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Advancing Membrane Electrodes and Optical Ion Sensors

Eric Bakker*, Gastón Crespo, Ewa Grygolowicz-Pawlak, Günter Mistlberger, Marcin Pawlak, and Xiaojiang Xie

Abstract: While potentiometric sensors experienced a golden age in the 1970s that drove innovation and implementation in the clinical laboratory as sensors of choice, it has been only fairly recently that a theoretical understanding coupled with modern materials approaches transformed the area of membrane electrodes from a playful, yet empirical field to one firmly rooted in scientific understanding. This paper summarizes key progress in the field during the past two decades, emphasizing that the key impulses at the time originated from the emerging field of optical ion sensors. This simplified and transformed the underlying theory of their potentiometric membrane electrode counterparts, where subsequently substantial progress was made, including the realization of ultra-trace detection limits. The better understanding of zero-current ion fluxes and transport processes in turn allowed the development of approaches utilizing dynamic electrochemistry principles, thereby drastically expanding the field of membrane electrodes and making available a range of new methodologies that would have been difficult to predict only a few years ago. These significant developments are now starting to come back and influence the field of optical sensors, where the control and triggering of dynamic processes, away from simpler equilibrium principles, are becoming a highly promising field of research.

Keywords: Analytical chemistry · Chemical sensors · Membrane electrodes · Optodes · Potentiometry

Eric Bakker is professor of analytical chemistry at the University of Geneva since 2010, with an interest in electroanalysis, membrane transport, molecular recognition, fluorescent sensors, and photoactivatable sensor principles, with applications in intracellular analysis and the environmental sciences. He studied chemistry and earned his doctorate degree at ETH Zürich and subsequently pursued his academic career in the United States at the University of Michigan, Auburn University and Purdue University. After a three year period as institute director and professor in Perth, Western Australia, he moved to his present position in Geneva. He has published about 200 papers.

*Correspondence: Prof. E. Bakker
Department of Inorganic, Analytical and Applied Chemistry
University of Geneva
Quai E.-Ansermet 30, 1211 Genève 4
E-mail: eric.bakker@unige.ch

1. Introduction

Chemical sensors represent a highly desirable, yet difficult to achieve goal in the analytical sciences. They aim to translate chemical information into a detectable signal in a direct fashion, possibly without the work intensive and error prone steps in traditional analytical approaches that involve sampling, homogenizing, preconcentration, the forming of aliquots, separation and detection, and, of course, calibration of the process.

Chemical sensors are today invaluable tools in the area of clinical diagnostics, especially point of care analysis at the bedside of patients, where rapid information on constituents in blood and other body fluids is required for diagnosis and treatment. Glucose monitoring in diabetic patients with the help of compact biosensors has also become a reality, while continuous *in vivo* sensing with implantable sensors is a more difficult goal to achieve, but research continues in this important direction. Other applications that are in need of chemical sensor systems are environmental sensor networks to identify fluxes of nutrients, electrolytes or toxic substances in oceans, estuaries and river systems, and the identification and control of nutrients in soil.

Today, electroanalytical sensing principles are the most widely used for aqueous sample analysis. Electrolytes, including hydrogen ions, are assessed with potentiometric sensors. Redox active transition metals at trace levels are typically detected by stripping voltammetric techniques, and glucose and lactate are measured with enzyme biosensors. While much current research is underway to expand this palette to other species and recognition principles, the three types of sensors mentioned above still dominate the current market.

The potentiometric sensor on the basis of an ion-selective membrane is the quintessential chemical sensor, since it is able to translate chemical information directly into an electrical signal. While traditional pH electrodes contain a lithium or sodium doped glass membrane as the sensing element, much progress has been made in the past decades by developing solvent polymeric membrane electrodes that contain lipophilic ion receptors, also called ionophores. The sensors are governed by ion extraction principles and hence form a bridge between the analytical sciences and host-guest chemistry. This particular field has experienced a significant resurgence in recent years, with a simplified theoretical understanding giving rise to improved analytical characteristics and new modes of operation. This progress is briefly summarized in this review.

2. Understanding Membrane Electrodes

The earliest ionophore based membrane electrodes consisted of an organic solvent membrane doped with an electri-

cally neutral antibiotic ionophore such as valinomycin,[1] giving a Nernstian response for potassium ions, typically written as

$$E = B_i + \frac{RT}{z_i F} \ln a_i \quad (1)$$

where E is the observed electromotive force, B_i is a constant, a_i is the activity of the analyte (in this case, potassium) with charge z_i, while F, R and T have their established meanings as the Faraday constant, the universal gas constant and the absolute temperature.

Importantly, valinomycin based membranes exhibited a selectivity that had been unattainable with other membrane materials such as glass. While this triggered the successful application of the technology to measure potassium in blood samples, it was more difficult to understand the operational principles of these unusual systems.

One may use the Nernst Planck flux equation to summarize the early disagreements on the response mechanism of ionophore based ion-selective electrodes, which can be written for a unidimensional process within a given phase as:

$$J_i(x,t) = -D_i \frac{\delta c_i}{\delta x} - z_i D_i c_i \frac{F}{RT} \frac{\delta \phi}{\delta x} + c_i v \quad (2)$$

where J is the flux of the ion i (with charge z_i) in space and time, D_i is the diffusion coefficient of i, c_i its molar concentration, ϕ the electrical potential and v the velocity of the medium. The right hand side exhibits three terms, diffusion (first term), migration (second term) and convection (third term). While convection can normally be neglected for polymeric membranes, the origin of the observed membrane potential was subject to heated debate in the past.[2] Some scholars proposed the predominant effect due to diffusion processes (caused by concentration gradients) within the membrane, while others advocated that the potential principally develops at the interface between the aqueous phase and the membrane. The former model is based on Eqn. (2), where a diffusion potential develops whenever ions from a concentration gradient within one phase. The second model assumes an electrochemical equilibrium across the interface and can be described in general form by the following phase boundary potential (also called Nernst potential), E_{PB}:

$$E_{PB} = \frac{\mu_i(m) - \mu_i(aq)}{z_i F} + \frac{RT}{z_i F} \ln \frac{a_i(aq)}{a_i(m)} \quad (3)$$

where a_i(aq) and a_i(m) are the so-called free activities of i in the denoted phase,

