gold microelectrode before etching; and, D) side view of the electrode after etching to yield the desired recess.

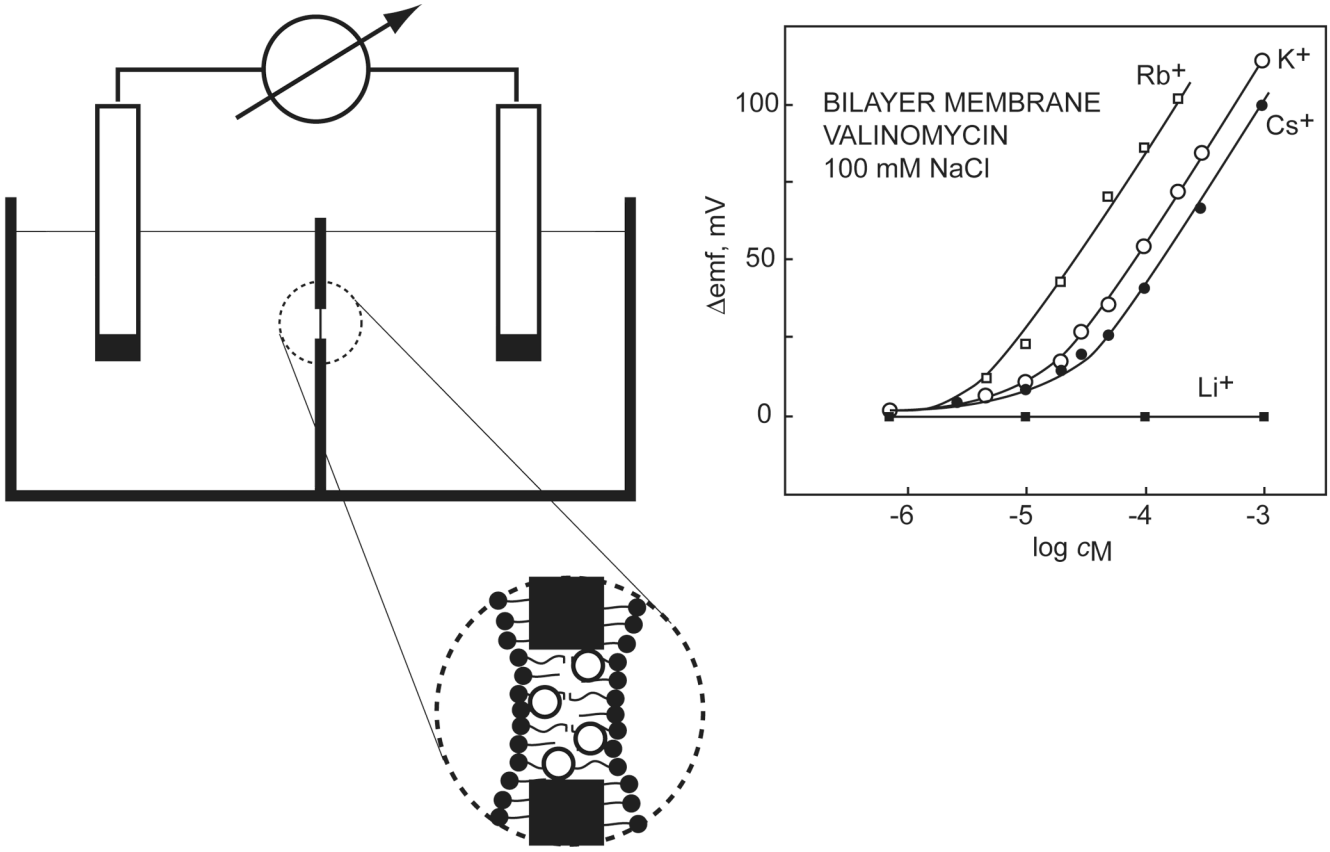

Figure 3.

Free-hanging bilayer membranes doped with potassium ionophore valinomycin (symbolized with open circles) may lead to Nernstian response slopes and a selectivity pattern comparable to that of thick-film polymeric ion-selective electrode membranes [41]. Left: experimental set-up. Right: potentiometric responses to the ions indicated in a background of 100 mM NaCl.

