

Bio Currents Research Center Protocol

Electrode Construction

Potentiometric: Making an Ion Selective Microelectrode (ISM)

Introduction

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

References:

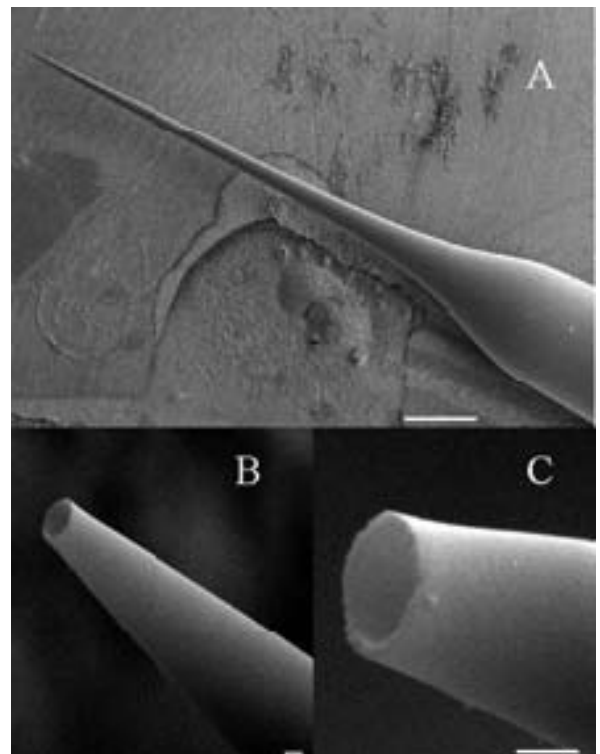
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2. Smith, P.J.S. and Trimarchi, J. R. (2001) Non-invasive measurement of hydrogen and potassium ion flux from single cells and epithelial structures. *Am. J. Physiol. Cell Physiol.* 280: C1-C11.
3. Smith, P.J.S., Sanger, R.S. and Messerli, M.A. (2007) 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.
4. 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.
5. Bakker, E. and Meyerhoff, M.E. (2000) Ionophore-based membrane electrodes: new analytical concepts and non-classical response mechanisms, *Anal. Chim. Acta.*, 416, 121-137.
6. Sokalski, T., Ceresa, A., Zwickl, T. and Pretsch, E. (1997) Large improvement of the lower detection limit of ion-selective polymer membrane electrodes, *J Am. Chem. Soc.*, 119: 11347-11348.
7. Mathison, S., and Bakker, E. (1998) Effect of transmembrane electrolyte diffusion on the detection limit of carrier-based potentiometric ion sensors, *Anal. Chem.*, 70: 303-309.

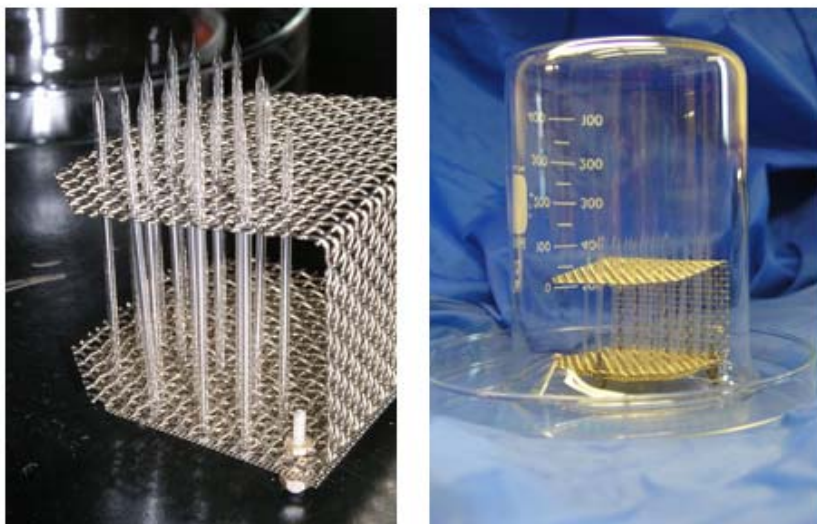
An electrode is made ion selective by the incorporation of an ionophore (ion exchanger or carrier) dissolved in a lipophilic cocktail. A selection of these ionophores, with details on their performance, is given in Table 1 and 2. A critical caveat to be aware of with any ISM is that they are **selective not specific**. Further, they can detect or be ‘poisoned’ by compounds used to target different transporters^{ref 4} (see selectivities section). The detection of chloride fluxes, or apparent chloride fluxes, is a particularly good example. These problems are discussed below and in the reviews cited above. These reviews should be used as a source of the original literature.

Pulling the pipettes

In the BRC pipettes are pulled with a Sutter P-97 puller using untreated 1.5mm borosilicate glass, without filament (cat. # TW150-4 World Precision Instruments). The single step pull program produces a stubby electrode with a shank of 8 mm and a tip with an aperture diameter of approximately 2 μ m (see figure 1). Pulling parameters will vary on each instrument in accordance with variables such as the filament geometry, but approximate values might be helpful. When the ramp value is measured to be 497, the other settings are adjusted such that; pressure is 500, heat is 580, pull is 0, velocity is 40 and the time is set to 200.