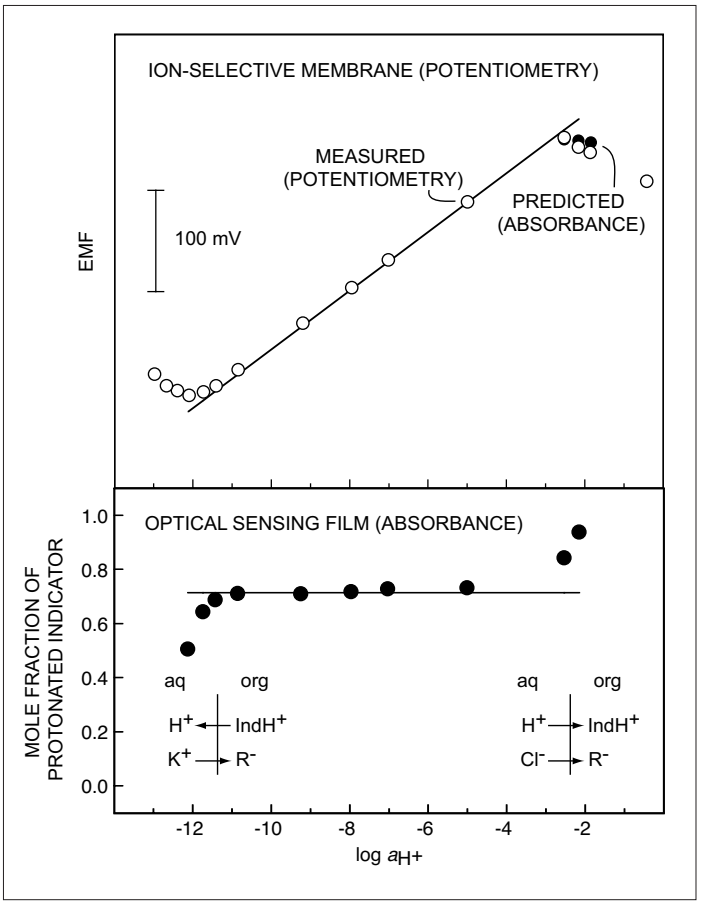


Fig. 1. Top, open circles: potentiometric calibration curve for a plasticized poly(vinyl chloride) membrane containing a lipophilic ion-exchanger and an electrically neutral, lipophilic pH indicator.[4] Bottom: mole fraction of protonated indicator for an identically formulated film spin coated onto a glass support, determined spectrophotometrically in the same sample. Ion-exchange of hydrogen ions with interfering potassium ions occurs at high pH, while hydrogen ion extraction with chloride ions is observed at low pH. The corresponding change in hydrogen ion activity in the membrane is responsible for the deviation from the Nernstian slope in the corresponding potentiometric experiment, shown as solid circles that are calculated from the spectroscopic data and Eqn. (3).[4]

and μ_i is the standard chemical potential of i in the indicated phase and comprises the solvation energies for the ion of interest. It can be clearly seen that Eqn. (3) reduces to the Nernst Eqn. (1) if the activity of i in the membrane, $a_i(m)$ is kept independent of the sample composition. This is achievable with a membrane exhibiting ion-exchanger properties, for example by adding the salt of a lipophilic organic ion and its hydrophilic counterion to the membrane.

A number of key experiments helped to fully establish the second model (Eqn. (3)) and to relegate the influence of the diffusion potential to a more negligible role. Optical sensors containing the same type of membrane materials, but additionally being doped with a suitable ion-exchanger and an indicator dye, and solvent cast onto solid supports, gave rise to systems where the response behavior was directly correlated to ion extraction equilibria (ion-exchange and electrolyte coextraction).[3] Fig. 1 (bottom) shows the response behavior (based on absorbance measurements) of such an optode film as a function of pH.[5] The activity change of the hydrogen ion in the organic sensing phase may be directly calculated from buffer equilibrium considerations, using the experimental absorbance values, and inserted into Eqn. (3) to obtain the expected phase boundary potential change of the corresponding ion-selective electrodes. As Fig. 1 (top) shows, the correspondence is convincing and supports the phase boundary potential model. Another key experiment was performed by Bühlmann and Umezawa, who showed that valinomycin membranes containing no added ion-exchanger no longer responded to potassium in a Nernstian fashion once all membrane components were carefully purified.[6] Much of the early success of neutral carrier based membranes (and the very early theoretical focus on diffusion potentials) seemed to have been aided by

membrane materials that contained a cation-exchanger impurity.

Numerous other approaches that had been successfully developed for the emerging field of optical ion sensors were subsequently extended to the more established area of potentiometric membrane electrodes. This allowed both fields of research to be more directly compared to each other, so that each could benefit from advances in materials and fundamental understanding of the other. This cross-fertilization focused primarily on thermodynamic characteristics.

As an example, a theory of selectivity was developed for optical ion sensors by considering ion-exchange equilibria, thereby relating the optical response in the presence of an interfering ion to that in the absence of interference.[7] This resulted in compact mathematical formulations that, however, deviated significantly from established (and IUPAC approved) approaches for their potentiometric counterparts.[8] This discrepancy, and the fact that potentiometric sensors were characterized with a semi-empirical formulation that is inconsistent if the interfering ion exhibits a different charge as the analyte ion, resulted in renewed efforts to describe the selectivity of potentiometric membrane electrodes on the basis of thermodynamic characteristics.[9] The resulting equations were an extension of earlier efforts by Morf[10] and could now be directly compared to those for optical sensors. They showed that the selectivity of both types of sensors are fundamentally identical for comparable experimental conditions, including equilibrium concentrations in the membrane.[11]

This work on ion-selective electrode selectivity had important consequences. With a description of selectivity on the basis of thermodynamic ion-exchange considerations, one was able to establish adequate experimental conditions to determine correct selectivity coefficients.[12] The phase boundary potential model could then be used to predict how the selectivity coefficient may be optimized with respect to the concentrations of membrane components, complex stoichiometries and ionophore complex formation constants.[13] Fig. 2 shows how the selectivity coefficient is expected to depend on membrane concentrations for two divalent ions that form different complex stoichiometries with the ionophore (1:1 complexes for the primary ion and 1:2 for the interfering one).