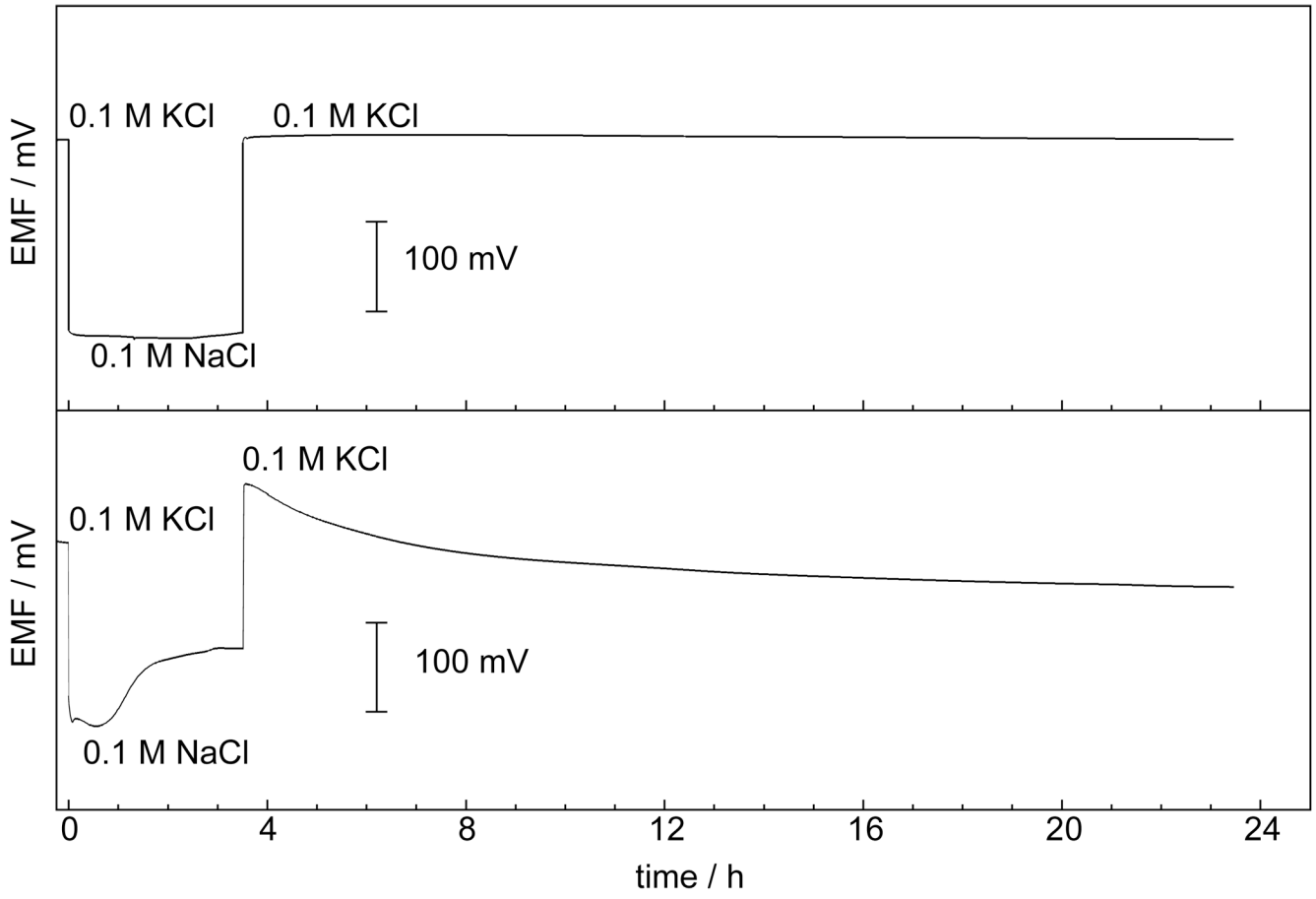

Figure 4.

Potentiometric reconditioning procedure to evaluate the presence of a nanoscale layer of water between the ion-selective polymeric membrane and the metallic support [50]. Top: In the absence of a water layer, replacing the electrolyte on the sample side of the membrane results in stable potentials. Bottom: a thin water underlayer will change its composition as a function of the sample solution outside on the basis of counter-diffusion fluxes across the polymeric membrane and result in characteristic potential drifts.