*Figure 1: Scanning electron micrographs of the electrode used for ion detection, demonstrating the overall shape of the electrode and the geometry of the tip. Shape is important in producing a stable column of cocktail. Scale bars are 1mm (A), 1 μ m (B) and 1 μ m (C). From Smith et al. (1999) *Micros. Res. Tech.* 46: 398-41.*





Drying

Freshly pulled electrodes, 50 at a time, are placed upright, **base down**, in a stainless steel and brass rack, on a glass petri dish under a 500ml presilanized glass beaker (figure 2) and baked in an oven at 200 °C overnight or for at least 4-6 hrs.

Figure 2: Micropipettes in the BRC storage rack and silanizing chamber.

Silanizing

Why is silanization necessary? Ion-selective liquid membranes are organic cocktails and hydrophobic. Freshly pulled electrodes are, in comparison, hydrophilic due to the density of surface hydroxyl groups (4.6 free OH groups per 10nm²). Silanization of these charged surfaces produces a monomolecular hydrophobic finish allowing the organic liquid membrane to bed comfortably in the tip of the electrode, no longer being displaced by the electrolyte. Although in principle silanization is extremely easy, in practice the results can be irritatingly variable. The golden rules, however, appear to be cleanliness in handling the glass both before and after pulling, keeping storage containers and baking ovens dust free, keeping the silanizing agent in a dry environment and baking the glass to at least 200 °C. Several different approaches to silanizing and filling ISMs are available in the literature. We have found that the vapor phase treatment with a reactive aminosilane compound is the most reliable in our circumstances.

To produce the hydrophobic silane coating, the rack of dried electrodes is exposed to 0.05- 0.1ml of N,N-dimethyltrimethylsilylamine (Sigma Fluka, cat # 41716). The silane is injected under the beaker through a syringe fitted with a bent needle. The silane vaporizes on contact with the beaker wall. Covered electrodes are left in the oven at 200 °C for 30-45 min. After this and in order to discard any additional vapor, the beaker is opened to the air for about 30s.

Recovered, the silanized electrodes are then baked at 200 °C overnight or for at least 4-6 hrs.

Storing

The electrodes can be stored for 2-3 weeks at 200 °C. Up until this time the electrodes remain stable on use but after this period we see a gradual increase in the fail rate. When electrodes are being used on a daily basis an additional rack of treated electrodes is stored in a desiccating chamber, which in our laboratory is kept close to the filling station. We have not found it necessary to acid wash our glass, nor store our electrodes under vacuum, and although we store our stocks of ionophores and cocktail in low light, we do not cover them with an inert gas.

Filling the ISM

A dispensing reservoir is first constructed using a silanized broken back pipette (tip diameter approximately 100µm) which is dipped into the stock cocktail taking up a column of cocktail of approximately 1mm. This pipette is placed into an assembly that allows controlled positive pressure, through tubing attached to a 20ml syringe, and the cocktail is pushed out to form a convex surface. This is done under a microscope adjusted to focus on the bright edge of the cocktail. A second electrode is back filled with a short column (5mm) of electrolyte and is mounted on a micromanipulator and connected to a syringe. The back filling solution is used to provide electrical contact between the back surface of the ion-selective solvent and the voltage-sensing node, via a Ag/AgCl wire. Usually a 100 mM solution of the ion being measured is used as the backfill to ensure that the ionic concentration between the back of the ion-selective solvent and the backfilling solution does not change significantly. However, it is now apparent that regulating this parameter can significantly alter the performance of the ISM ^{ref 3}.

Bakker and Meyerhoff review the phenomenon that the ion of interest from the back filling solution can easily move from the solution through the membrane into the bulk^{ref 5}. This will raise the local ion concentration effectively 'blinding' the electrode to small activity changes at the tip. Clearly, there is a need to re-examine the use of more dilute solutions, and/or the use of ion buffers, in the electrolyte. Sokalski et al. show that the addition of buffer to the inner solution yielded picomolar detection levels for Pb^{2+} .^{ref 6} The influence of the electrolyte composition has also been demonstrated for K^+ detection using valinomycin.^{ref 7} Additionally, the backfilling solution should be matched osmotically to the bath solution. Osmotic gradients across the ion-selective solvent can lead to its movement into or out of the electrode tip, either causing the electrode to fail or an increase in noise (unpublished observation - Mark Messerli).

Both ionophore dispensing pipette and the electrode are lined up to the same focal plane under a binocular compound microscope. The column of electrolyte in the electrode is now pushed forward to the tip (using positive pressure) and, within seconds of arrival, the tip of the electrode is immersed in the protruding ionophore. If the electrolyte is allowed to rest at the tip before insertion it dries and blocks the aperture.

