Pure Appl. Chem., Vol. 76, No. 4, pp. 839–845, 2004.

Potentiometric detection for high-performance liquid chromatography is a reality: Which classes of organic substances are the targets?*

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Abstract: Potentiometric detection in high-performance liquid chromatography (HPLC) is shown to be an underexploited technique. The technique can be of great use to less classical potentiometry targets, such as bioorganics, of low as well as of high molecular weight. The understanding of non-faradaic potentiometry is, however, still problematic. Predicting the selectivity and sensitivity of a potentiometric electrode for organic ionizable substances can be done to a certain extent using QSAR methods. Although many new polymer materials and synthetic receptor molecules for organic ionics are being synthesized, few of them are applied in potentiometric membrane coatings. Hydrophilic organics form an interesting target group for these new materials.

DEVELOPMENT OF ELECTROCHEMISTRY IN SEPARATION METHODS AND HPLC/POTENTIOMETRY IN PARTICULAR

Whereas amperometric and conductometric detectors for high-performance liquid chromatography (HPLC) are commercially available, potentiometric detectors are still in the research phase. The first reports on the use of potentiometric detection in liquid chromatography occured in the 1970s [1–4], mostly with liquid membrane electrodes. The total number of publications that we found on the potentiometry/HPLC subject from 1970 to date was 50. A group with extensive experience in the field is Haddad's group, using a copper electrode [5–8]. Later, other groups such as those of Manz and Simon [9], Isildak and Covington [10–12], Hong [13,14], and Trojanowicz [15] explored potentiometric HPLC detection. The mentioned 50 articles mostly deal with the classical potentiometry analytes such as metal cations and small inorganic anions. Our group started with the technique in 1993 [16] and obtained very sensitive and reproducible results in the latest years [17–20], especially with coated-wire electrodes based on PVC. We focused on organic ionic substances, as this important area seemed unexploited. At present, the drive toward application of potentiometric sensing in miniaturized techniques seems stronger than the drive toward application in HPLC (see next paragraph). In a 10-year period, Tanyanyiwa [21] reports 90 references on conductivity, plus potentiometric detection applied in CE and microchip capillary, most of them being quite recent. Some 20 of these publications are on potentiometric detection. For comparison: Wang [22] reports 23 applications of amperometry in miniaturized separation systems from 1998 to 2001. FIA/potentiometry is by far the most used combination [23].

*Plenary lecture presented at the Southern and Eastern Africa Network of Analytical Chemists (SEANAC), Gaborone, Botswana, 7–10 July 2003. Other presentations are published in this issue, pp. 697–888.

Potentiometry coupled to separation methods can give access to many bioorganics, which are available in minute quantities only, and often in admixture with other substances. Many questions arose however on the working mechanism of the electrodes and electrode materials used, the membrane-substrate electrode interface, selectivity- and sensitivity-determining factors, choice of eluents, response times, and the type of compounds and samples the technique could handle. How these topics are dealt with in the literature is explored in the next paragraphs.

SEPARATION METHODS REQUIRE "NON-FARADAIC" POTENTIOMETRIC DETECTORS AND NON-FARADAIC REASONING

Electrode potentials measured in potentiometric methods express the driving force (Gibbs energy) behind a redox reaction or behind a physicochemical process. The first type of potentiometry is called redox potentiometry [24] or faradaic potentiometry [25]. In separation methods of analysis, redox potentiometry is seldom used. For redox active substances, amperometric detection (not to be discussed here) is the accepted technique. Electrode potentials can be developed, however, also by a multitude of physicochemical processes. Mostly, such a physicochemical process is driven by intermolecular attractions, eventually by mixing [26]. The latter potentials are sometimes referred to as "non-faradaic" [25]. Recently, Cheng [25] stated that this kind of potentiometry is "plagued by fundamental errors and lack of conceptualization". Many electroanalytical chemists indeed try to explain non-faradaic potentiometric responses by faradaic reasoning: The potential is generated by a chemical (redox) reaction. The potentiometric membrane/reference electrode system behaves as a galvanic (voltaic) cell. At each interface with an electronic conductor (e.g., a metal substrate electrode), there must be ion-to-electron conversion. Moreover, the Nernst equation for galvanic cells is applied:

$$E = E^0 + \text{cst.} \times \log Q \tag{1}$$

where Q is the reaction quotient.

