Nanoscale Potentiometry

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Potentiometric sensors share unique characteristics that set them apart from other electrochemical sensing principles. Potentiometric nanoelectrodes have been reported and successfully used many decades ago, and these developments are here reviewed. Current research is chiefly focused on nanoscale films at the outer or inner side of the membrane, with outer layers for increasing biocompatibility characteristics, expansion of the sensor response, or improvement of the detection limit. Inner layers are mainly used for stabilizing the response and eliminating inner aqueous contacts or undesired nanoscale water layers. This report also discusses the ultimate detectability of ions with such sensors and the power of coupling the ultra-low detection limit of ISEs with nanoparticle labels for attractive bioassays that can compete with the state of the art in electrochemical detection.

Keywords: Ion-selective electrodes; Miniaturization; Nanolayers; Nanoparticles; Bioanalysis

1. Introduction

Nanoscale potentiometry comprises a variety of topics. Potentiometric micro- and submicroelectrodes have been known for decades; they were simply not named nanoelectrodes because the term "nano" was not en vogue that time. These devices and their modern counterparts are discussed in the first part of this review. The second part is dedicated to nanolayers that are of importance in all-solid-state ion-selective electrodes (ISEs) either as unwanted disturbing effects, such as 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 the potential stability in the presence of proteins. Miniaturized ISEs with extremely low detection limits are subsequently presented. Although these ISEs are not nanoscale devices, their accessibility is the prerequisite for diverse applications based on nanoparticles including quantum dots as labels for the potentiometric detection of proteins or DNA, as described in the last section of this review.

2. Ion-selective micro- 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 70s of the last century (cf. Fig. 1). More recently, nanometer-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 {Zhang et al., 2002, Anal. Chim. Acta, 452, 1-10}. Selective ionophores are known since the late 60ies of the last century but their use ~~The use of ionophores~~ in microelectrodes became only possible [5] after it was observed that ion exchangers, such as tetraphenylborate derivatives for cation-selective electrodes, must be added to the sensing membrane phases [6]. Then, within a few years, ion-selective microelectrodes based on glass micropipettes with diameters down to a few tens of nm [7] were described for a series of ions including H^+ [8], Li^+ [5], Na^+ [9], K^+ [10], Ca^{2+} [11], and Mg^{2+} [12]. Such electrodes were 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 [13] 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^- [14]. Microelectrodes were subsequently applied as detectors in capillary zone electrophoresis [15-19]. To avoid interferences by the high voltage, they were originally used as post-column detectors [15] but later on-column detection of cations [17] and anions [18,19] became possible with conically etched capillaries [16]. A lower detection limit of 5 x 10^{-8} M ClO_4^- has been obtained [19].

Potentiometric microelectrodes also serve as detectors in scanning electrochemical microscopy [20]. After first applications of Ag [21] and Sb microdisk electrodes [22] to assess Cl^- and H^+ activities, ionophore-based single- and double-barrel 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 in the electrochemical reduction of Zn^{2+} [23,24].

Astonishingly, not much effort has been invested into combining microelectrodes with miniaturized total analysis systems (μ-TAS). So far, the smallest membrane used in μ-TAS had dimensions of 20 x 20 μm^2 and the Ba^{2+}-selective electrode gave rather noisy and drifting signals [25].

Microelectrodes have also been used in the vibrating mode to measure ion fluxes. For example, a Cd^{2+}-selective microelectrode optimized in terms of its selectivity and lower detection limit [26,27] was used to characterize the Cd^{2+} flux near plant roots.

Since potentiometric measurements in pL volumes are possible and potentiometric lower detection limits 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 was already discussed more than 20 years ago [28].

First of all, it must be kept in mind that the potential at the membrane/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 ~~of~~ ions [29]. 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 6 x 10^5 ions. Also, the bulk and surface resistance 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. Not only the bulk resistance but also the surface resistance must 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- or super-Nernstian responses might be induced at low sample concentrations. Corresponding response curves were estimated by applying the Butler-Volmer equation [28]. 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 to 10^6 ions. Based on these considerations, it is not possible to determine single ions by potentiometry. The situation is, of course, 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 be measured by potentiometry. Thus, 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 {Durst, 1969, Ion-Selective Electrodes, Chapter 11; Vesely et al., 1972, Anal. Chim. Acta, 72, 1-12}.

The major disadvantage of conventional micropipette-based electrodes is their difficult handling, fragility, and short lifetime. Therefore, more recently, various efforts have been made to develop rugged microelectrodes. Robustness was increased by using an inner solid contact, i.e., a 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 [30]. 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 (cf. Fig. 2) [31]. A membrane with 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 the polymer membrane [32]. These electrodes were used as selective probes in scanning electrochemical microscope experiments [31,32].

In another approach, arrays of silicon nitride micropipettes with diameters ranging from 250 nm to 6 µm were prepared by microfabrication technology [33]. Arrays of 24 or 16 Ca^{2+} [34], K^+, and NH_4^+ micropipette electrodes were constructed for monitoring extracellular ion activities in cell cultures. Owing to the rather large internal membrane reservoir, excellent lifetimes of >1 month have been achieved [35].