With a minimal negative pressure the ionophore is drawn into the tip. The required column length (see Table 2) can be adjusted only while the tip is immersed in the ionophore source. Prepared electrodes are used immediately or within 2 hours. The majority of electrodes are Nernstian to within $\pm 2mV$, and stable for well over an hour. Some electrodes will last a working day. The setup, procedure and a finished ISM, next to the cocktail reservoir, are shown in figure 3.



Figure 3

Completing the circuit

For measurement an electrode is inserted into a half-cell microelectrode holder (cat #MEH3S15, WPI) fitted with a silver wire, plated electrolytically with silver chloride, which dips into the column of electrolyte back filling the electrode. Care should be taken not to scrape this wire as unstable junction potentials can result from unsatisfactory silver chloride coverage. The return pathway for the circuit is a bent glass bridge electrode filled with 3M KCl in 3% agar, inserted in a standard silver-silver chloride electrode holder (cat. #PDB1500, WPI) filled with 3M KCl. When using a K^+ -Seric probe the bridge uses 3M NaCl: for chloride, NaAcetate.

Bridge electrodes can be easily prepared in advance. Untreated glass capillary tubing (cat. # 34502, Kimble) is bent at one end under a gas flame and covered with a well dissolved and hot solution of 3M KCl and 3% agar. Multiple, short, exposures to microwaves helps eliminate air bubbles almost entirely. 100-200 pieces are made at one time. The finished electrode, although broad at the tip and stubby in design, has an extremely high resistance. For the calcium-selective electrode, with a 30 μm cocktail column, this resistance is around 2-3 Giga Ω . This places restrictions on the design of the experimental set-up – in particular the need to conduct experiments within Faraday Boxes as opposed to the normal Cage.

Calibration

ISM should be calibrated in different concentrations of the targeted analyte made up in the physiological solution to be used in the experiment. A good practice is to use three concentrations, each an order of magnitude apart, that bracket the

expected concentration in the saline to be used. For example, calcium might be used at 0.1 mM, 1.0 mM, and 10 mM. For H^+ sensors, unless there is a reason to expect problems from an unusual saline, calibration in commercially available buffer solutions are adequate provided a good pH value is determined on immersion in the saline at the start of the experiment. Under these conditions the output from any ion selective solvent-based ISM should be close to Nernstian (see Table 1). Electrodes should be calibrated before and after each experiment. Extreme deviations indicate a failed electrode or the presence of a competing analyte or 'poison' in the saline. Adding pharmacological compounds to these solutions is a simple and straightforward way to check for the latter type of interference (see selectivities section).

An additional calibration step is to compare the self-referenced output to a semi-empirical, modeled, gradient.

***See Equipment and Software Protocol for equipment requirements**

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:	
$\text{Log}K_{\text{CaNa}}^{\text{Pot}}$	-5.5
$\text{Log}K_{\text{CaK}}^{\text{Pot}}$	-5.4
$\text{Log}K_{\text{CaMg}}^{\text{Pot}}$	-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 $2\text{-}3 \cdot 10^9\Omega$. 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 $1.1 \cdot 10^{11}\Omega$. 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 $\text{log} a_K = -5.0$ 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

Table 2

Electrode	Column length	Tip Size	Medium	Response times ($t_{95\%}$ msec) for concentration ranges			
				10-1 mM	1-0.1 mM	0.1-1 mM	1-10 mM
K ⁺	100 μ m	1 μ m	1	195 \pm 59	376 \pm 86	165 \pm 41	114 \pm 26
				369 \pm 91	516 \pm 86	247 \pm 54	191 \pm 46
	1000 μ m	1 μ m	1	41 \pm 3	77 \pm 4	53 \pm 4	44 \pm 3
	100 μ m	2-3 μ m	1	64 \pm 9	225 \pm 6	91 \pm 8	69 \pm 15
w/ 450 mM Na ⁺	100 μ m	2-3 μ m	2				
H ⁺	30 μ m	1 μ m	3	pH 6-7	pH 7-8	pH 8-7	pH 7-6
				209 \pm 12	220 \pm 15	214 \pm 27	202 \pm 13
	300 μ m	1 μ m	3	251 \pm 21	269 \pm 14	245 \pm 11	244 \pm 17
	30 μ m	2-3 μ m	3	135 \pm 23	130 \pm 32	126 \pm 30	124 \pm 35
Ca ²⁺	30 μ m		4	10-1 mM	1-0.1 mM	0.1-1 mM	1-10 mM
				58 \pm 9	81 \pm 10	48 \pm 7	53 \pm 10

Table 2. Characteristics and response times determined for a variety of electrochemical microsensors under different conditions. All tip diameters and reactive areas are approximate.

Medium 1. 100 mM HEPES (pH 7.0) with 0.1, 1.0 or 10 mM KCl.

Medium 2. Medium 1 with 450 mM NaCl.

Medium 3. 100 mM MES (pH 6), 100 mM HEPES (pH 7,8) set with KOH

Medium 4. in mM, 120 NaCl, 5 KCl, 2 CaCl₂, 2 MgCl₂ 10 HEPES (pH 7.4)