few years ago, leading scientists advised their younger colleagues not to work in the field of potentiometric sensors. Researchers viewed potentiometry as a mature field; all the basic facts were well understood, and no possibilities existed for significant new achievements.

In fact, although ion-selective electrodes (ISEs) had been described for more than 50 analytes, their practical use was limited to only a few fields. They were especially well established in clinical chemistry for the measurement of $K^+$, $Na^+$, $Ca^{2+}$, and $Cl^-$ concentrations (activities) in whole blood, serum, or plasma. The two main limitations were insufficient detection limits and selectivities. Indeed, most potentiometric sensors had detection limits in the micromolar range, and interfering ions were discriminated by, at most, a factor of $10^3$–$10^4$. Such detection limits and selectivities were not acceptable for trace-level measurements, that is, for assessing submicromolar activities in the presence of large concentrations of interfering ions. Applications such as monitoring heavy metals in drinking water or blood seemed like hopeless ideas.

This situation has dramatically changed in the past few years. Today, it is feasible to construct ISEs for a number of analytes with detection limits down to low parts-per-trillion levels ($10^{-10}$ M). Accurate monitoring of heavy metals in unspiked environmental samples has been demonstrated and compared with routine analytical methods. This breakthrough certainly came as a surprise to most researchers working with chemical sensors and has fueled renewed interest in pursuing active research in this field. Unlike most other analytical methods for trace-level determinations, these advances were not achieved by improving the instrumental technique but by studying the underlying chemical principles and eliminating experimental biases. In this article, we describe how the detection limits and selectivities of poten-

Thanks to recent advances, ISEs are tackling trace-level measurements.
Electrodes
Ion-selective sensors are essentially passive electrochemical devices, in which changes in the electromotive force (emf) are monitored under virtually zero current conditions. In so-called direct potentiometry, the emf is ideally a function of the activity of only one chosen sample ion so that it can be selectively assessed in the presence of other ions. Achieving sufficient selectivity to measure one ion in the presence of others has long been the main focus of research in this field (1). The basic concept is perhaps best illustrated with ISEs containing polymeric membranes, which are assumed to behave as organic liquids of high viscosity. Today, this class of sensors is by far the most versatile and widely established (2).

If this membrane contains a lipophilic cation exchanger, its counterion can freely exchange or equilibrate with the two contacting aqueous solutions (Figure 1a). Assume that these solutions contain the same hydrophilic electrolyte, $M^+$, and $M^-$ is the counterion of the exchanger in the membrane. A chemical driving force that depends on the activity of this ion in both phases causes $M^-$ to partition from the organic to the aqueous phase. Because $M^-$ carries a charge and no net current can flow, this driving force is counterbalanced by the phase boundary potential. It builds up spontaneously as $M^-$ starts to cross the interface and leads to interfacial charge separation. The relationship between this interfacial potential, $E_{PB}$, and the ion activities on either side of the phase boundary is

$$E_{PB} = \frac{RT}{zF} \ln \left( \frac{a_{M(aq)}}{a_{M(org)}} \right)$$

in which $a_{M(aq)}$ and $a_{M(org)}$ are the activities of the ion, with charge $z$ in the aqueous and organic phases, respectively; $k_M$ is a constant that incorporates the standard Gibbs’s free energy of ion transfer from the aqueous to the organic phase; and $R$, $T$, and $F$ are the gas constant, the absolute temperature, and the Faraday constant, respectively.

Figure 2a illustrates this simple mechanism. In the case shown here, the composition of the membrane phase ($a_{M(org)}$ in Equation 1) remains essentially unchanged as the concentration in the sample phase is altered, and a response according to the so-called Nernst equation is observed:

$$E_{PB} = K + \frac{RT}{zF} \ln a_{M(aq)}$$

in which $K$ is a constant for each $M$. If interference from other sample ions is significant, the membrane concentration of $M^+$ changes, which alters the observed emf (Equation 1). To obtain highly selective ISEs on the basis of polymeric membranes, selective complexing agents (ionophores) have been synthesized and doped into these membranes. Their purpose is to selectively incorporate only one type of ion into the membrane and to suppress competition from interfering ions (3, 4). The synthesis and application of novel ionophores were the primary focuses of research in this field for many years (5).