3. Nanolayers in potentiometry

There have been a limited number of studies about the functionality of nanoscale membranes in potentiometry. In fact, there was some anecdotal concern that ISE membranes require a minimum thickness beyond the so-called Debye length in order to possess permselectivity [36]. This notion appears to be rebutted by early results of the Eisenman group {Szabo et al., 1969, J. Membr. Biol., 1, 346-382} and, more recently, by the group of Umezawa, who observed similar potentiometric behavior of bulk PVC membranes and hanging lipid bilayer membranes doped with valinomycin ~~showed Nernstian response slopes when interrogated potentiometrically~~ (cf. Fig. 3) [37]. ~~If so,~~ Thus, potentiometric sensors based on nanoscale films may indeed become a reality.

In most research, however, the actual ion-selective membrane is on the µm-scale. Recent work by the group of Neshkova introduced thin electrodeposited films of chalcogenide ISE membranes on platinum supports for ion sensing in flow-injection analysis [38]. The actual thickness of the films was, however, not reported in detail. As discussed in the previous section, microelectrodes usually have thicknesses in the µm range as well. Clearly, nanoscale layers were most often applied for potentiometric sensors either at the front or back side 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 readout is not affected by the presence of this nanoscale layer [39]. In contrast, other electrochemical excitation experiments such as 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 a higher level of robustness of potentiometric sensors as compared to other electrochemical principles in real-world applications.

For this reason, outer nanoscale films deposited onto ISE membranes have had important roles in improving indirect characteristics of these sensors, rather than attenuating the actual ISE response. Imato and coworker used with a perrhenate ISE an outer anionic polyelectrolyte layer to achieve an improvement of its detection limit by two orders of magnitude {Imato and Nakamura, 1996, Denki Kagaku oyobi Kogyo Butsuri Kagaku, 64, 1334}. 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 have also played a role.

The group of Cha used a thin hydrophilic coating of cellulose acetate on a hydrophobic polyurethane Cl^--ISE membrane with the purpose of reducing the response to salicylate in comparison with uncoated membranes [41]. 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 groups of Meyerhoff and Brown applied thin outer hydrophilic coatings (hydrophilic polyurethane) to hydrophobic polymeric ISE membranes as a platform to immobilize enzymes such as urease and adenosine deaminase [42]. This was done in view of 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 [43]. A competitive assay involving an anti-human IgG conjugated to urease gave rise to the potentiometric response to the released ammonia in the presence of urea.

The group of Bachas used heparinized outer coatings on cellulose acetate membranes and found that the resulting membrane selectivity and potentiometric response characteristics were not altered significantly [44]. However, the biocompatibility was markedly improved, supposedly from the anticoagulant action of the immobilized heparin coating, and measurements in undiluted serum samples were performed.

3. 2. Inner layers at ion-selective membranes

In view of achieving small, mass fabricated potentiometric sensors, so-called solid-contact or all-solid state ISEs are an important direction of modern sensor research. Cattrall and Freiser coined the term coated-wire electrode, which typically suffered from an ill-defined inner interface and hence exhibited long-term potential instabilities and was suitable only for special applications [45]. It was much later postulated that a nanoscale water layer 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 counterdiffusion fluxes [46]. 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 [47].

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 by potentiometric reconditioning protocols showed convincingly that an inner water layer was absent (cf. Fig. 4) [48]. More recently, the group of Malinowska explored ferrocene-terminated thiols as more convenient inner monolayer coatings and also indicated the absence of a water layer [49]{Grygolowicz-Pawlak et al., 2007, Sens. Actuators, B, 123, 480-487}.

As an alternative route, conducting polymers can be employed as inner layers for ISEs. These efforts have recently been reviewed [50]. While some materials such as polypyrrole [51] and poly(3,4-ethylenedioxythiophene) (PEDOT) [52] allow for convenient electropolymerization, alternative compounds, as e.g., poly(3-octylthiophene) are often solvent-cast but exhibit desired lipophilicity characteristics as well as reduced redox interference from potentially interfering solution species [53-55].

Recently, the group of Bühlmann 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 the fabrication of all solid state ISEs [53]. The much larger effective surface area at the inner membrane side was shown to render that interface essentially nonpolarizable, even though no defined ion to electron transduction mechanism was involved. The resulting potential drifts were on the order of mere 11 μV/h. Similarly, both porous silicon {Zhu et al., 2007, IEEE Sensors J., 7, 38-42} and single-walled carbon nanotubes {Crespo et al., 2008, Anal. Chem., 80,

1316-1322} seem to make the presence of a redox couple at the membrane-solid interface unnecessary.

