

Bio Currents Research Center Protocol Electrode Construction

Amperometric Electrodes

Introduction

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.

The amperometric microelectrodes are solid-state, with a core of platinum, gold or carbon as the electrolytic surface. The BRC has developed and published the application of amperometric, self-referencing electrodes for the detection of oxygen, nitric oxide, hydrogen peroxide and ascorbate. Oxygen electrodes produced by depositing thin layers of gold on optical fibers are also possible^{Ref 4}. Positional information can also be acquired while measuring oxygen through self-referencing and impedance feedback^{Ref 3}.

Selectivity for amperometric electrodes is usually defined by the conditioning, operating voltage and excluding membranes. The basic body of the electrode is frequently reused with modification in these parameters to detect different chemicals. The version most popularly used within the BioCurrents Research Center has a platinum core based roughly on Whelan's original design for oxygen detection. This core electrode is discussed in depth below. Subsequent sections discuss the adaptation of this design to target different molecules.

1. Making an Oxygen Electrode

Platinum wire, 25 μm in diameter, (1.5 cm length: Johnson Matthey, Ward Hill, MA) is straighten by rubbing with cardboard against a flat surface. These wires are then glued into 27 gauge hypodermic tubes (5 cm long: Small Parts, Inc., Miami Lakes, FL) with silver epoxy (Epoxy Technology, Inc., Billerica, MA) so that the Pt wire extends ≈ 1 cm from the tip. The wire is then electrochemically etched in an aqueous solution of 3 M KCN and 1 M NaOH by applying square waves of 4.0 V amplitude and a 4 ms period (pulse generator model 114 Tektronix, Inc., Portland, OR) until the tips are reduced to a fine point of < 1 μm . The etched Pt wires are rinsed with water and isopropyl alcohol and inserted into borosilicate glass capillaries (1B150, WPI) pulled to outer tip diameters of ≈ 3 μm using a Sutter P-97, so that the wire protrudes slightly from the pipette tip. The electrodes are dipped into epoxy (Epon 828 with m-phenylenediamine curing agent; Miller-Stephenson, Danbury, CT) and heated to 90 ± 6 °C on a hot plate. The electrodes dry overnight. At this stage the epoxy has sealed the Pt to the glass. A thin layer of epoxy probably coats the Pt protruding from the glass. Briefly dipping into acetone cleans the Pt. The epoxy is then cured by baking at 100 °C for 2 h and 150 °C for 2 h. The exposed Pt electrodes are etched again (same conditions as above) to form a recessed electrode with a cavity 2-3 μm deep. This etching takes no more than 5 min and can be monitored by examining the electrode under a microscope every 60 sec. Finally, the electrodes are coated by dipping in a solution of 10% cellulose acetate (30 kDa: w/v in tetrahydrofuran, THF) for 60 s and drying for 10 min. In a more recent paper THF is replaced by acetone. A machined pin (Allied Electronics: part no. 900-0510) is attached to the tubing and inserted into a BNC connector with its pin removed. The finished assembly is coupled to the headstage (Fig.1).

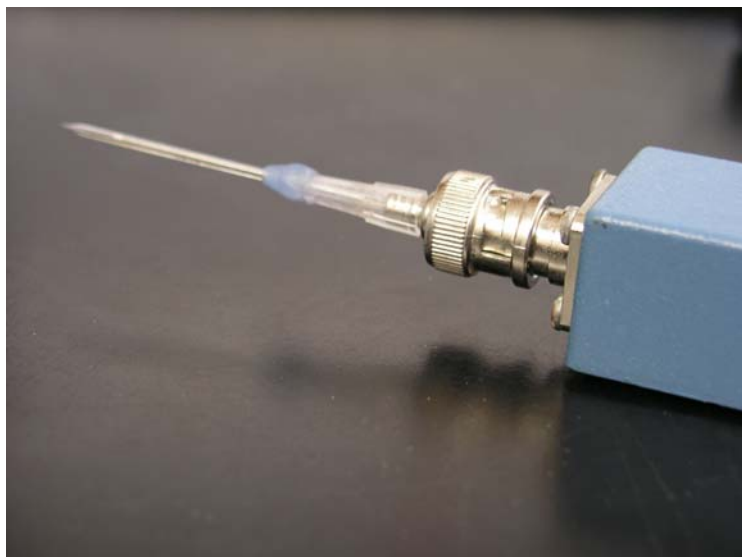


Figure 1: The final construction of the oxygen electrode attached to the BRC amperometric headstage via a modified BNC. A small piece of dental wax (blue) seals the glass to a trimmed pipette tip providing good stability.

Completing the circuit

A Ag/AgCl reference electrode, connected via a 3 M KCl/5% agar bridge and placed in the bulk solution, completes the circuit.

