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SHORT COMMUNICATION

A SIMPLE METHOD FOR CONSTRUCTION OF FLEXIBLE, SUBMINIATURE ION-SELECTIVE ELECTRODES

BY M. J. O'DONNELL

Department of Biology, McMaster University, Hamilton, Ontario, Canada

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Liquid membrane electrodes have greatly facilitated the measurement of pH and ion activities in intra- and extracellular fluids. Ion-selective microelectrodes (ISMEs) can be constructed by introducing a short column of a neutral carrier or ion-exchanger cocktail into the tip of a glass micropipette. The inner surface of the glass is first made hydrophobic by exposure to compounds containing silane (Thomas, 1978; Amman, 1986). This process, termed silanization, prevents subsequent displacement of the hydrophobic cocktail, either by entry of aqueous biological fluids through the tip of the micropipette or by migration of the backfilling solution. Macroscopic electrodes (5–10 mm diameter) can be produced by first incorporating appropriate neutral carriers into solvent polymeric membranes of polyvinyl chloride (PVC) or silicone rubber. Membranes can then be stamped out with a cork borer and glued or fused onto PVC tubing or commercially available electrode bodies (Meier *et al.* 1980). Electrodes with outer diameters of 1.5–1.7 mm have been formed by dipping tubing into a cocktail containing the ionophore, PVC and solvents. Solvent evaporation forms a membrane about 25 μm thick on the tip of the tubing (Oesch *et al.* 1987). A technique for the construction of miniature double-barrelled K^+ electrodes with a total o.d. of 600 μm and a shank 1.3 cm long has been described by Hill *et al.* (1978).

Although the latter type of electrode is suitable for intramyocardial and intravascular recording from animals the size of dogs and pigs (Hill *et al.* 1978), flexible electrodes with smaller diameters and longer shanks are required for recording ion activity in the circulatory system or body cavities of smaller animals such as insects. This paper describes techniques for the simple and rapid construction of flexible pH- and ion-selective electrodes with external diameters as small as 40 μm. Double-barrelled electrodes with total outer diameters of 80–100 μm and shanks as long as 50 cm can be inserted into the body cavity of insects such as locusts through syringe needles as fine as 26 gauge.

The electrode body is made from a disposable polyethylene 1 ml syringe (Becton-Dickinson & Co., Rutherford, NJ). A 1–2 cm region of the barrel is heated over a low flame until it softens and the diameter increases slightly (Fig. 1A). The syringe is then drawn out into a fine tube about 0.1 mm in diameter

Key words: ion-selective electrodes, pH, sodium, potassium, calcium, ammonium.