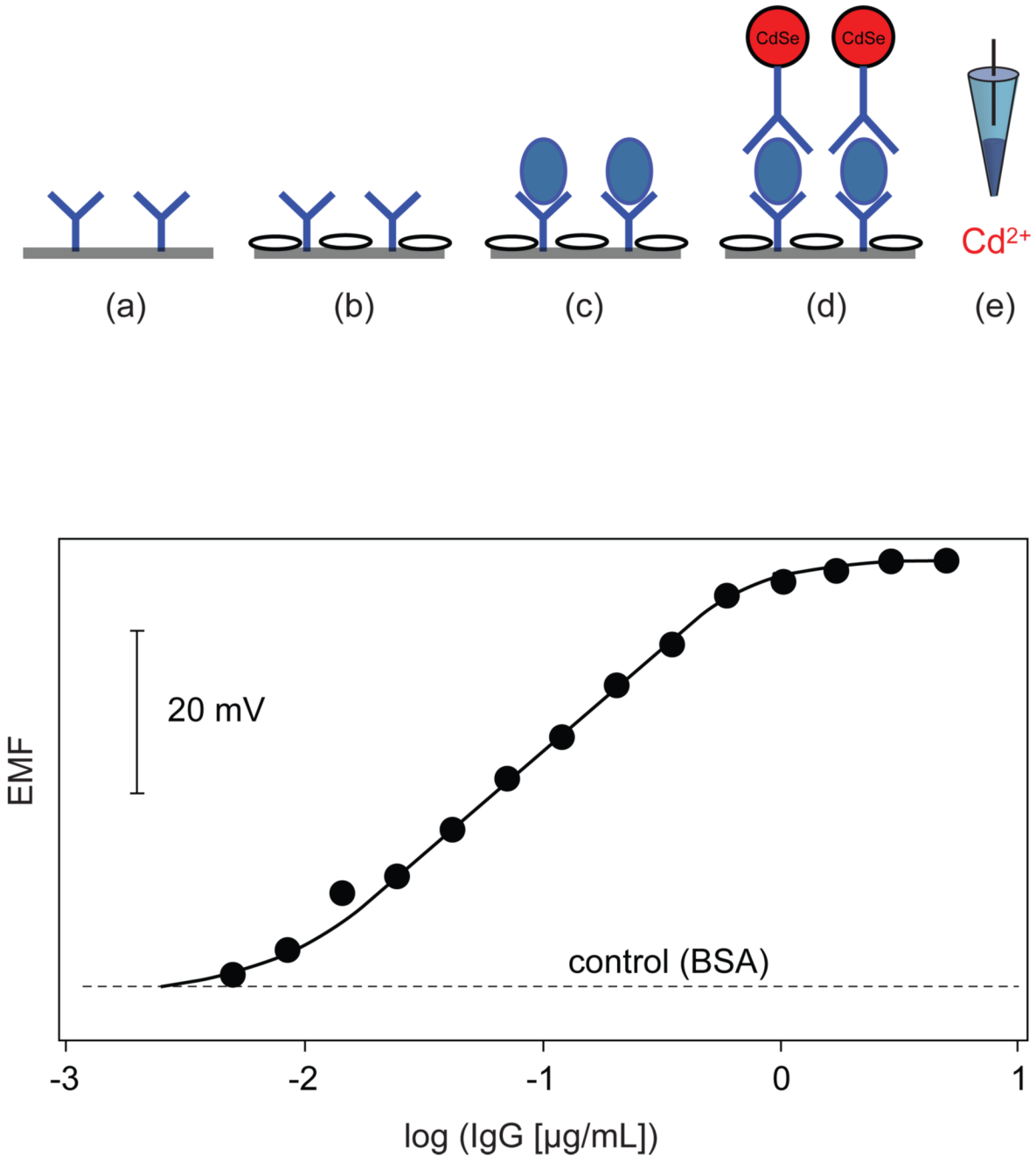

Figure 5.
Cadmium-selenide-nanoparticle-labeled sandwich immunoassay, performed in microtiter plates and detected at trace level with a potentiometric microelectrode [73]. Top: assay sequence, which includes: a) immobilizing capture antibodies; b) passivation of unreacted surface; c) affinity binding to the analyte IgG; d) binding with a secondary antibody labeled with CdSe quantum dots; and, e) potentiometric detection of the cadmium ions released with hydrogen peroxide. Bottom: concentration response of the assay, as recorded by the microelectrode.