This approach was extended to electrically charged ionophores, demonstrating that the corresponding membrane electrodes benefit from addition of an ion-exchanger of the same charge sign as the analyte ion.[14] This type of equilibrium theory was also used by Amemiya and Bühlmann to understand unusual response

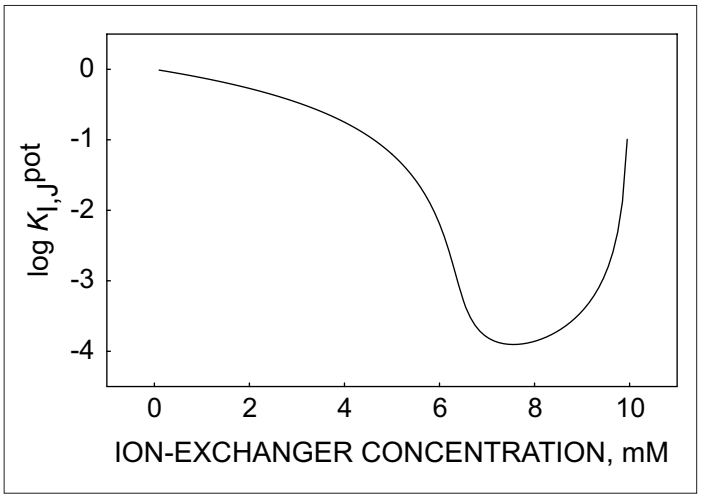


Fig. 2. Predicted dependence of the logarithmic selectivity coefficient on the lipophilic ion-exchanger concentration in the membrane if the primary and interfering ion have the same charge, but the primary ion forms a 1:1 stoichiometry with the ionophore while the interfering one forms a 1:2 complex.[9c] The ionophore concentration is here 5 mM.

slopes (of half or double the Nernstian value) of membranes containing compounds that interact also with other ions, as for metalloporphyrins that may be able to form hydroxy-bridged dimers.[15]

Approaches to determine apparent complex formation constants in the membrane phase and developed originally for optical sensors were also extended to potentiometric membrane electrodes. In an early strategy, such formation constants were estimated by comparing the ion-exchange characteristics of two membranes, one with and one without the ionophore of interest, and both containing a lipophilic H^+-indicator and a cation-exchanger.[16] The approach assumes that the exchanging cation does not chemically interact with the H^+-indicator. The methodology was first introduced for optical sensors, and the subsequent extension to potentiometric sensors showed good correspondence of the observed formation constants,[17] again supporting the phase boundary potential model. In later work, a range of alternate methodologies were introduced for potentiometric sensors that are now more commonly used than the two-ionophore method described here.[18]

3. Non-equilibrium Processes with Membrane Electrodes

While the phase boundary potential model outlined above provided a firm basis for the thermodynamic characteristics of potentiometric membrane electrodes, one must acknowledge that an ion-selective membrane is normally contacted by two aqueous solutions of unequal composition, and cannot be at equilibrium. A measurement at zero current does not equate to a zero transmembrane ion flux. Indeed, concentration polarizations can be observed on the basis of counterdiffusion processes (ions of the same charge sign diffusing in opposite directions) or co-diffusion (electrolyte diffusing across the membrane without net charge transport).[19]

For permselective polymeric ion-selective membrane exhibiting ion-exchanger properties, counter-diffusion properties are often more prevalent since they originate in unequal levels of ion-exchange at either side of the membrane.[20] If one assumes that the interfacial ion-exchange process occurs at local equilibrium, the mole fraction of analyte ions at the sample–membrane phase boundary is a direct function of the selectivity of the membrane:[20b,21]

$$\frac{c_{iL_n}}{R_T} = \frac{a_i(aq)}{a_i(aq) + K_{ij}^{pot} a_i(aq)} \qquad (4)$$

where c_{iLn} is the concentration of analyte ions in the membrane (which is in its complexed form if an ionophore is present) and R_T is the ion-exchanger concentration. For Eqn. (4), all ions are monovalent. The activities shown on the right hand side for the analyte and interfering ion are phase boundary activities, and are not necessarily equal to the bulk sample values.

Note that Eqn. (4) may yield a different concentration of analyte ion at the inner membrane side because the composition of the inner solution does generally not match that of the sample. If, for example, the level of ion-exchange is more significant at the sample side of the membrane than at the inner solution side, an outward concentration gradient of analyte ion develops across the membrane, resulting in a net flux of these species in direction of the sample. At steady-state, one may assume linear concentration gradients and by considering only diffusion as the predominant mode of transport, Eqn. (2) is rewritten and related to concentration gradients in the membrane and aqueous phase as follows:

$$J_i(x) = -D_{i,m} \frac{\Delta c_{iL_n}}{\delta_m} = -D_{i,aq} \frac{\Delta c_{i,aq}}{\delta_{aq}} \qquad (5)$$

where δ_m and δ_{aq} are the diffusion layer thicknesses in the two indicated phases. For convectively agitated samples, δ_{aq} is the Nernst diffusion layer, while δ_m is equal to the membrane thickness. Eqn. (5) may be rewritten to describe the analyte concentration at the aqueous phase boundary as a function of its bulk concentration and the gradient in the membrane (Eqn. (6)).[20b]

Clearly, this process may give rise to concentration polarizations and associated ion fluxes that may effect potentiometric measurements. The first application of this principle was the explanation of the unusual response behavior of potentiometric sensors for the polyions heparin and protamine.[22] Equilibrium theory was found to be inadequate to rationalize the large (super-Nernstian) response slopes of these membrane electrodes, as well as other behavior (effect of plasticizer content and geometry of the electrode) that clearly hinted at a system where transport processes were relevant.[23] Despite their promise of direct detection of polyions in undiluted whole blood samples, these systems were not adequate for continuous heparin monitoring because of the essentially irreversible extraction behavior of the polyion.

Subsequently, Eqn. (6) was successfully used to explain why the response of ion-selective electrodes exhibited detection limits that were orders of magnitude inferior than their optical sensor counterparts containing exactly the same underlying chemistry.[24] Indeed, optical film or particle based sensors function on the basis of a two-phase equilibration step and hence can yield detection limits that are dictated by thermodynamic ion-exchange with interfering ions.[24b,25] Ion-selective membranes of similarly high selectivity, on the other hand, are bound by Eqn. (6), where even a comparably small concentration gradient can result in an elevated concentration of analyte ions at the membrane surface that masks the true response to the bulk composition of the sample.