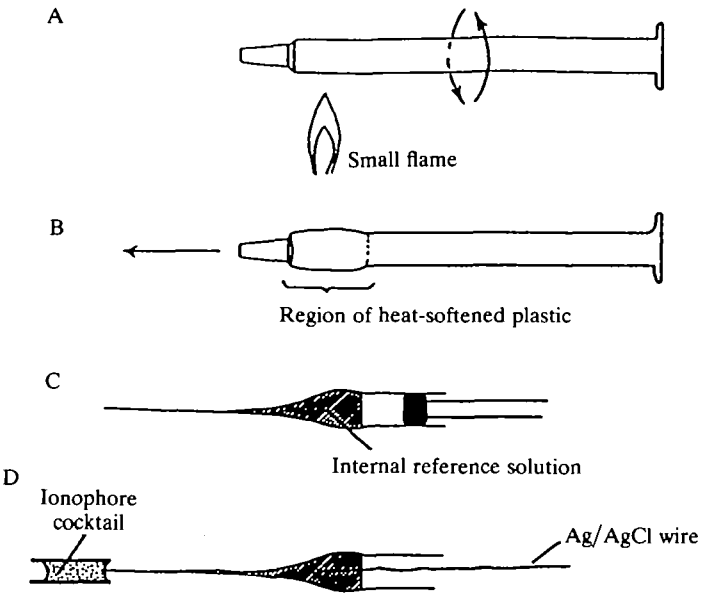

Fig. 1. Steps in the construction of a flexible subminiature ISE. Further details in text. (A) A disposable plastic syringe is rotated (arrows) as it is heated over a low flame. (B) The heated region bulges slightly and becomes more translucent when the plastic is near its melting point. For double-barrelled pipettes, the melted regions of two identical syringes are pushed together so that they fuse. Single or fused double syringes are then drawn out (arrow) by holding vertically and pulling in the direction of the arrow. (C) Internal filling solution is forced to the tip of the fine tubing, which is of nearly constant diameter over lengths up to 50 cm. (D) The tip of the tubing is placed into a glass capillary tube containing ionophore cocktail, and allowed to fill by capillarity until a 1–2 mm long column is present. A chloride-coated silver wire is then introduced into the back of the internal reference solution.

(Fig. 1B). The procedure to this point is that described previously for producing a disposable syringe with integral plastic needle for use in backfilling glass micropipettes (see Fig. 10 of Thomas, 1978). During or after cooling, the fine tube can be pulled out further by hand to a diameter as small as 40–50 μm and cut to length with a razor blade. The barrel is then cut to within about 1 cm of the top of the shank. About 0.2 ml of an appropriate internal reference solution is injected into the back of the syringe, and forced to the tip of the tubing by the application of pressure either by mouth or by re-inserting the plunger and pressing lightly (Fig. 1C). Under a dissecting microscope, the tip is then inserted into a fine glass capillary tube (Fig. 1D) containing about 1 μl of the appropriate ionophore cocktail (Table 1). The cocktail can be used as supplied if the electrodes are to be used within 1 week. Modifications to provide electrodes with longer lifetimes are described below. After a column of about 1–2 mm (approximately 20 nl) of ionophore cocktail has entered the tip by capillarity, the tubing is retracted from the glass tube. Silanization is not required because the syringe tubing is

Table 1. *Selectivity factors ($\log K_{ij}$) for flexible, subminiature ion-selective electrodes*[a]

Primary ion (i)	H^+	H^+	Ca^{2+}	Ca^{2+}	Na^+	K^+	NH_4^+
Ionophore	TDDA[b]	ETH 1907[c]	ETH 129[d]	ETH 1001[e]	ETH 157[f]	Val[g]	Nonactin/monactin[h,k]
Interfering ion (j)							
H^+	0	0	−7.9 (5.0)	−5.7 (5.0)	–	–	−3.9 [−3.8,−2.2]
Li^+	–	–	−8.5	−4.9	−2.0	−5.1	−4.5 [−3.6,−3.6]
Na^+	<−8.0 (−8.7)	<−9.0 (−8.7)	−6.9 (−4.2)	−5.0 (−4.2)	0	−3.9 (−3.6)	−3.0 [−2.9,−2.0]
K^+	<−8.5 (−7.5)	<−8.7 (−7.5)	−12.3 (−4.0)	−6.4 (−4.0)	−0.5 (−0.9)	0	−0.9 [−0.8,−0.6]
Ca^{2+}	–	–	0	0	−0.9 (−0.02)	−4.2 (−1.6)	−4.1 [−4.8,−4.2]
Mg^{2+}	–	–	−10.4 (−2.3)	−6.0	−3.1 (−0.02)	−3.7 (−1.6)	−3.9 [−5.5,−4.4]
Cho[l]	–	–	–	−8.0	−2.8	−3.2	−4.0
Tris[m]	–	–	–	−8.7	–	−3.0	−4.0
TEA^+[n]	–	–	–	–	–	−2.7	–

[a] The primary ions sensed by the ionophores (b–k) are listed horizontally, while selectivity factors for various interfering ions are listed vertically.

Details of the individual ionophore cocktails are given in the references listed below.

Selectivity factors for Ca^{2+}, K^+, Na^+ and NH_4^+ were determined by the separate solution method (Ammann, 1986) using 0.1 mol l^{-1} solutions of the chloride salts. Selectivity factors for H^+ were determined with the fixed interference method (Ammann, 1986) in solutions of 0.01 mol^{-1} Tris(hydroxymethyl)aminomethane and 1 mol l^{-1} NaCl or KCl over the pH range 6–9.