4. Improved detection limits with miniaturized electrodes

After understanding the adverse effect of transmembrane ion fluxes [54,55], which may bias the primary ion concentration in the vicinity of the membrane, ISEs with lower detection limits in the range of 10^{-8}–10^{-11} M total ion concentrations have been developed for more than ten ions [56,57]. Although, due to spherical diffusion, microelectrodes should be advantageous in this respect, so far, they have not shown such low detection limits. On the other hand, various designs of miniaturized electrodes with excellent detection limits have been described {Vigassy et al., 2005, Anal. Chem., 77, 3966 – 3970; Malon et al., 2006, J. Am. Chem. Soc., 128, 8154-8155; Rubinova et al., 2007, Sens. Actuators, B, 121, 135-141}. They allow the measurement of low concentrations in small sample volumes and are a prerequisite of the application of nanoparticles as labels for protein and DNA analysis (see part 5).

In a first approach, lipophilic monolithic capillaries of 2–5 mm in length and an inner diameter of 200 µm were filled with the polymer-free membrane material. Due to the hindered diffusion through the monoliths, the sensor responses did not depend on the composition of the inner solution and excellent lower detection limits comparable with the best optimized liquid-contact electrodes were achieved [58]. Later, similar excellent performances were obtained both with hard PVC membranes in polypropylene micropipette tips of the same diameter [59] and with methylmethacrylate/*n*-decylmethacrylate copolymer-based miniaturized solid-contact electrodes [60]. With such ISEs, trace-level measurements in confined samples are possible. For example, the 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 similar 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 larger than the noise. The estimated detection limit according to the 3 sigma rule was found for the three ions to be 2.5 attomoles, 25 attomoles, and 1 zeptomoles, respectively {Malon et al., 2006, J. Am. Chem. Soc., 128, 8154-8155}.

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 detection limit and a good scope for miniaturization, further chemical amplification should lead to highly attractive detection limits.

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 the microsomal hormone thyroxine, which was measured at sub-micromolar concentrations with an I^--selective electrode [61]. Some analogy to this approach is found in a later work by the group of Meyerhoff who utilized chemical amplification steps involving polymeric membrane-based polyion-selective electrodes and polyion-cleaving enzymes as biochemical labels [62]. Another early pioneering approach of potentiometric bionsensors made use of liposomes loaded with marker ions. Antigenes were dissolved in the lipid bilayer memranes of the liposomes, which were then destroyed by the immunoreaction the marker ions were lysed {Shiba et al., 1980, Anal. Chem., 52, 1610-1613}. Indirect potentiometric detection of bioreactions is also possible by measuring the modulation of ion fluxes through nanopores due to such reactions {Gyurcsányi, 2008, Trends Anal. Chem., 27, in press (this volume)}.

To couple potentiometric sensors exhibiting ultra-trace detection limits 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 [63]. 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 {Authier et al., 2001, Anal. Chem., 73, 4450-4456}. The liberated silver ions were detected with a solid-contact Ag^+-selective microelectrode, yielding promising results.

Shortly afterwards, a lower detection limit 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 (cf. Fig. 5) [64]. A Cd-ISE with liquid inner contact served as the detecting system. The improved detection limit achieved with these quantum dots 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 and is, therefore, not always beneficial. Further optimization of the assay may, however, improve the detection limit by this approach.

The use of cadmium sulfide quantum dots as amplification labels was recently expanded towards aptamer-based potentiometric assays [65]. For this purpose, a solid contact Cd-ISE exhibiting a nanomolar detection limit in 200-µL microwells was developed and used to detect thrombin with aptamer-based chemistries in analogy to the sandwich immunoassay principle utilized above. The detection limit for thrombin was found as ca. 5 ppb, which competes favorably with other 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 readout yielded a 10 pM (2 fmol) detection limit, which competes well with comparable stripping voltammetric techniques.

6. Conclusions

Nanoscale potentiometry is a natural progression in the history of ion-selective electrodes that makes use of nanoscale materials to improve their characteristics and expand on 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 detection limits 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. Without amplification, however, it appears to be theoretically impossible for potentiometric sensors to reach single ion detection capability.

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Figure Captions

Fig. 1 Early examples of nanoscale potentiometric sensors based on micropipettes filled with ion-selective membrane materials, adapted from A) [66], B) [66], C) [67].

Fig. 2 All-solid state microelectrodes based on a recessed gold microelectrode covered with a conducting polymer PEDOT and an ion-selective liquid membrane [31]. A) schematic of the electrode assembly; B) bottom view and C) side view of the gold microelectrode before etching; D) Side view of the electrode after etching to yield the desired recess.

Fig. 3 Free-hanging bilayer membranes doped with the 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 [37]. Left: experimental setup. Right: potentiometric responses to the indicated ions in a background of 100 mM NaCl.

Fig. 4 Potentiometric reconditioning procedure to evaluate the presence of a nanoscale water layer between ion-selective polymeric membrane and metallic support [46]. 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 outside sample solution on the basis of counterdiffusion fluxes across the polymeric membrane and result in characteristic potential drifts.

Fig. 5 Cadmium selenide nanoparticle labeled sandwich immunoassay immunoassay, performed in microtiter plates and detected at trace level with a potentiometric microelectrode [64]. 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.

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