

Bio Currents Research Center Tutorial

Potentiometric Ion Selective Microelectrodes (ISMs)

The material below has been adapted from several primary papers and reviews originating from the BRC. These should be referred to in publications and for the original source materials.

References:

1. *Smith, P.J.S., Sanger, R.S. and Messerli, M.A. (2007) Principles, Development and Applications of Self-Referencing Electrochemical Microelectrodes to the Determination of Fluxes at Cell Membranes. In: Methods and New Frontiers in Neuroscience. Ed. Adrian C. Michael. CRC Press. Ch. 18: 373-405.*
2. *Messerli, M.A., Robinson, K.R. and Smith, P.J.S. (2006) Electrochemical sensor applications to the study of molecular physiology and analyte flux in plants. In: Plant Electrophysiology - Theory and Methods. Ed. Alexander G. Volkov. Springer-Verlag. 73-107.*

Introduction

Several studies have contributed to our understanding of the factors that influence ISM performance. Bakker et al. provide a review.³ A key point discussed in that paper is the importance of the internal electrolyte on detection limits, a matter dealt with below. It is not the purpose of this tutorial to review methods of electrochemical detection so readers are referred to other reviews.^{3,4} Here we will focus on the use of these designs in a self-referencing mode.

The ISEs are based on an ion-selective solvent or liquid membrane, immobilized in a silanized micropipette with tip diameters of 1-4 μm . For an ideal electrode the measured voltage, E , is related to the activity of the ion by the Nernst equation:

$$E = E_o + S \log a_i \quad (1)$$

where E_o is an offset potential, S is the Nernstian slope = $\frac{2.3RT}{z_i F}$ (eq. 2) and a_i is the activity of the primary ion. R , T

and F have their usual meaning. Z is the relevant valence of i , the measured ion. The offset potential is composed of the boundary potentials and liquid junction potentials that exist across any circuit made up of a reference and measuring electrode. Through calibration of each ISM a value for the slope of the line describing the voltage output and the change in ionic activity is collected. Since ionic activity is directly proportional to ion concentration, via the activity coefficient, and the changes that occur to this coefficient due to changes in ionic strength are usually negligible during self-referencing, we will use concentration in place of activity.

Minimizing drift and noise for a potentiometric sensor operating in a self-referencing mode (termed SERIS⁵) can be explained by introducing a formulation of the Nernst equation. The voltage difference at the two poles of excursion within an ionic gradient is related to the concentrations at the two poles by the following equation:

$$E_1 - E_2 = (E_o + S \log C_i)_1 - (E_o + S \log C_i)_2$$

$$\Delta E = (S \log C_i)_1 - (S \log C_i)_2$$

$$\Delta E = \log C_{i(1)}^{S_1} - \log C_{i(2)}^{S_2}$$

$$\Delta E = \log\left(\frac{C_{i(1)}^{S_1}}{C_{i(2)}^{S_2}}\right) \quad (3)$$

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E_1 , $C_{i(1)}$ and S_1 are the measured voltage, primary ion concentration and slope of the voltage-log plot for the near pole of excursion. Subscript 2 labels the same parameters for the far pole of excursion. By calculating the difference in potentials over short periods of time the impact of slow drift, due to the constant potential differences throughout the system, are reduced. This places the emphasis of the potential difference measurement on the concentration difference of the ion.

Equation 3 can be simplified to determine the relationship between the ionic concentrations at the two points of excursion:

$$C_{i(1)} = C_{i(2)} \frac{S_2}{S_1} \bullet 10^{\frac{\Delta E}{S_1}} \quad (4)$$

The slopes at two points of the calibration curve are included to address measurement of the primary ion under different circumstances; 1) differential concentration of the primary ion in the absence of an interfering ion; 2) differential concentration of the primary ion in a constant concentration of an interfering ion and; 3) differential concentration of a primary ion coexisting with a gradient of an interfering ion.