Required selectivity factors for a maximal interference of 1 % by other cations at concentrations given in the text are given in parentheses.

For NH_4^+, the first and second values in the square brackets are selectivity factors for a macroscopic solvent polymeric membrane electrode (Ammann *et al.* 1983) and microelectrode (Buhrer *et al.* 1988), respectively.

[b] Tridodecylamine; pH range 5.5–12; Ammann *et al.* (1981).

[c] 4-Nonadecylpyridine; pH range 2–9; Chao *et al.* (1988).

[d] Schefer *et al.* (1986).

[e] Lanter *et al.* (1982).

[f] Ammann and Anker (1985). Low Ca^{2+} interference, but K^+ interference of 2.5 % in 10 mmol l^{-1} K^+, 135 mmol l^{-1} Na^+.

[g] Valinomycin; Oehme and Simon (1976); low sensitivity to quaternary ammonium compounds such as TEA^+. Corning ion exchanger 477317 (Walker, 1971), an alternative sensor, is more selective for TEA^+ than for K^+.

[h] Ammann *et al.* (1983).

[k] Buhrer *et al.* (1988).

[l] Choline.

[m] Tris(hydroxymethyl)aminomethane.

[n] Tetraethylammonium.

hydrophobic. Electrodes can be maintained in physiological saline or internal reference solution until use. With practice, the time of fabrication and filling can be reduced to less than 3 min per electrode.

The following ionophore cocktails were obtained from Fluka Chemical Corp., Ronkonkoma, NY: (1) Ca^{2+} ionophore I (ETH 1001), cocktail B; (2) Ca^{2+} ionophore II (ETH 129), cocktail A; (3) H^+ ionophore I (tridodecyl amine, TDDA), cocktail B; (4) H^+ ionophore II (ETH 1907), cocktail A; (5) K^+ ionophore I (valinomycin), cocktail A; (6) Na^+ ionophore II (ETH 157), cocktail A. For NH_4^+ electrodes, a cocktail was made by mixing 10 % NH_4^+ ionophore I (75 % nonactin, 25 % monactin) with 89 % 2-nitrophenyl octyl ether and 1 % sodium tetraphenylborate. Internal reference solutions for each ion (in parentheses) were as follows: (Na^+), 0.5 mol l^{-1} NaCl; (K^+), 0.5 mol l^{-1} KCl; (H^+), 0.1 mol l^{-1} NaCl and 0.1 mol l^{-1} sodium citrate, adjusted to pH 6 (Thomas, 1978); (Ca^{2+}), 0.2 mol l^{-1} KCl, 5 mmol l^{-1} $CaCl_2$ and 10 mmol l^{-1} EGTA, yielding pCa 7 (Alvarez-Leefmans *et al.* 1981).

Recording from the subminiature ISE was accomplished by placing silver chloride-coated silver wires into the internal reference solution and connecting the wires to an operational amplifier. Noise due to electrical interference was reduced by filtering the amplifier output through a low-pass RC filter with a time constant of 1 s. Electrode response times were of the order of 2–4 s. The resistance of the subminiature ISEs varied from 1×10^8–$5\times10^8\,\Omega$, comparable to or less than the $10^9\,\Omega$ typical for solvent polymeric electrodes and the 10^9–$10^{11}\,\Omega$ of ISMEs. The potentials generated by the subminiature ISEs, therefore, can accurately be measured by operational amplifiers with input impedances of $10^{11}\,\Omega$ or more. These are typically used for recording from standard glass microelectrodes filled with 3 mol l^{-1} KCl, and are cheaper and more readily available than the high-impedance ($>10^{14}\,\Omega$) operational amplifiers typically used for ISMEs.