Polarizing the electrode

For oxygen detection the electrodes are polarized to -0.6 V. Electrodes should be calibrated before and after each experiment by making measurements in nitrogen-bubbled (2-5 pA dark current) and normal air saturated salines (70 pA: $210 \mu\text{mol.l}^{-1}$). When polarized incrementally from 0 to -1.0 the output in air-saturated saline should demonstrate a plateau between -0.6 and -0.8 V at ≈ 70 pA. Oxygen electrodes have a rapid response time (Table 1) and are normally used at a modulation frequency of 0.5 – 0.3 Hz over 10 – 20 μm . These parameters are, however, case specific. Land et al. (1999) present a measured versus a semi-empirically derived gradient. ^(see Ref. 1)

2. Making an Hydrogen Peroxide Electrode

H_2O_2 microelectrodes are prepared as described above for oxygen sensors. The final tip diameter of the electrode is about 3 μm . The microelectrode is polarized to +0.6 V against a Ag/AgCl reference electrode with a sensitivity of 0.85 ± 0.12 pA/ μM (mean \pm SD, $n = 4$). Although the electrode can potentially detect other reactive oxygen intermediates beside H_2O_2 (such as nitric oxide and O_2^-), in the BRC applications to date H_2O_2 is predicted to be the major component of the concentration gradient.

3. Making an Ascorbate Electrode

This electrode design introduces the second major body design used at the BRC – a carbon fiber core. This involves sealing a 5 μm carbon fiber (Amoco, Greenville, SC) in a glass microcapillary. Heating and pulling the capillary draws it down to make contact with the fiber. The fiber is stabilized and sealed in the pulled glass pipette using epoxylite (Epoxylite Corp., Westerville, OH). After the epoxylite is cured in an oven at 110°C for 5-10 hours, the electrode is backfilled with a graphite/epoxy paste (PX-grade Graphpoxy, Dylon Industries, Cleveland, OH). A copper wire can then be inserted to make electrical contact with the carbon fiber through the paste. The paste is cured (110°C for 5-10 h), and the excess carbon fiber chopped with a scalpel and beveled to 30° using a Narishige Model EG-44. This generates a comparatively large reactive surface area of $\approx 30 \mu\text{m}^2$. The electrodes are then electrochemically treated, based on previous studies. A 70 Hz triangle wave that cycles between +3.0 V and +1.5 V (7 sec) is followed by a constant potential of +1.5V (7 sec), then held at 0.0V for 30 min. The electrochemical treatment decreases the polarization voltage required for oxidation of ascorbic acid down to the 0.15 to 0.3V range and delivers a reasonable selectivity for ascorbate under the correct conditions. Experimentally the ascorbate microelectrodes have an applied voltage of 0.3V, conferring good selectivity for ascorbic acid, and are oscillated over a distance of 30 μm at a frequency of 0.3 Hz.

4. Making a Nitric Oxide Electrode

The construction of the electrode body is the same as for the ascorbate electrode described above.

To impart selectivity for the oxidation of NO, the carbon fibers are treated with Nafion and OPD. Nafion is a polysulfonated Teflon carrying an intrinsic negative charge that repels electrochemically active anions (e.g., nitrate, nitrite, and ascorbate). OPD is an electrochemically active material imparting a degree of selectivity for NO by size exclusion of non-charged interferents, such as catecholamines. The exposed carbon fiber surface is first coated with Nafion (5% in aliphatic alcohols, Aldrich cat #27,470-4) and dried at 110°C for 5-10 min. After two additional Nafion coatings, the fiber is plated using a 5 mM OPD plating solution containing 0.1 mM ascorbic acid in 100 mM PBS (pH 7.4). The OPD, prepared fresh for each use, is plated on the fiber at a constant +0.9V until a stable current is obtained.

The modified carbon fiber electrodes have final tip diameters of 7-8 μm , but after beveling have a reactive surface area of approximately 30 - 40 μm^2 , and are operated at +0.90 V vs a Ag/AgCl return electrode, which completes the circuit in solution via a 3 M KCl/5% agar bridge. While the sensitivity of the NO electrodes for sodium nitrite is 1.43 ± 0.16 pA/mM, the sensitivity for NO is 730 ± 0.11 pA/mM. Therefore, the NO electrodes are ≈ 500 times more sensitive to NO than nitrite.

NO electrodes can be calibrated with a standard 2 mM NO solution prepared by bubbling HEPES buffered saline with argon gas for 30 min and then compressed NO for 40 min in a fume hood. The saturation concentration of NO in aqueous media at 22°C is 2mM (see Porterfield et al., 2001). Porterfield et al. present a comparison of the modeled versus measured NO gradient generated using the NO donor S-nitroso-N-acetylpenicillamine (SNAP). The modeling equation includes a correction for the $\frac{1}{2}$ life of NO in an aqueous solution. The electrode is operated at 0.3 Hz over a translational distance of 10 μm .

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

Electrode	Column length	Surface area	Medium	Response times ($t_{90}\%$ msec) to different partial pressures.	
				Air – N ₂ saturated	N ₂ – Air saturated
O ₂	n.a.	2 μm^2	4	17.95 \pm 5.26	17.03 \pm 5.75
NO	n.a.	30 –40 μm^2	4	49.63 \pm 13.21	61.00 \pm 14.33

Table 1. Characteristics and response times determined for two amperometric microsensors. All tip reactive areas are approximate.

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

Literature Cited:

- Osourn, D., Sanger, R.H. and Smith P.J.S. (2005) Determination of single cell oxygen consumption with impedance feedback for control of sample-probe separation. *Anal. Chem.* 77, 6999-7004.
- Smith P.J.S., Haydon P.G., Hengstenberg, A. and Jung, S.K. (2001) Analysis of cellular boundary layers and their modulation by plasma membrane transporters: Application of electrochemical microsensors. *Electrochimica Acta* 47, 283-292.