The two groups of Pretsch and Bakker formed a collaborative effort to offer theoretical insights into the detection limits of ion-selective electrodes and to subsequently design potentiometric sensor systems that exhibit detection limits at ultra-trace levels, sometimes down to 10^{-10} M (see Fig. 3, left).[28] While early systems were targeted to environmental analysis, for example the measurement and speciation of lead ions in drinking water samples.[21,29] the work was later extended to bioanalysis. For this purpose, it was established that ion-selective electrodes are capable of detecting ultra-low concentrations in microliter sample volumes, demonstrating that hundreds of attomoles can be detected, essentially without altering the sample and any delicate equilibria that may be present.[30] This attractive sensing technology was subsequently applied to affinity sandwich assays. Chemical amplification was achieved with nanoparticle labels on the secondary binding compound that were subsequently oxidatively dissolved to yield a burst of ions for each biological binding event. Such signal enhanced systems were demonstrated to function in potentiometric assays on the basis of antibody-antigen interactions,[31] DNA hybridization,[32] and a DNA Aptamer assays for thrombin (see Fig. 3, right),[33] with detection limits that rival those of other state of the art technologies and that appear to be given by the selectivity of the assay, not the detection limit of the electrochemical sensor.

$$c_i(aq, bp) = c_i(aq, \text{bulk}) + \frac{D_{i,m}}{D_{i,aq}}\frac{\delta_{aq}}{\delta_m}\left(c_{iL_n}(\text{inner solution side}) - c_{iL_n}(\text{sample side})\right) \quad (6)$$

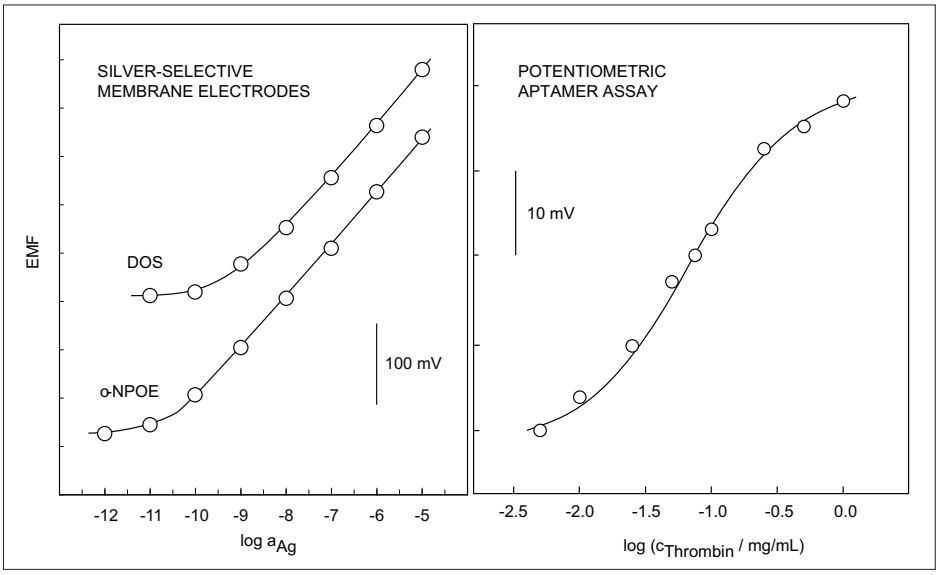


Fig. 3. Left: Potentiometric calibration curves for optimized neutral carrier based silver-selective membranes on the basis of two different plasticizers, o-nitrophenyl octyl ether and dioctyl sebacate, demonstrating ultra-trace detection limits.[26] Right: dose response for a thrombin aptamer assay with CdS nanoparticle labels, read out potentiometrically by a cadmium selective microelectrode after dissolving the nanoparticles and liberating the cadmium ions.[27]

While zero current ion fluxes are undesirable for reaching trace level detection limits, they may be exploited to realize new types of sensors. The polyion sensors mentioned above fall in this category. Another example includes increased potential changes at the endpoint in potentiometric titrations if membrane electrodes are chosen that exhibit inward ion fluxes.[34] Membrane electrodes with different flux behavior can be measured against each other to achieve highly sensitive sensing systems without the need for a traditional reference electrode, as demonstrated by Pretsch and coworkers.[35]

Among these methodologies, the concept of backside calibration potentiometry is perhaps the most thought provoking[36] (see Fig. 4). Here, a reasonably thin membrane (*ca.* 20 μm) is chosen with relatively rapid diffusion characteristics and contacted on both sides with an identical sample solution. The membrane is at equilibrium, and hence concentration polarizations are unimportant as evidenced by the absence of a stir effect (a change in the sample stirring rate does not alter the observed membrane potential). Conversely, an unknown sample composition can now be evaluated by performing stir effect experiments by changing the composition of the backside solution, as illustrated in Fig. 4. The approach benefits from the facts that the sample composition does not have to be changed in the calibration/evaluation step, which is highly attractive in a number of scenarios such as *in vivo* diagnostics or environmental monitoring, and that a classical reference electrode appears unnecessary. Note, however, that the underlying counter-diffusion processes are also governed by a second ion (such as the hydrogen ion), which needs to be known and selectively extracted into the membrane as well.

4. Dynamic Electrochemistry with Membrane Electrodes

Potentiometric membrane electrodes where ion fluxes are relevant have placed the field closer to voltammetric sensors, especially electrochemistry at the interface of two immiscible electrolyte solutions (ITIES),[37] which traditionally have

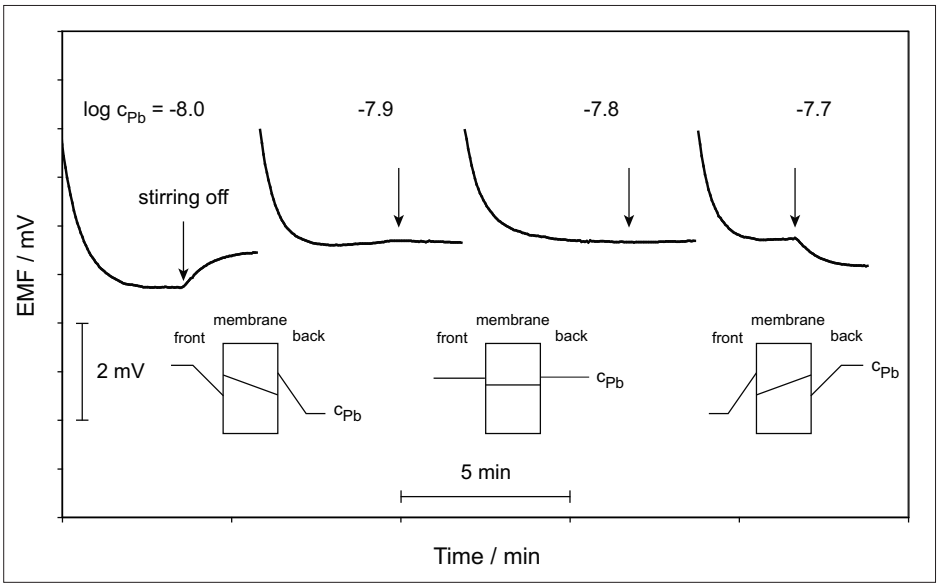


Fig. 4. Demonstrating the concept of backside calibration potentiometry with a lead ion-selective membrane.[36a] The presence of transmembrane ion fluxes is evaluated potentiometrically with a stir effect. The electrolyte composition at the back side of the membrane is varied until the stir effect disappears, indicating a matched sample composition. The nature and concentration of counterdiffusing ion must be known for this concept to work reliably.