Whether a potentiometric sensor is faradaic or non-faradaic, it will always respond as described in the equation below:

$$E = A + B \log c_i \tag{2}$$

c_i being the concentration (activity) of the analyte ion, and A and B are constant values. Even if the potential of a potentiometric sensor would derive from diffusion phenomena, the generated potential is described by an equation such as eq. 2 [27].

Both eqs. 1 and 2 are linked to the more general thermodynamic eq. 3, via the well-known $\Delta G = -nFE$ translation. The $\Delta G = -nFE$ equation is normally used to relate the potential of a galvanic cell (a faradaic process) to the Gibbs free energy of the redox reaction taking place in the cell. It can as well be used in the case of non-faradaic phenomena [28].

$$\Delta G = \Delta G^0 - RT \ln Q \tag{3}$$

Equation 3 itself derives from the Boltzmann factor, which is valid as well for chemical reactions [29] as for physicochemical phenomena. Physiologists derive "their" Nernst equation for (obviously non-faradaic) membrane potentials directly from the Boltzmann factor [30].

USE OF CLEAR MODELS SHOULD ACCELERATE APPLICATION OF POTENTIOMETRY IN SEPARATION METHODS

Analyte ions can provoke potentials at surfaces of different materials. In the following discussion, we will restrict ourselves to "coated-wire" electrodes. Coated-wire electrodes have a robustness that is compatible with the requirements of classical and miniaturized separation systems. Materials used in coated-wire electrodes such as polymer-based liquid membranes and conducting polymers have ion-ex-

changer properties (see Fig. 1). They possess ions or ionic sites that are quasi-immobile, and small mobile water-soluble counter ions. They are either electronically or ionically conductive. The most successful material is based on plasticized PVC. This material is in a rubber phase, a state with restricted molecular movements that allow ionic conductivity [31], while still giving mechanical stability. In a former publication on the subject [32], we used a practical model to explain the potential change at the coating-solution interface of such materials. Figure 1 visualizes this model: An analyte ion present in the solution tends to distribute itself in the membrane phase (ion extraction). As ion-exchange materials exclude the ion types with the same charge as the immobile ion [33], only the ion with opposite charge can enter the membrane. It will do so until the developed potential matches the tendency of the ion to be extracted in the membrane. The physicochemical process (ion extraction) does not proceed, as its driving force is counterbalanced by the build-up of the potential. Just as a balance expresses the force exerted on a substance, without the substance falling to earth.

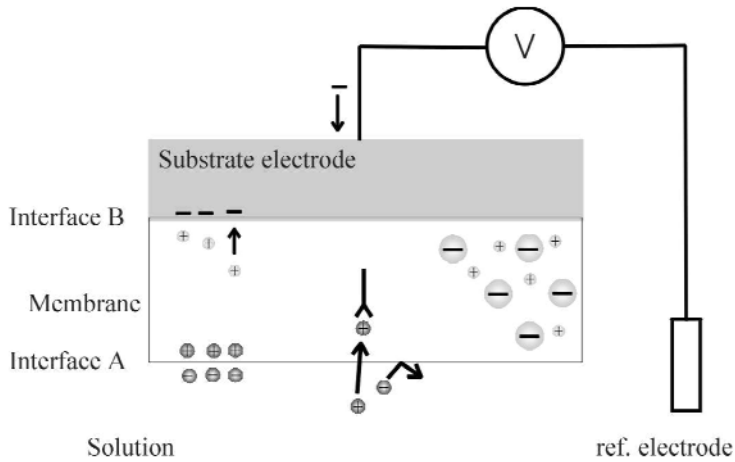

Fig. 1 Better models for coated-wire electrodes should accelerate potentiometric detection in HPLC.

DO NON-FARADAIC POTENTIOMETRIC SENSORS NEED ION-TO-ELECTRON CONVERSION?

It is a general belief in potentiometry that an ion-to-electron conversion (a faradaic process) must occur at interface B (Fig. 1) to generate current through the voltmeter. Non-faradaic processes like the discharge of an ion–ion capacitor (interface A) or an ion–electron capacitor (interface B) may also be considered. The discussion on these phenomena was reopened very recently by Cheng [34]. The strong belief in the necessity of ion-to-electron conversions at interface B (Fig. 1) always raised suspicion on the reliability of coated-wire electrodes. It was not clear which redox process was occurring. Some electrochemists argued that the redox active impurities present in the coating materials (PVC) were involved. Membrane-internal solution interfaces are generally believed to be more "correct", as the internal solution contains a perfect ion-to-electron converter, i.e., a reference electrode with a reversible redox system (see Fig. 1).