For an ideal electrode, used in the absence of interfering ions, the slope is constant over a wide range of the primary ion concentration and close to Nernstian. Under these conditions eq. 4 simplifies to:

$$C_{i(1)} = C_{i(2)} \bullet 10^{\frac{\Delta E}{S}} \quad (5)$$

Under many circumstances the average concentration of the ion at the far pole, position 2, is not that different from the average concentration of the ion in the bulk solution. Therefore, the difference in ion concentration between the two points of excursion can be described as follows:

$$\Delta C = C_{i(1)} - C_{i(2)} = C_{bath} 10^{\frac{\Delta E}{S}} - C_{bath} \quad (6)$$

A primary assumption here is that the concentration difference measured between the two excursion points is linear. This is only true if the excursion distance is small compared to the extent that the gradient extends out into the bulk solution. For small cells an excursion of 10 μm will most likely sample over a distance in which the concentration difference is not linear and could, therefore, lead to an underestimate of the flux. An incorrect estimation of flux could also occur during a two-point measurement in an intense extended gradient, where the concentration of the ion at the far pole is substantially different from the background concentration of that ion. The solution to these issues is the use of a three-point measurement to; 1) more carefully map the concentration gradient with a third point to either ensure a linear relationship or determine a more accurate nonlinear relationship and; 2) to determine the concentrations in the gradient relative to the background concentration of the ion in the bath. For slow drift conditions the electrode can simply be moved from position 3 (outside of the gradient) to position 2 (slightly into the gradient) to position 1 (deeper into the gradient). In general, regular measurements at all three positions should be attempted.

Measurement of a concentration difference for a primary ion in the presence of a constant concentration of an interfering ion presents us with a more difficult situation. This has been dealt with in detail by Messerli et al.² and will be summarized here. Two variables in eq. 4 now need to be determined, specifically the concentration of the ion in the near pole $C_{i(1)}$ and the new slope of the calibration curve in the near pole, S_1 . Therefore, a relationship between the change in voltage and the change in slope needs to be established in order to measure two points in a concentration gradient of a primary ion in the presence of a constant concentration of an interfering ion. The Nicolsky-Eisenman equation provides a means for predicting the change in slope due to the change in voltage in the presence of an interfering ion, assuming the electrode responds to the interfering ion in a Nernstian manner.

Rearranging the Nicolsky-Eisenman equation to get primary ion concentration in terms of voltage we can determine the relationship of the slope at any point along the curve in terms of voltage:

$$E = S_N \bullet \log(C_i + K_{ij} \bullet C_j^{\frac{z_i}{z_j}})$$

$$C_i = 10^{E/S_N} - K_{ij} \cdot C_j^{z_i/z_j}$$

$$\log(C_i) = \log(10^{E/S_N} - K_{ij} \cdot C_j^{z_i/z_j})$$

$$\frac{1}{S} = \frac{d \log(C_i)}{dE} = \frac{10^{E/S_N}}{S_N (10^{E/S_N} - K_{ij} \cdot C_j^{z_i/z_j})} \quad (7)$$

S_N is $\frac{2.3RT}{z_i F}$ and S is the slope along the curve. Z is the relevant valence and i is the measured ion and j the competing.

In order to achieve this sort of measurement we need to know the starting position (voltage point) along the theoretical curve. We can calculate the starting voltage point on the theoretical curve by determining the slope in the bath empirically and solving for E in eq. 7.

$$E = S_N \log\left(\frac{S_N \cdot K_{ij} \cdot C_j^{z_i/z_j}}{S_N - S}\right) \quad (8)$$

The unknown S in the denominator is determined by calculating the slope between two different concentrations of the primary ion in the working solution. More points can be used to validate the starting position along the curve. From this point on the theoretical curve, a small change in measured voltage, ΔE , either up or down the curve, can be used to determine both the new slope at the near pole from eq. 7, and the concentration in the near pole from eq. 4. The differential concentration can now be determined as the concentration in the far pole and near pole are known.

The final case to be considered is measurement of a gradient of a primary ion coexisting with a gradient of an interfering ion. If a relatively ideal ISM can be used to measure the interfering ion then the situation discussed above with eq. 7 can be used to solve for the primary ion flux. However, if the ISM used to measure the primary ion can also sense the interfering ion then no simple electrochemical method to determine the absolute flux of either ion exists. Selective pharmacological block of targeted transporters can go some way to solving this problem by removing ion specific flux values. However, this is dependent on prior knowledge of the system.

Types of Potentiometric Ion-Selective Microelectrodes

Many more ion-selective microelectrode designs, based on liquid membranes, exist than have been used in a self-referencing mode. Lists are available in Umezawa et al.⁶ for inorganic cations, and Bühlmann et al.⁷, with 30 available commercially from Sigma/Fluka. Calbiochem is another source. Fluka published data sheets for the ion selective solvents they offer.⁸ As this publication is no longer generally available Table 1 gives the details for the ion-selective solvents that have been used in a self-referencing mode and unless stated otherwise this material comes from Fluka.⁸ These data are also included to highlight the fact that all sensors are **selective** not **specific**.