had limited interactions with each other. This is a welcome change that has resulted in fruitful cross-pollinations in recent years.

As stated above, ion fluxes can be driven by concentration gradients that originate in unequal levels of ion-exchange at either side of the membrane. But ion fluxes may also be imposed instrumentally by an applied current as follows for a unidimensional system:

$$j = F\sum_i z_i J_i \qquad (7)$$

where j is the current density and J_i the flux for an ion i. Lindner (and earlier, Buck) was a key researcher advocating the use of current perturbation for chronopotentiometry with ion-selective membranes. In particular, he showed that a well defined applied low amplitude current may compensate the concentration gradient driven spontaneous ion flux (see Eqn. (6)) to yield an ultratrace level detection limit.[38] Later theoretical work suggested that this optimal current may be found by a stir effect experiment, similar to that introduced for the backside calibration methodology explained above, but that the detection limits achievable with this methodology cannot be significantly better than by chemical optimization alone.[39]

On the other hand, a high amplitude current may be used to drive ions from the sample into the membrane and hence control the membrane composition and the associated sensor behavior by instrumental means. Early experiments on reasonably concentrated samples suggested that classical membrane formulations containing an ion-exchanger for permselectivity give essentially featureless chronopotentiometric responses.[40] To render the interface polarizable, therefore, membranes were chosen that do not contain added ion-exchanger but an inert lipophilic salt in addition to the ionophore.[40] If all membrane components are very lipophilic, as necessary for practical applications, current can be passed through the membrane only by extracting a counterion at the inner membrane side for each ion at the sample side. This concentration perturbation at each interface results in a transient potential change. It can be made reproducible by electrochemically regenerating the membrane under controlled potential conditions, for example by applying the previously measured open circuit potential. Such a sensor therefore is operated by alternating between an applied current pulse (for measurement) and applied potential pulse (for regeneration).[41] Alternatively, a zero current pulse may follow the applied current pulse to allow for potential measurements that is not influenced by any ohmic drop.[42]

It was found that such a pulstrode (pulsed galvanostatic membrane electrode) exhibits near-Nernstian electrode slopes in complete analogy to zero current potentiometry if sample concentrations were high.[41] However, the sign of the applied current dictates the charge sign of the ion that is measured, and sample anions as well as cations can now be assessed with the same membrane. The amplitude of the current controls the interfacial concentration of extracted ions, and hence exhibits a selectivity modifying influence on the membrane, in analogy to variations of the ion-exchanger salt concentration in potentiometry.[41] This is an attractive level of flexibility that allows one to explore new modes of operation and to gain fundamental information about membrane material and ionophore properties.

At lower concentrations where mass transport of the analyte ion to the membrane may be rate limiting, a more abundant background ion may need to be extracted into the membrane as well to fulfill Eqn. (7). This results in a super-Nernstian response slope akin to potentiometric measurements, but under kinetically controlled conditions that give highly reproducible sensor behavior where potentiometry exhibits strong potential drifts.[41] For the first time, operationally reversible and reproducible polyion sensors became achievable.[43] The direct and reproducible detection of the polycation protamine in undiluted whole blood samples was subsequently demonstrated with this promising new methodology (see Fig. 5).[44]

A fundamental study involving the casting of polyelectrolyte multilayers onto ion-selective electrode membranes shed light into the role of interfacial transport on the sensor response.[45] While the presence of polyelectrolyte multilayers were confirmed by atomic force microscopy and zeta potential measurements, the potentiometric response to the analyte ion calcium did not exhibit a measurable change relative to uncoated membranes.[45] This may be surprising at first sight, but establishes once more that a Nernstian electrode response is observed under essentially thermodynamic conditions. Interestingly, however, the pulsed galvanostatic measurement protocol in the super-Nernstian response region showed important signal changes upon multilayer coating. The results suggest that transport of the analyte ion across the polyelectrolyte membrane is inhibited relative to the more abundant background ion and causes a shift in the super-Nernstian chronopotentiometric response. This insight was further exploited for the establishment of an affinity biosensor approach, using biotin groups covalently attached onto an ion-selective membrane.[46] Depending on the sample concentration of avidin, the chronopotentiometric ion response changed, suggesting that surface bound avidin exhibited a similar inhibition of ion transfer as the polyelectrolyte multilayers in the earlier study. This affinity biosensor platform is especially attractive since it avoids metal electrodes and uses non-polar membranes as substrates that may chemically mimic the lipid bilayers present in biological systems.

Another approach focused on the analysis of the shape of a transient chronopo-

tentiometric pulse in a pulsed galvanostatic measurement protocol.[47] Indeed, diffusion theory predicts a critical time at which the analyte concentration at the membrane surface will deplete, akin to a dynamic titration endpoint. This critical time depends on the applied current density, the sample concentration and diffusion coefficient, as described by the Sand equation:

$$|j| = \frac{F}{2}\left(\frac{\pi D_{aq}}{\tau}\right)^{1/2} c_i \qquad (8)$$

where τ is the critical time. This approach was successfully established for membrane electrodes selective for hydrogen ions,[47] calcium[48] and the polyion protamine,[49] and gave critical times on the order of 1–2 s for concentrations up to *ca*. 1 mM. Higher concentrations seem feasible for membrane materials exhibiting larger mobilities or smaller thicknesses, otherwise analogous transitions in the membrane phase will mask the response.