Non-faradaic phenomena should indeed be included in the discussion. The electrical potential energy is built up at interface A as an ion–ion capacitor (Fig. 1). This potential will also provoke some ion–electron capacitance at interface B. If we want to measure the membrane potential with a (high-impedance) voltmeter, this voltmeter will use part of the electrical potential energy of the capacitors at interfaces A and B. In other words, we will have to disturb (discharge) the capacitors at least partly. Discharge can take place by coupling electrically to interface C (mostly a reference electrode) where a redox reaction (faradaic) or a physicochemical process (non-faradaic) takes place. In such a case, elec-

trons move through the connecting wire. At interface A, these electrons provoke a negative electric field, which attracts cations from the interior of the membrane. This would result in a (partial) depolarization of interfaces A and B. One can calculate roughly that it would take days for a 10^{13} MΩ impedance voltmeter to discharge even partly an electrode surface polarized at 100 mV, in contact with, e.g., a 10^{-6} M monovalent analyte solution. More experimental data will be needed on this topic. Our experiences with coated-wire electrodes with membrane-metal interfaces (Fig. 1) in separation methods are very positive. Non-faradaic descriptions of the phenomena occurring at interface B may explain this positive impression.

FOR COATED-WIRE ELECTRODES, THE SENSITIVITY AND SELECTIVITY ARE DETERMINED BY INTERMOLECULAR ATTRACTIONS

The methods used to measure selectivity (selectivity coefficients) and sensitivity (detection limits) are thoroughly discussed in potentiometric literature [35,36]. It is less clear, however, which factors determine this selectivity and sensitivity. For the type of potentiometric membranes shown in Fig. 2, a model can be given (see ref. [32]). The driving force for the ion to be extracted into the membrane is the difference in Gibbs free energy of the ion between the solution and the membrane phase. This energy difference will be great when the membrane-phase components have good intermolecular attractions to the analyte ion. Yu [37] splits this free energy difference in different terms, an idea that was adapted somewhat by our group [32]:

$$\Delta G_{tr} = \Delta G_{hydr} - (\Delta G_{solv} + \Delta G_{ex} + \Delta G_{complex}) \quad (4)$$

ΔG_{tr} stands for the difference in Gibbs free energy for the analyte ion in the water phase and the membrane phase. ΔG_{hydr} is the hydration energy of the ion in the water phase containing the analyte ion. ΔG_{solv}, ΔG_{ex}, and $\Delta G_{complex}$ are respectively the solvation energy of the ion in the membrane components, the ion–ion interaction energy of the ion with the ion-exchange sites (e.g., lipophilic ions) in the membrane, and the complex formation with an eventual neutral ionophore.

The Gibbs free energy difference of an ion in the eluent phase and the membrane phase, ΔG_{tr}, is the important sensitivity- and selectivity-determining factor. It determines the developed potential E:

$$\Delta G_{tr} = -qE = -nFE \quad (5)$$

The tendency for the ion to be extracted into the membrane (ΔG_{tr}) is translated into an electrical potential (E).

Equation 4 is very practical to estimate the important factors in the design of electrode membranes of the type shown in Fig. 1. We have to optimize the membrane so as to obtain maximum $\Delta G_{solv} + \Delta G_{ex} + \Delta G_{complex}$ values. ΔG_{solv} can be optimized by developing membrane coatings with high intermolecular interactions with the analyte substances.

We replaced the mostly unknown ΔG_{hydr}, ΔG_{solv}, ΔG_{ex}, and $\Delta G_{complex}$ values from eq. 4 by physicochemical molecular descriptors which can be estimated and which are free energy-related. Using QSAR calculations, we have shown that especially log P and P_{vol} (polarizability) are important descriptors to predict the sensitivity (detection limits) [18]. In membranes without added receptor molecule (ionophore), an equation of the type of eq. 6 shows good correlation between the logarithm of an observed property of a compound, e.g., the detection limit (DL), and such free energy-related descriptors.

$$\text{Log DL} = a - b \log P + cP_{vol} \quad (6)$$

As most existing membrane materials used (polymer + plasticizer) are very lipophilic, the lipophilicity log P of the analyte is very important [17,18]. For organic analytes, this very practical factor can be easily calculated with software available on the Internet [38]. Most of the organic substances that can be detected very sensitively have large positive log P factors.