Ionophore cocktail tended to leak out of the tip of the electrode if use involved inadvertent contact between the tip and a hydrophobic surface such as an acrylic or polyvinyl chloride experimental chamber. This problem was eliminated by filling the tip with a mixture of one part of an ionophore cocktail with two parts of 15 % (w/v) polyvinyl chloride in tetrahydrofuran (Fluka). Evaporation of the solvent resulted in a tough gel-like membrane that was not easily displaced or dislodged by contact of the tip with hydrophobic surfaces. Additional advantages were that the lifetime of electrodes stabilized with PVC exceeded 4 weeks, and that hydrostatic pressures within very long (>50 cm) electrodes did not force out the sensor, as sometimes occurred in the absence of PVC. Electrode resistance and response times were not noticeably altered by the addition of PVC.

For reference electrodes, plastic syringes were heated and pulled as for ISEs and were backfilled either with 3 mol l^{-1} KCl (for pH, Na^+, Ca^{2+}) or with NaCl (for K^+ measurements) in 3 % agar. Double-barrelled subminiature ISEs were fabricated by heating two plastic syringes, as described above, then joining the barrels while the plastic was near its melting point. The fused barrels could then be pulled out to the desired total diameter (80–100 μm), as for single-barrelled ISEs,

and one barrel used for a reference while the other was used for measurement of ion activity.

Typical slopes per decade change in ion activity were 56–58 mV for H^+, 52–55 mV for Na^+ and K^+, 50 mV for NH_4^+ and 27–29 mV for Ca^{2+}. Selectivity of an electrode for an interfering ion relative to the primary ion sensed by the ionophore is described by selectivity factors (K_{ij}), usually given as $\log K_{ij}$, where 'i' refers to the primary ion and 'j' to the interfering ion. A value of −2, for example, indicates that the electrode is 100 times more selective for the primary ion, i, than for the interfering ion, j. Measured selectivity factors of subminiature ISEs are summarized in Table 1, together with the calculated selectivity factors required for a maximal interference of 1 % ($K_{ij,max}$). The latter were calculated for a representative extracellular fluid containing 135–150 mmol l^{-1} Na^+, 3.5–10 mmol l^{-1} K^+, 0.45–2 mmol l^{-1} ionized Mg^{2+} and 1–2 mmol l^{-1} ionized Ca^{2+}, at a pH of 6.5–7.5. Required selectivity factors were calculated from the equation of Oesch *et al.* (1986):

$$K_{ij,max} = \frac{a_{i,min}}{(a_{j,max})^{Z_i/Z_j}} \cdot \frac{p_{ij}}{100},$$

where $K_{ij,max}$ is the highest tolerable value of the selectivity factor, $a_{i,min}$ is the lowest expected activity of the primary ion i, $a_{j,max}$ is the highest expected activity of the interfering ion j, p_{ij} is the highest tolerable error in measured activity of i due to interference by j, and Z_i, Z_j are the valencies of i and j, respectively. Providing that $\log K_{i,j}$ is more negative than $\log K_{ij,max}$, the selectivity is sufficient to measure the primary ion with less than 1 % interference. Table 1 shows that with currently available ionophore cocktails, subminiature ISEs are suitable for measurement of H^+, Ca^{2+} and K^+ in typical extracellular fluids. For 135 mmol l^{-1} Na^+, 10 mmol l^{-1} K^+ results in 2.5 % interference. However, in fluids in which the ratio a_K/a_{Na} is less than 0.03, the interference of K^+ on the response of subminiature Na^+-selective electrodes is less than 1 %.

Flexible subminiature ISEs may be suitable for recording near or on mucosal surfaces, such as gills, bronchial passages or the oesophagus. Levels of pH and NH_4^+ in the micro-environment near the gills of trout, for example, are different from those in the bulk water (Playle and Wood, 1989), and such local ion gradients might be detectable using flexible subminiature ISEs. An example of measurement of the surface pH of moistened filter paper is shown in Fig. 2A. The flexible double-barrelled electrode was advanced until slight bending of the tubing indicated contact with the surface of the filter paper. This type of ISE may also be useful for the measurement of bathing fluid pH or ion activity in small experimental chambers used for *in vitro* perfusion, particularly if holes or passageways, which need not be straight, are milled in the chamber. They can also be used for analysis of nanolitre droplets of biological fluids collected in micropipettes and expelled under paraffin oil.