Note that in most such dynamic methods where sample ions effectively deplete near the membrane surface, the sensor response becomes a function of the available ion concentration, and not the ion activity as in the Nernst equation. This must be taken into account when designing or utilizing different methodologies involving the same basic materials. There are situations where a combination of various protocols will allow one to yield a more complete picture of the sample. Calcium ions in whole blood, for example, are either reported as ionized calcium (free calcium) or total calcium, and the two values are different by typically a factor of two, depending on the sample pH and the availability of calcium binding proteins in the sample. Potentiometry with calcium-selective electrodes in undiluted blood gives ionized calcium levels, while acidification of the sample transforms bound to free calcium and hence yields total calcium levels with the same methodology.[50] The use of membrane electrodes that employ dynamic electrochemistry in conjunction with zero current potentiometry may allow one to report on both values without sample manipulation, as reported recently.[48] This elegant distinction of the speciation of an ion without bulk sample manipulation may also be valuable for the study of dynamic biological and environmental processes.

The most recent direction of dynamic electrochemistry with membrane electrodes in our group has focused on the design of sensing principles for absolute measurements by thin layer coulometry.[51] If successful, this would present a platform to design robust chemical sensors that would be attractive for field deployable monitoring systems, handheld analyzers and perhaps even implantable sensor units. Absolute measurements are in principle recalibration free and would perhaps not suffer from signal drifts or fluctuations in temperature as traditional sensing principles. We aim to develop membrane electrode principles for thin layer coulometry, in which an applied potential forces the depletive transport of analyte ions from a thin layer sample through the selective membrane into a receiving solution. This contrast to work by other groups such as Kihara,[52] who effected the assisted transfer into a bulk organic solvent, which appears less practical for the envisioned applications. Early work in our group suggested to utilize permselective membranes for this purpose,[51] allowing one to transport only one type of ion across the membrane. Such membranes, moreover, can be interrogated potentiometrically before any electrochemical perturbation. The decaying current during electrolysis is integrated to give the coulomb number, which, if the volume is known and constant, directly relates to the number of moles of analyte ion present in the sample by applying Faraday's law. Fig. 6 illustrates this principle with a tubular calcium selective membrane as example. Currents unrelated to the analyte extraction process can be partially compensated for by recording a second current transient after an intermediate baseline pulse.[53] While we are only at the beginning of this project, the resulting calibration curves were found to be independent of temperature and allowed for excellent reproducibility on the order of 1% within a two week period without recalibration.[53]

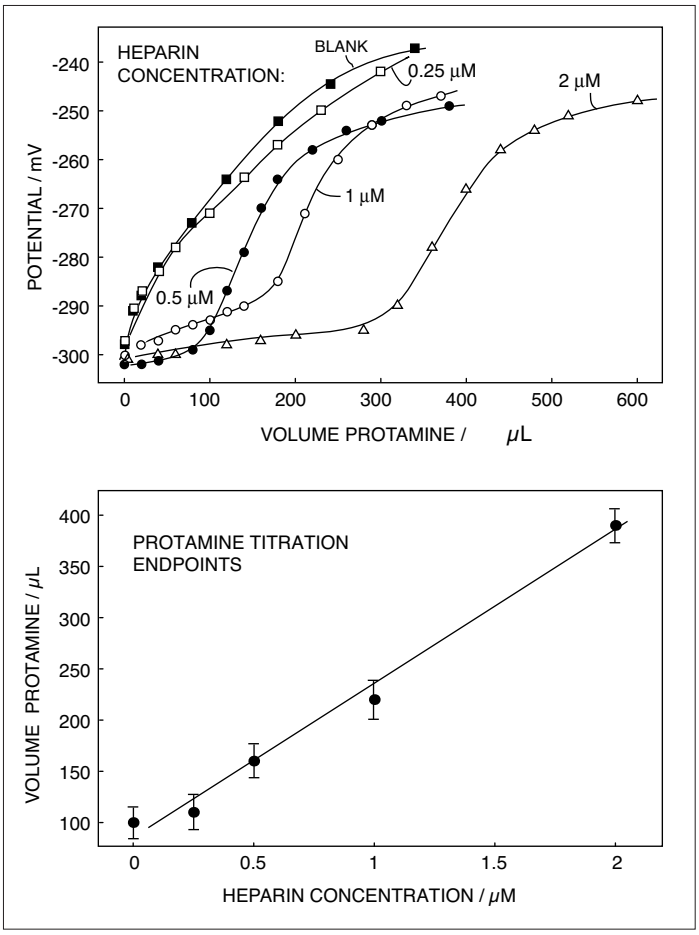


Fig. 5. Operationally reversible membrane electrodes for protamine can be constructed by controlling the polyion extraction by a multipulse electrochemical excitation sequence.[43,44] Top: chronopotentiometric responses of a protamine selective membrane electrode in undiluted whole blood sample containing the indicated levels of the anticoagulant polyanionic drug heparin.[44] Bottom: the corresponding heparin-protamine titration endpoints correlate linearly with heparin concentration.

5. Optical Ion Sensors

Traditional optical sensor approaches directly mimicked the design of their electrochemical counterparts by physically attaching an optical sensing layer to a solid support.[54] This support either served to just physically stabilize the sensing film, or was designed to consist of the optical transducer (such as a optical fiber or an attenuated total reflectance crystal). Indeed, the working definition of a chemical sensor originally demanded a physical attachment between sensing layer, transducer and detector. The resulting sensor devices were aptly named optodes or optrodes, referring to the design similarity to ion-selective electrodes, and hence were based on the somewhat unscientific term 'optical electrodes'.

There is, however, no clear reason why sensing chemistry and transducer cannot be physically detached, as electromagnetic radiation possesses the attractive property of penetrating soft matter and numerous solvents with relative ease. One example