Not dealt with in Table 1 are interferents frequently used to block or activate molecular transport. This is a complex issue with no simple solution; examples are given for calcium detection⁵ and for chloride detection.^{9,10} Detecting a chloride flux over the actions of blockers or interferents is particularly interesting and has led to erroneous data getting into the literature. See references 2 and 11 for a critique. The BioCurrents Research Center is compiling a web site database highlighting known problems (www.biocurrents.org) but any investigator is advised to conduct tests using their own system and solutions.

An interesting design for the detection of Cu fluxes has also been published.¹² As in Gow et al. self-referencing movement was manually controlled.¹³

Table 1: Selectivities, concentration dependent slopes and response times for the LIXs used in a self-referencing mode.

Calcium	
Fluka Calcium Ionophore I-Cocktail A. It has the following selectivity values determined by the fixed interference method:	
$\text{LogK}_{\text{CaNa}}^{\text{Pot}}$	-5.5
$\text{LogK}_{\text{CaK}}^{\text{Pot}}$	-5.4
$\text{LogK}_{\text{CaMg}}^{\text{Pot}}$	-4.9
Electrode function: Slope of the linear regression is 28.1+/- 1.8mV (10^{-2} to 10^{-7} CaCl ₂ determined in calcium buffered solutions at a constant background of 125mM K ⁺). With a tip diameter between 1-2µm the expected resistance is in the range of $2 \cdot 10^{10}\Omega$. Data from Fluka (1996). In our applications with larger tips (2-4µm) and short column lengths (30µm) we record a resistance of 2-3·10 ⁹ Ω. Response times evaluated at the BRC give a mean value of 48ms to 90% response (see below).	
Chloride	
Fluka Chloride Ionophore 1 - Cocktail A.	
It has the following selectivity factors as determined by the separate solution method:	
$\text{log K}_{\text{ClHCO}_3}^{\text{Pot}}$	-1.5 (Fluka, 1996)
	-0.9 (Garber et al., 2005)
$\text{log K}_{\text{ClAcetate}}^{\text{Pot}}$	-1.3 (Fluka, 1996)
	-1.2 (Garber et al., 2005)
$\text{log K}_{\text{ClGlutamate}}^{\text{Pot}}$	-3.2 (Garber et al., 2005)
$\text{log K}_{\text{ClGluconate}}^{\text{Pot}}$	-3.0 (Garber et al., 2005)
$\text{log K}_{\text{ClCitrate}}^{\text{Pot}}$	-1.6 (Garber et al., 2005)
$\text{log K}_{\text{ClThiosulfate}}^{\text{Pot}}$	-0.8 (Garber et al., 2005)
$\text{log K}_{\text{ClSO}_4}^{\text{Pot}}$	-2.6
$\text{log K}_{\text{ClSCN}}^{\text{Pot}}$	3.4
$\text{log K}_{\text{ClSalicylate}}^{\text{Pot}}$	3.0
Electrode function: Slope of the linear regression is 57.5+/- 0.5mV (0.001 to 0.5M NaCl on 0.01M Tris/H ₂ SO ₄ , pH 7.4). The resistance for an approximately 1µm tip will be in the region of $7 \cdot 10^{10}\Omega$. These data are taken from Fluka (1996). The 90% response time is given as 1.8 s (Garber et al., 2005).	
Hydrogen	
Fluka Hydrogen Ionophore 1 - Cocktail B	
It has the following selectivity factors determined by the fixed interference method:	
$\text{log K}_{\text{HNa}}^{\text{Pot}}$	-10.4
$\text{log K}_{\text{HK}}^{\text{Pot}}$	-9.8
$\text{log K}_{\text{HCa}}^{\text{Pot}}$	<-11.1
Electrode function: Slope of the linear regression is 58.0+/-0.4mV pH range 5.5 - 12.0. The resistance for an approximately 1µm tip will be in the region of $1 \cdot 10^{11}\Omega$. These data are taken from Fluka (1996).	
Response times evaluated at the BRC give a mean value of 88ms to 90%.	
Potassium	
This cocktail has the following selectivity values based on the separate solution method:	
$\text{log K}_{\text{KLi}}^{\text{Pot}}$	-4.2
$\text{log K}_{\text{KNa}}^{\text{Pot}}$	-3.9
$\text{log K}_{\text{KMg}}^{\text{Pot}}$	-4.6
$\text{log K}_{\text{KCa}}^{\text{Pot}}$	-4.9
$\text{log K}_{\text{KAcetylcholine}}^{\text{Pot}}$	-4.9
Electrode function: Slope of the linear regression is 58.8+/-1.2mV (20°C: 10^{-4} to 10^{-1}). The detection limit of this ionophore is given as $\text{log } a_{\text{K}} = -5.0$ against 140mM Na ⁺ . These data are from Fluka (1996). The response time to 95% is 41-77ms depending on the change in concentration (Messerli et al., 2005).	
Cadmium	
These data are taken from Pineros et al. (1996). Reference should be made to this paper for Ism preparation and testing. Selectivity based on the separate solutions method:	
$\text{log K}_{\text{CdZn}}^{\text{Pot}}$	-2.9
$\text{log K}_{\text{CdPb}}^{\text{Pot}}$	-4.6
$\text{log K}_{\text{CdCu}}^{\text{Pot}}$	-4.8
$\text{log K}_{\text{CdMn}}^{\text{Pot}}$	-5.2
$\text{log K}_{\text{CdFe}}^{\text{Pot}}$	-8.6
$\text{log K}_{\text{CdNi}}^{\text{Pot}}$	-10.4
$\text{log K}_{\text{CdCa}}^{\text{Pot}}$	-10.8
$\text{log K}_{\text{CdMg}}^{\text{Pot}}$	-12.2
$\text{log K}_{\text{CdNH}_4}^{\text{Pot}}$	-6.2
$\text{log K}_{\text{CdNa}}^{\text{Pot}}$	-7.1
$\text{log K}_{\text{CdK}}^{\text{Pot}}$	-7.9