Log P seems logically related to $\Delta G_{hydr} - \Delta G_{solv}$ (see eq. 4). "Hofmeister series" descriptions often take into account a less accessible quantity such as ΔG_{hydr} only (ΔG_{solv} is not known). P_{vol} is related to ΔG_{ex}. $\Delta G_{complex}$ is more difficult to estimate. An approach as used by Abraham is possible [17,39], and/or molecular modeling. The mentioned techniques (QSAR, Abraham's approach) are also used to estimate drug activity in medical research. It seems possible that the estimation of the activity of a medicinal drug, and the estimation of the potentiometric "activity" of an organic ion can be based on similar approaches [40]. Both phenomena are related to uptake of compounds by lipophilic membranes.

CHROMATOGRAPHIC ELUENTS SHOULD RESPOND AS LITTLE AS POSSIBLE TO THE SENSOR

In the presence of a second ion, eq. 2 is no longer valid. This situation occurs also at low analyte concentrations, where interfering ions become important, or in chromatographic eluents, where we always have interfering buffer ions. In this case, the Nicolski–Eisenmann equation gives an understandable description of the E vs. c_i behavior:

$$E = E^0 + \mathrm{RT}\ \ln(c_i + K_{ij}^{\mathrm{pot}} c_j) \tag{7}$$

This equation was never derived analytically. In eq. 7, we give the most simple form, for the case of a monovalent interfering ion j. It is clear from this equation, that if the buffer that is chosen for the chromatographic eluent contains an interfering ion j with high K_{ij}^{pot} value or with a high concentration of ion j, the calibration curve (E vs. ln c_i) will be independent of c_i, and the detector becomes insensitive.

IN CHROMATOGRAPHY, RAPID RESPONSE TIMES ARE NEEDED

The time required to obtain 90 % of the maximum response when a concentration pulse passes the detector is called the response time. It should be in the order of a few hundred ms in chromatographic systems. For the small organic ions investigated in our lab, response times are still in the order of a few seconds. For larger molecules such as oligonucleotides (to be published), response times may still be in the order of minutes. The mobility of the analyte ion in the membrane may be important in this respect, but also the kinetics of intermolecular attractions (complex formation) between analyte ion and membrane components.

WHICH ANALYTE SUBSTANCES ARE TO BE DEALT WITH IN THE FUTURE?

At this moment, potentiometry is commercially (very) successful for batch determination of a number of metal cations, halogen anions, and some other small inorganic anions like S^{2-}, SCN^-, NO_3^-, NO_2^- and ClO_4^- (taken from a commercial supplier catalog). Sensors developed in research laboratories extend the applications to larger ionic organics including organic acids, amines, and basic pharmaceutical drugs. Especially for these larger organic ionics, the potentiometric sensors used will not be highly selective, and successful use will require coupling to a separation technique. The above collection is very limited, especially if we know that a large and important part of the bio-world is ionic. At least part of the reason for the absence of potentiometry in bioorganics is the high water solubility (negative log P) of many ionic bioorganics. In principle, noradrenalin (log $P = -1.54$) could be detected as sensitively by potentiometric detection as by amperometry or fluorimety. At this moment, however, its response is 100 to 1000 times smaller than the response of an equimolar solution of clenbuterol (log $P = 2.91$) or bromhexine (log $P = 4.37$). The obvious solution for this problem is optimization of $\Delta G_{solv} + \Delta G_{ex} + \Delta G_{complex}$ (see eq. 4): get the analyte molecule interacting with the polymer plus plasticizer (ΔG_{solv}),

with the immobile ion (ΔG_{ex}), or with an added receptor (ionophore, $\Delta G_{complex}$). This requires the development of new coating materials and receptor molecules. The development both of new coatings and of new receptors [41] seems to shift into a higher gear. Another reason for the absence of potentiometry in bioorganic ionics is the nonavailability of many of these substances in pure form, and their high price for minute quantities. Application of potentiometry in HPLC and CE will change this, as only minute amounts are required, and the purity is less important. We studied the behavior of oligonucleotides in potentiometry using the HPLC/potentiometry system (to be published), which would be impossible in batch, and complicated in FIA. Also practically absent from the potentiometry scene are the other larger biomolecules such as proteins. Feng [42] has shown, however, that they can be determined via a potentiometric immunosensor, an approach which has not yet been frequently applied, but which may be very promising [43].

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