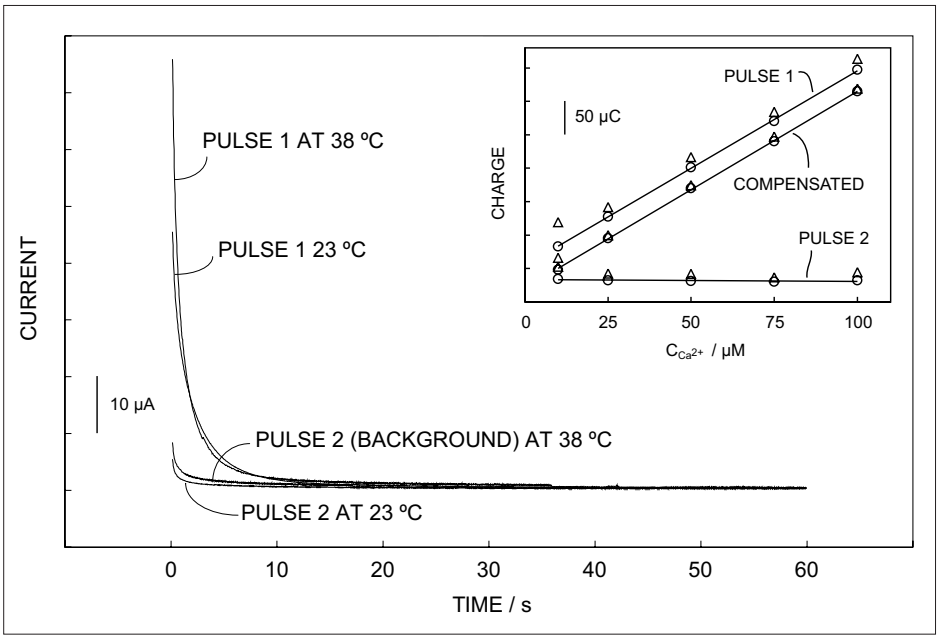
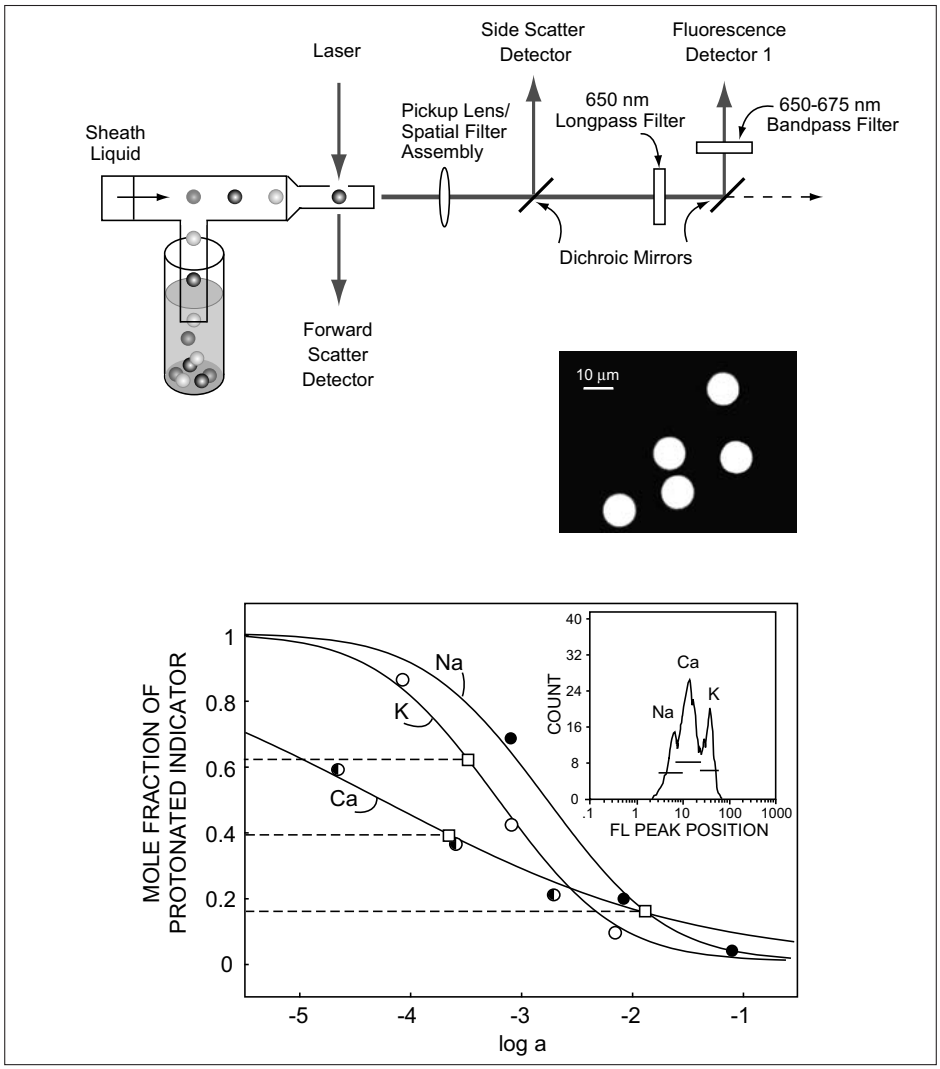


Fig. 6. Tubular membrane electrodes in contact with a thin layer sample can be designed to quantitatively and selectively deplete the analyte ion by voltammetric ion transfer.[51,53] This results in a current decay that yields, after integration, the transferred charge and, by applying Faraday's law, the available concentration of calcium in the sample. Shown here are the coulometric detection of calcium ions at two different temperatures.[53] Non-Faradaic charging currents can be partially compensated by performing a second excitation pulse whose charge is subtracted from that of the first pulse. Inset: Corresponding calculated charges, demonstrating the independence of the signal on temperature for this absolute charge counting technique.



explored in our group involved the integration of optical sensor approaches into bead based chemical analysis schemes.[55] Analytical flow cytometry, originally developed for counting and sorting cells by means of fluorescence spectroscopy, is a technology that is readily adapted to microbead suspension array assays. If selective chemistries become available to detect most key analytes in a target sample such as blood, analytical flow cytometry may become a highly attractive platform for clinical diagnostics or environmental analysis. Fig. 7 illustrates such optical sensing beads and their use in analytical flow cytometry.[56] The bead based optodes were fabricated by a sonic casting apparatus, in which an inner core stream containing all sensing ingredients is focused by a sheath flow and destabilized into droplets by vibration.[57] Flow cytometry of the cured particles may monitor the fluorescence properties of thousands of particles, hence allowing the use of statistics to narrow the confidence interval of the resulting fluorescence signal.[56] This general bead based sensing approach was extended to the use of imaging arrays in which the beads were dropped into etched wells that can be individually addressed with fluorescence microscopy.[58]

The volume of a 10-µm microparticle sensors is about 1 picoliter and hence drastically smaller relative to their traditional thick film counterparts.[59] A net extraction of even 1 millimolar concentration into such a particle corresponds therefore to just a quantity of about 1 fmol. This should result in extremely low achievable detection limits without perturbing the surrounding bulk sample. Indeed, as shown in Fig. 8, detection down to the picomolar level was demonstrated with such beads,[61] which is much lower than the corresponding ion-selective electrodes, even after optimization for low detection limits.[62] Clearly, miniature optical sensing beads not only benefit from their small size but also from their ability to be read out by light: they do not require any solid support and can be made to obey true two-phase equilibria.