The relationships outlined earlier can be adequately described with thermodynamic models. Indeed, the composition of the organic-phase boundary layer can be related to the composition of the adjacent sample layer on the basis of ion-exchange and electrolyte coextraction equilibria between the two phases and complexation and ion-pair formation equilibria in the organic membrane phase (2). Today, the theoretical background has matured to a stage that it can be used to optimize the membrane composition and describe how ion sensors behave in mixed electrolyte solutions. The lower detection limit, for example, is reached in theory when interfering ions displace 50% of the ions to be measured from the membrane. As a consequence, one established experimental protocol to determine the selectivity of the sensor toward an ion calls for the measurement of the lower detection limit in the presence of a constant ionic background (6). If the detection limit is low, the selectivity toward the background ion is good (Figure 1b).

Unfortunately, the thermodynamic treatment of ISE function has apparently failed for dilute solutions. The observed emf has often been affected by sample stirring, for example, and selectivities determined under such conditions have turned out to be grossly underestimated. It is now well established that the observed detection limit is often not dictated by changes in the membrane concentration of $M^+$, as expected from the thermodynamic model alone. Rather, nonthermodynamic processes may polarize the concentration of $M^+$ in the contacting sample solution and give a higher value at the sensing surface than in the sample bulk. A thermodynamic treatment is still valid for the local interfacial
processes, even though the sensed concentration at the phase boundary may deviate from the concentration in the bulk. This is very surprising. After all, potentiometry is assumed to be a static method and should give thermodynamic information about a sample. How can we locally polarize the concentration if no current is passed across the cell?

**Ion fluxes in potentiometry**

Generally, the sample and the inner solution do not have identical compositions. Because ions can diffuse across the organic membrane phase according to various mechanisms, this concentration asymmetry induces a process toward a true equilibrium. This process is so slow that it can be ignored if the solution concentrations of the relevant species are higher than ~1 µM. However, if lower concentrations are involved, transmembrane ion fluxes may have a decisive influence on the response of ISEs. According to the equilibrium model (Figure 2a), no gradients arise in the system. This model is very close to reality, even if the composition of the inner solution is different from that of the sample. The ion-exchange property of the hydrophobic membrane largely blocks the incorporation of the aqueous cation and its counterion into the organic phase. Furthermore, the high selectivity largely hinders the exchange of the analyte ion with an interfering ion. The key to understanding the processes at low sample concentrations is the word “largely”. A very small fraction of the primary ions is exchanged by interfering ions already at concentrations that are orders of magnitude larger than the lower detection limit (Figure 1b).

For clarity, the situation is represented in Figure 2b with exaggerated gradients (only the unstimred layer of the sample is shown). The exchange of a small portion of the sample ions (blue) with interfering ions (red) induces opposite gradients of the primary (blue lines) and interfering ions (red lines) in the membrane and in the contacting stagnant aqueous layer. These gradients generate ion fluxes without net charge transport and cause an elevated primary ion concentration at the sensor surface. Because this phase boundary concentration determines the emf, these diffusions processes may cause a strong bias when trying to measure lower detection limits and apparent selectivities. Such elevated concentrations near the membrane surface were indeed recently observed by scanning electrochemical microscopy (7).

Fortunately, the phase boundary potential model described above is also valid when the whole system is out of equilibrium because phase boundary processes are much faster than transmembrane ion fluxes. Because the thickness of the concentration gradient in the stagnant aqueous layer and the membrane are comparable (Figure 2b), the ratio of the two gradients is roughly equal to the ratio of the corresponding diffusion coefficients (8). Diffusion coefficients in the membrane phase vary according to polymer and plasticizer content but are ~1000-fold smaller than those in water. Therefore, the concentration difference between the two surface layers of the membrane is ~1000-fold higher than between the bulk of the sample and in the aqueous layer near the membrane. Because ordinary ion concentrations are on the order of 10⁻² M in the membrane phase, a concentration polarization of only 1% in the membrane produces a concentration difference of ~10⁻⁷ M between the surface layer and the bulk of the sample. The analyte will have this concentration in the aqueous layer adjacent to the membrane even if the bulk contains virtually no sample ions. Because the phase boundary concentration determines the emf, it is the fundamental reason the lower detection limits of innumerable polymer membrane electrodes were reported to be on the order of 10⁻⁶–10⁻⁷ M (3).

The same process has also dramatically biased the quantification of the selectivities of many membrane electrodes. To quantify selectivities correctly, the measurements must be done under conditions in which only one of the ions is potential-determining. If the membrane were highly selective, the emf of a concentrated solution of an interfering ion would be lower than the emf of a 10⁻⁶-M solution of the analyte ion (Figure 1b). In other words, the small amount of leached primary ions, not the discriminated interfering ions, determined, in many cases, the measured selectivities (6).

