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A simplified method for silanization of doublebarrelled ion-sensitive microelectrodes

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Summary. A simplified procedure is described for silanization of one shank of a doublebarrelled microelectrode. this procedure makes it possible to make more functioning electrodes in a shorter time.
Key words. Microelectrode, ion-sensitive; silanization.

For recording the intracellular ion activity with ion-selective microelectrodes it is necessary to make one shank of the doublebarrelled microelectrode hydrophobic. For this reason the hydrophile groups of the inner glass surface must be masked by silane compounds. This procedure ensures that the ion exchanger or related compound remains in the tip during the experiment. But the treatment of the electrode with silanes dissolved in organic solvents is a very delicate process, and after baking the tip of the electrode is often blocked by solid plugs of polymerized silanes[1–8].

An alternative treatment is to handle the ion-selective shank with vapor of silane instead of dissolved compounds[9]. But this method is tedious, for one shank of the doublebarrelled electrode must be perfused with N_2 while the other is perfused with silane and then heated within the same apparatus[9,10]. Therefore a quicker and simpler method is suggested which allows the production of more functioning electrodes in a shorter time, according to the following steps:

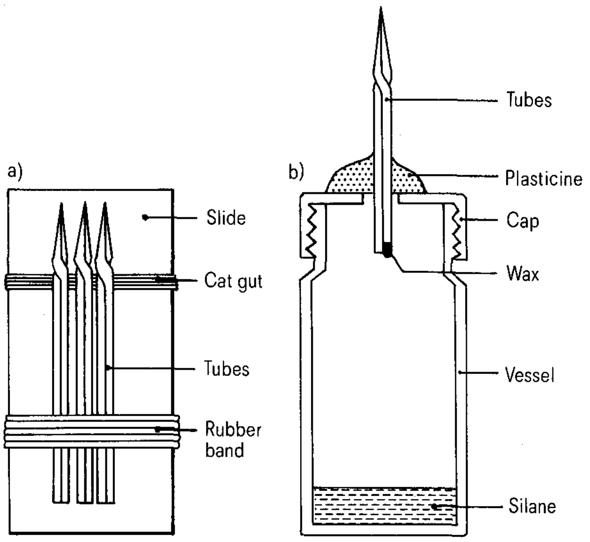
1. Use doublebarrelled tubes with inner filament only. Twist them in the middle (180–200°) and pull electrodes with a resistance of about 20 MΩ when they are filled with 3 M KCl.
2. Store the electrodes in a dry atmosphere. The inner surface is then free of water vapor.
3. One stem is blocked with dental wax (Deiberit 502, Ludwig Böhme KG, D–3423 Bad Sachsa).
4. Insert the doublebarrelled electrode into a perforated cap of a closeable vessel such as a scintillation vial, and seal it with plasticine (fig.).
5. The bottom of the scintillation vessel is covered with a silane, which has a high vapor pressure (i.e., di-methyl-di-chlor-silane; Sigma Chemie GmbH, D–8028 Taufkirchen)[11].
6. Screw the cap on to the vessel and expose the tube's free stem for 50–60 sec to the vapor of the silane (total length of the capillary about 5 cm)[12]. This procedure masks the hydrophilic groups of the inner surface[12].
7. Break off the waxed end of the capillary and be sure that both wax and plasticine are completely removed! Fire polish the broken ends to prevent damages to the Ag/AgCl electrode.
8. Bake the tubes (tip down) for about 1 h at 120–140 °C.
9. Fill them from the stem with the different solutions. First the reference stem then the ion-selective stem. If the solution does not run into the tip use a cat's whisker to push it slightly down. The whiskers are also suitable for eliminating air bubbles.

The filled electrodes can be stored for some days in a test solution. The transfer of the electrodes between the different steps should be done on microscope slides which have a piece of cat gut wrapped round them at one end and rubber bands at the other (fig.). The cat gut protects the tips from damage, and the rubber bands fix the capillaries. The microscope slides with the tubes are stored in beakers with silica gel on the bottom to keep the atmosphere dry.

The electrodes produced in this way should have a resistance of 20–30 M Ω on the reference side. If a K$^+$-exchanger of the type Corning 477317 is used they will then have a resistance of 2000–5000 M Ω on the ion-selective side.

Using amplifiers of 10^{12} M Ω input resistance, there will be enough resistance to ensure a correct recording on the ion-selective channel. The slope of the ion-selective stem is usually about 50 mV/decade using the K$^+$-exchanger from Corning. The ideal value of 58 mV is only reached sometimes.

Such electrodes were used very successfully to measure the intracellular K$^+$-activity of Malpighian tubules of *Drosophila hydei*[13].



a Object slide for transfer handling; *b* scintillation vessel for silanization.

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11 Caution; flammable and very toxic; avoid contact with skin and do not breathe fumes. Therefore use a fume-cupboard and gloves.
12 Work in a warm place to prevent condensation of silane vapor in the tip (e.g. use an infra-red lamp).
13 Rönnau, K.C., Verh. dt. zool. Ges. (1983).

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An apparatus for the calibration of electromagnetic flowprobes on small veins in situ

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Summary. Electromagnetic flowprobe calibration must be done under controlled conditions similar to those encountered experimentally. This in situ calibration apparatus is simple in design, inexpensive, and provides pressure and flow conditions analogous to those found in small veins in vivo.

Key words. Vein, gracilis, dog; dog gracilis vein; blood flowmetry, electromagnetic; calibration in situ.

For cuff-type, non-cannulating electromagnetic blood flowprobes, probe fit (electrode contact), vascular constriction, hematocrit, method of zero baseline determination (occlusive vs non-occlusive zero), flow profile, intraluminal pressure, and vessel conductivity and wall thickness (i.e., vessel type: artery or vein) are all important determinants of probe sensitivity that must be controlled during probe use[2,3]. As such, any calibration system should closely mimic experimental conditions regarding all these factors. Considering this, it has previously been suggested that an in situ apparatus is the best practical way of simulating the in vivo probe environment, and calibration of larger probes on large vessels has been accomplished in this way[4]. However, no previously described in situ method was suitable for calibrating our small flowprobes (1.0–2.0 mm internal diameter) on small veins (1.0–3.0 mm external diameter) with low luminal pressures and low flow rates. We have previously demonstrated that, given an appropriate calibration method, probes of this size are accurate for measuring blood flow rates from 0–30 ml/min[3]. Here, we describe a calibration apparatus that provides constant flows at low luminal pressures in small veins in situ.

Materials and methods. All calibrations were done on in situ gracilis veins in dogs anesthetized with sodium pentobarbital (30 mg/kg). After dissection to clear a suitable length for flowprobe placement, the gracilis vein on one side was cannulated proximally and distally to the probe with polyethylene intravenous catheters (16 gauge or larger). The remainder of the apparatus was then assembled (fig. 1). A constant pressure reservoir from a liquid chromatography column was positioned at the proper height to provide a hydrostatic pressure of 10–20 mm Hg (1.33–2.67 kPa) during flow in the vein. This was monitered using a pressure transducer as shown. Tygon tubing with the largest internal diameter possible (5 mm) was used to allow high flow at low pressure. All glassware and tubing were siliconized to inhibit blood clotting and to improve flow. After an i.v. bolus injection of 4000 units of sodium heparin, 200 ml of blood was withdrawn from the dog at a separate site and used to fill the apparatus as shown. Hematocrit was determined by the standard centrifuge method. Stepwise changes in the flow rate were obtained by adjusting the distal roller clamp. A 1-min timed collection into a graduated cylinder quantified the actual blood flow. Constancy of flow over the