Literature Cited:

3. Bakker, E., Bühlmann, P., and Pretsch, E. (1997) Carrier-based ion-selective electrodes and bulk optodes. 1. General characteristics, *Chem. Rev.*, 97, 3083-3132.
4. Bakker, E. and Meyerhoff, M.E (200) Ionophore-based membrane electrodes: new analytical concepts and non-classical response mechanisms, *Anal. Chim. Acta.*, 416, 121-137, 2000.
5. Smith, P.J.S., Hammar, K., Porterfield, D.M., Sanger, R.H. and Trimarchi, J.R. (1999) A self-referencing, non-invasive, ion selective electrode for single cell detection of trans-plasma membrane calcium flux. *Micros. Res. Tech.*, 46, 398-417.
6. Umezawa, Y., Bühlmann, P., Umezawa, K., Tohda, K. and Amemiya, S.(2000) Potentiometric selectivity coefficients of ion-selective electrodes: Part 1. Inorganic cations, *Pure Appl. Chem.*, 72, 1851-2082.
7. Bühlmann, P., Pretsch, E. and Bakker E. (1998) Carrier-based ion-selective electrodes and bulk optodes. 2. Ionophores for potentiometric and optical sensors, *Chem. Rev.*, 98, 1593-1687.
8. Fluka (1996) Selectophore, Ionophores for Ion-selective Electrodes and Optodes. Fluka Chemie A.G. Ronkonkoma, New York.
9. Doughty, J.M. and Langton, P.D (2001) Measurement of chloride flux associated with the myogenic response in rat cerebral arteries, *J. Physiol.*, 534, 753-761.
10. Garber, S.S., Messerli, M.A., Hubert, M. Lewis, R. Hammar, K., Indyk, E., and Smith P.J.S. (2005) Monitoring Cl⁻ movement in single cells exposed to hypotonic solution, *J. Memb. Biol.* 203, 101-110.
11. Messerli, M. A., Danuser, G., and Robinson, K. R.. (1999) Pulsatile fluxes of H⁺, K⁺, and Ca²⁺ lag growth pulses of *Lilium longiflorum* pollen tubes, *J. Cell Sci.*, 112, 1497-1509.
12. Papeschi, G., Mancuso, S. and Marras, A.M. (2000) Electrochemical behavior of a Cu/CuSe microelectrode and its application in detecting temporal and spatial localization of copper(II) fluxes along *Olea europaea* roots, *J. Solid State Electrochem.*, 4, 325-329.
13. Gow, N.A.R., Kropf, D.L. and Harold, F.M. (1984) Growing hyphae of *Achlya bisexualis* generate a longitudinal pH gradient in the surrounding medium, *J. Gen. Microbiol.*, 130, 2967-2974.