**How can ion fluxes be reduced?**

The key to the recent spectacular improvements toward lower detection limits and better selectivities has been the reduction of transmembrane ion fluxes (9, 10). As shown in Figure 2b, opti-
by excessively rapid diffusion through the membrane and excessively slow diffusion in the aqueous phase. Any measure that reduces ion fluxes in the membrane is, therefore, beneficial (14, 15). For example, membranes are more rugged if the diffusion coefficients are reduced by increasing their polymer content; if the ionophore is covalently bound to the polymer backbone; if the total amount of ions (i.e., the concentration of the ion exchanger in the membrane) is reduced; if the thickness of the membrane is increased; if its effective surface is reduced relative to water by adding inert lipophilic particles; or if the stirring of the aqueous phase is increased (e.g., by using a flow-through system or a wall-jet configuration) (15, 16). By combining two of these measures (i.e., adjusting the inner solution and making the membrane less sensitive to concentration gradients), scientists have designed potentiometric sensors that are adequate for monitoring heavy metals such as lead in drinking water (17).

What’s going on now?
A series of ISEs have recently been developed with lower detection limits (Figure 3) that are far better than what was expected only a few years ago (18). These ISEs can now compete with the most sophisticated methods of trace analysis. More importantly and parallel to these developments, a sound theoretical understanding of the basic mechanisms has been achieved, which makes it possible to optimize the electrodes very efficiently (12, 17, 19). Once their selectivity has been determined, the composition of the inner solution can be predicted (12).

The most important practical applications seen today are heavy metal analyses of drinking water, other environmental samples, and biological samples, which demand high selectivity, ruggedness, and short response and calibration times. For lead in drinking water, the detection limit should be at least 10 times lower than the 15-ppb (7.2 \times 10^{-8} \text{ M}) action limit imposed by the U.S. Environmental Protection Agency (20). In Figure 4b, the action limit is marked by the color transition between the green and red zones of the plot. The detection limit of 7.2 \times 10^{-9} \text{ M} must be reached in the presence of 10^{-5}–10^{-4} \text{ M} levels of the abundant interfering ions Na^+, Ca^{2+}, and H^+.

Screening of several existing ionophores determined that the basket-shaped, thioamide-derivatized calix[4]arene was the most appropriate host molecule for lead because of its excellent selectivity (21). In fact, the characteristics of the resulting Pb^{2+} ISE exceeded the earlier stated requirements, and alkali earth metal ions were found to be discriminated by as much as 14 orders of magnitude. This left some room for reducing the ion exchanger concentration in the membrane, which resulted in a further reduction in ion flux and more rugged behavior, even though the selectivity was somewhat reduced. The detection limits in typical ionic backgrounds for tap water were \sim 1 \text{ nM}, and they were perfectly in line with theoretical expectations. Because response times during such low activities are rather long, calibration solutions in the range 10^{-5}–10^{-4} \text{ M} were used, indicated by the colored points in Figure 4b and coded with the same colors in Figure 4a. The 5-point calibration by standard addition required \sim 10 \text{ min}. It may seem dangerous to rely on calibration solutions that are 1000–10,000-fold more concentrated than the samples. How-

Table 1. Direct potentiometric determinations of Pb^{2+} in tap water (17).

<table>
<thead>
<tr>
<th>Uns spiked tap water</th>
<th>ISE (µg/L)</th>
<th>ICPMS (µg/L)</th>
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<tbody>
<tr>
<td></td>
<td>0.71 ± 0.04</td>
<td>0.54 ± 0.07</td>
</tr>
<tr>
<td>2.0 µg/L</td>
<td>2.61 ± 0.06</td>
<td>2.60 ± 0.04</td>
</tr>
<tr>
<td>10 µg/L</td>
<td>11.5 ± 0.3</td>
<td>11.50 ± 0.06</td>
</tr>
<tr>
<td>100 µg/L</td>
<td>22.2 ± 1.2</td>
<td>22.0 ± 0.2</td>
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ever, thanks to excellent reproducibility of the response slopes, the observed uncertainties were acceptably small (Table 1).

Figure 4b shows a typical measurement of untreated tap water at its native pH 8, indicated as the lower blue point on the calibration curve. Clearly, the assessed level of lead (2 x 10^{-10} M) is extremely low. The lead concentration of the same sample determined with inductively coupled plasma MS (ICPMS), indicated as a filled circle on the calibration curve of Figure 4b, is an order of magnitude higher than the one obtained with the potentiometric sensor. This is not surprising if one realizes that potentiometric measurements assess free ion activities, not total ion concentrations. At elevated pH, most of the lead ions are complexed, mainly by carbonate, and <10% remains in the free, uncomplexed form. It is this form that the potentiometric sensor observes, hence the lower value. If the sample is buffered to pH 4.0, where lead is mostly in its free form, the values obtained by potentiometry and ICPMS are indeed nearly identical (Figure 4b).