A major advantage of both single- and double-barrelled subminiature ISEs is

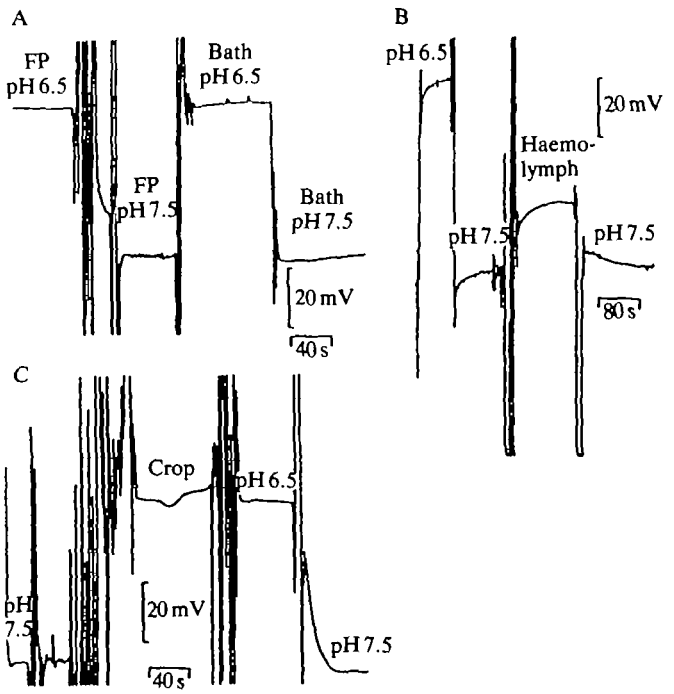

Fig. 2. Representative recordings obtained with flexible, double-barrelled subminiature pH electrodes based on the ionophore ETH 1907. A different electrode was used for each recording. (A) The electrode tip was pressed against filter paper (FP) moistened with insect saline (O'Donnell, 1985) adjusted to pH 6.5, then transferred through the air to a second piece moistened with saline at pH 7.5. The recording went off-scale during the transfer (through air) between filter papers. For comparison, recordings when the electrode was dipped into baths (Petri dishes) filled with the same salines (bath pH 6.5 and 7.5) are shown. (B) Recording of haemolymph pH from an adult male locust, *Locusta migratoria*. The electrode was inserted through a 26 gauge needle, which pierced the arthrodial membranes of the ventral surface of the abdomen. The electrode was calibrated in pH 6.5 and 7.5 solutions before and pH 7.5 solution after haemolymph pH measurement. (C) Recording of crop pH of an adult male locust. The digestive tract was dissected and pinned out in a dish of saline. The double-barrelled pH electrode was transferred through the air from a dish of pH 7.5 saline and introduced into the crop through a 2 cm length of fire-polished glass pipette (1.9 mm o.d., 0.6 mm i.d.), 2–3 mm of which was pushed into the cut end of the oesophagus. The pipette was then withdrawn. Small changes in crop pH were coincident with muscular contractions and resulting movements of crop contents. After withdrawal from the crop, the electrode was calibrated in saline at pH 6.5 and 7.5.

that they can be inserted into a blood vessel or other tissue compartment through syringe needles as small as 26 gauge (Fig. 2B) or through a fire-polished glass capillary tube (Fig. 2C). The syringe needle or glass capillary can be withdrawn after the subminiature ISE and reference electrode are in place. The flexibility of the subminiature ISE not only prevents damage to the electrode itself but also limits damage to the preparation, for example to the gut wall during peristalsis.

Although suitable for haemolymph pH measurements in insects over short periods (5–10 min), longer-term use resulted in blockage of the ISE tip by haemocyte clotting. Electrodes blocked in this way could be restored by simply cutting off about 0.2 mm of the tip with a razor blade, thereby exposing a fresh surface of the ionophore cocktail.

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