Fig. 7. Top: Analytical flow cytometry is an attractive option to read out large populations of fluorescent chemical sensor particles.[57] Middle image: fluorescence microscope image of ion sensing microspheres fabricated by a sonic stream preparation instrument.[56] Bottom: A mixture of sensing particles for the ions sodium, potassium and calcium can be reliably detected by analytical flow cytometry, even when containing the same indicator dye, as evidenced by the visible peaks in the histogram.[56] The variations of peak position as a function of the logarithmic ion activity correspond to theoretical expectations.

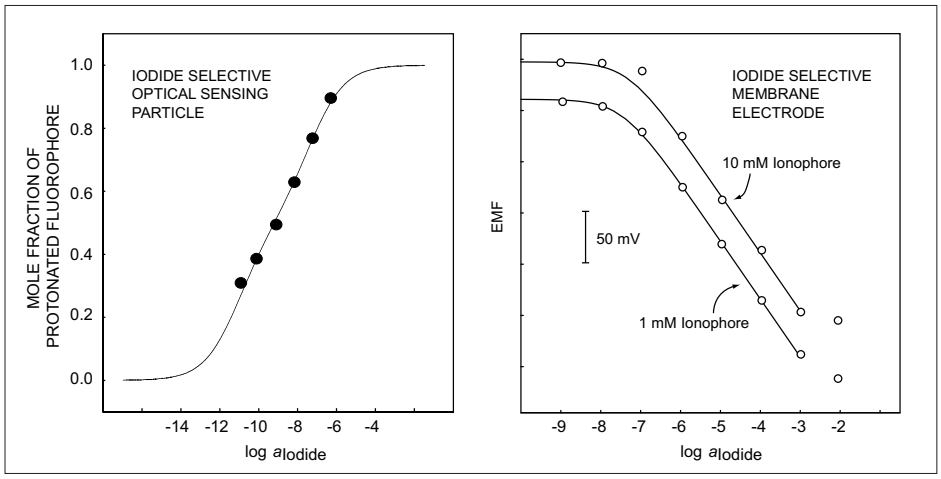


Fig. 8. Left: Response curve of a single iodide-selective optical sensing particle in a nitric acid background, demonstrating detectability of low picomolar concentrations.[60] Right: corresponding response of a similarly formulated and optimized iodide-selective electrode.[61] With the potentiometric sensor, ion fluxes cannot be completely eliminated and hence result in a significantly worse detection limit.

Electrochemical sensors cannot offer this characteristic.

The group of Kopelman pioneered the concept of intracellular detection with submicron-sized optical sensing particles,[63] which was an elegant extension on their earlier work with miniature optical fiber sensor probes.[64] Some of these beads mimicked the composition of classical hydrogel based indicator assays, but others were based on the ion optode principles discussed above.[65] In earlier approaches, indicator dyes such as calcium green or Fura-2 were simply dissolved within an intracellular environment,[66] resulting in chemical and biological degradation of the dye and the possibility of biotoxicity. The concept of localizing the chemical recognition into a small sensing sphere physically separates the sensing phase from the biological environment and avoids these limitations.[67] Moreover, these optical sensor strategies are closer to that employed traditionally with potentiometric microelectrode probes, but can avoid rupturing the cell walls for measurement as they do not require an electrical circuit to be completed. Kopelman and co-workers devised elegant materials approaches by the introduction of magnetic control of sensing spheres that are capped on one side by a metal coating.[67,68] Magnetically induced rotation of the sensing spheres results in blinking signals to eliminate background fluorescence.

The optical sensor concepts discussed above represent passive sensing systems, in which extraction and recognition processes occur spontaneously, without external control. In many ways, therefore, optical sensors mimic the function of corresponding potentiometric sensors. Once stable, the signal readout essentially represents an equilibrium response. Consequently, such optical sensors lack the ability to be externally triggered and to hence gain information about kinetic parameters of a sample whose composition is not changing rapidly. It would be highly desirable to achieve dynamic optical sensor concepts so that reactions can be triggered, fluxes to and from the sensor surface may be initiated, and relaxation after local perturbation can be monitored. Dynamic control may be offered by electrochemical means, as demonstrated elegantly by Heineman with the concept of spectroelectrochemical multimode chemical sensing.[69] Unfortunately, the principles set forth by combining electrochemical and optical sensing approaches necessarily require wired sensing films and are therefore not suitable for intracellular particle based detection. Instead, photoactivation of the sensing chemistry may be a promising research direction to achieve this goal. The first report on photoactivated optical sensors has recently been introduced by Shvarev, who used light to trigger the release of acid from a photosensitive dye embedded in a pH sensing film.[70] The kinetic signal recovery after photoactivation was found to be a direct function of the buffer capacity of the surrounding sample, hence yielding information that a static sensor approach cannot provide. Our group is now actively involved in further developing the concept of photoactivated chemical sensor approaches, especially in view of learning about the chemical behavior of local intracellular environments.

6. Conclusions

As in other fields of research, the initial exuberance of potentiometric sensors has eventually given way to a more measured progress that involve fewer and more focused groups. In this period, one could witness the consolidation and simplification of previously difficult to digest theoretical models that allowed the field to make bold predictions about attainable ion selectivities and detection limits. Indeed, subnanomolar detection limits are today a reality for many such sensors, and much of this was realized by understanding the underlying response mechanism. Recent progress in this field has involved combining the concepts of local phase boundary equilibria with transport kinetics within the bulk phases (sample and sensing membrane), giving adequate guidance for sensor design. The better understanding of transmembrane ion fluxes, combined with instrumental approaches to impose net ion fluxes by current control, has opened up this field further and brought it closer to the realm of dynamic electrochemistry. Many recent fruitful directions have come from this approach, yielding sensor concepts that are either more sensitive, operationally more reversible, more rapid, or more robust than traditional membrane electrodes. The application of this family of sensing principles to affinity bioassays and environmental monitoring has enriched the field and made the methodologies attractive to real world applications. While the emerging field of optical ion sensors was at the origin of quietly transforming the field of membrane electrodes, research in recent years focused more strongly on materials and miniaturization, rather on the development of novel sensing principles. This is now likely to improve with the advent of photodynamic optical sensors that is the topic of current research.

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