The unique characteristics of potentiometric sensors that allow speciation measurements are further illustrated in Figure 4c. A spiked tap water sample was adjusted to various pH values, and the potentiometry assessed lead values (open circles) agree well with theoretical expectations on the basis of two carbonate levels in the sample (dotted lines). Again, it can be seen that buffering to pH 4 transforms all the lead complexes into their free form, and potentiometric and atomic spectrometric methods yield comparable results under these conditions (17).

To compare total assessed lead levels determined by potentiometry and ICPMS, various samples were collected and buffered to pH 4.0 before measurement. Table 1 shows the observed lead concentrations for native and spiked samples and demonstrates excellent correlation and acceptably small standard deviations for the potentiometric data. The standard deviations were obtained from measurements with three different sensors on the same sample.

In the context of practical applications, it is important to remember that ISEs are quite unique in their capability to assess free ion activities. This is in contrast to the information obtained by other methods: Atomic spectrometry gives total concentrations, and volammetry yields concentrations of labile species and complexes. Depending on the problem at hand, one or the other value may be of primary interest. For speciation purposes, a comparison of several methods or potentiometric measurements under different conditions may be useful.

**What holds the future?**

It's been almost four years since the first papers on improving detection limits were published. The successes achieved since have been rather impressive. A series of ISEs have been described, showing how the lower detection limits of any polymeric membrane electrode of sufficient selectivity can be improved. The selectivities of a series of reinvestigated electrodes have been improved by many orders of magnitude over those determined in the original papers, if the biasing effect of ion fluxes is eliminated. Very probably, such improvements will be found for many other available systems.

A sound theoretical basis of the involved zero-current transport processes has been developed and the main predictions have been experimentally verified. A palette of measures is available that can be used to optimize membranes. The theory can be directly applied to predict the required composition of an inner electrolyte on the basis of the selectivity behavior of the membrane and the composition of the sample. Improving the detection limits of future electrodes will now be more routine. Lastly, the practical use of recently developed electrodes for trace-level determination has been demonstrated. On the basis of this, we expect that a series of potentiometric sensors with submicromolar detection limits for use in environmental and clinical analyses of trace metals will be described soon. Although the possibilities are promising, clinical trace metal determinations by potentiometry have not yet been the focus of research.

A very small external current can be used to compensate for zero-current ion fluxes (16, 22). This method is attractive because it is easier to alter the required current than the compositions of other available systems.
the membrane and the inner solution. However, determining the required current for an unknown sample is not a simple task, and further research is needed to show the practical applicability of this approach. Another related research endeavor is the development of ISEs without inner solution to eliminate one major source of transmembrane ion fluxes (23). This promises to eventually eliminate steady-state ion fluxes completely because only two phases would be involved (Figure 2). If successful, it should further improve detection limits by many orders of magnitude.

Sensor miniaturization will be necessary for achieving low detection limits because an ordinary membrane contains $\sim 10^{-7}$ moles of primary ions, which corresponds to a picomolar concentration in $10,000$ L of sample. If a sensing membrane is placed directly on a metal, there are two main concerns: how to guarantee a stable (redox) potential between the membrane and the metal (23–26) and how to prevent the spontaneous formation of a water film between the two (27, 28). Active research on different possible solutions is being pursued, so the development of low-detection-limit, solid-contact electrodes is likely.

Another promising field of research that will benefit from these developments is the design of optical sensors with small sensing volumes (29), whose response mechanism is closely related to that of potentiometric sensors (2). However, steady-state ion fluxes are not anticipated here because these sensors rely on a two-phase equilibration process. Therefore, low detection limits should be more easily obtained than with classical ISEs (30).

For most techniques, lower detection limits are either set by interferences or by the physical limitations of the instruments. The ultimate low detection limit of potentiometry is still not yet known. Highly selective systems exist and, in principle, they should be able to achieve such extremely low detection limits that they do not seem realistic. It is expected, but not yet shown experimentally, that the small currents on the order of $100$ fA required for zero-current potentiometric measurements will set the limits, and these might be somewhere between picomolar and femtomolar activities.

Compared with other methods of trace analysis, potentiometry is an extremely inexpensive technique. Because it can compete with the most sophisticated methods, it is very likely that we will encounter many practical applications in the future. Potentiometric sensors are known to only minimally perturb the sample, compared with most other analytical methods, and they should be especially well suited for integration into optimized fluidic and microfluidic systems for a variety of applications.

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