

## Bio Currents Research Center Tutorial

### Amperometric Ion Selective Microelectrodes

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.*

Amperometric electrodes have also been used in the self-referencing mode, taking advantage of the features discussed in the tutorial for potentiometric electrodes. To date all of the amperometric microelectrodes have been used in a constant potential mode. The electrode is clamped at a specific holding voltage to oxidize or reduce the analyte. This type of design and self-referencing application is termed SERP. Electrodes are built so that measurement of the analyte is limited by diffusion of the analyte itself, attempting to ensure a current that is dependent on the analyte concentration and not the concentration of a secondary factor or byproduct. The current generated by this redox reaction is, therefore, proportional to the concentration of the analyte. In the special case of enzyme-assisted electrodes (SERE) using glucose oxidase to measure a concentration difference of glucose, a by-product of the enzyme-substrate reaction,  $H_2O_2$ , is measured.<sup>3</sup>

For an ideal electrode under constant holding potential the measured current is proportional to the concentration of analyte. During self-referencing the differential current can be used to give a measurement of the differential analyte concentration:

$$I_1 - I_2 = (I_o + S \cdot C)_1 - (I_o + S \cdot C)_2$$

$$I_1 - I_2 = S(C_1 - C_2)$$

$$\Delta C = \frac{\Delta I}{S}$$

$I_1$  and  $C_1$  are the current and concentration of the analyte in the near pole of excursion. The subscript 2 has been used to identify the same parameters at the far pole of excursion.  $S$  is the slope of the calibration curve. Unlike potentiometric ISMs, knowing the background concentration of the analyte is not necessary to determine the differential concentration, due to the linearity of the current-concentration relationship.

### **Types of Amperometric Microelectrodes**

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, and enzyme-assisted, self-referencing electrodes for the detection of oxygen, nitric oxide, hydrogen peroxide, ascorbate, glucose and glutamate.<sup>3-9</sup> Mancuso et al. independently published this approach for oxygen detection.<sup>10</sup> Oxygen electrodes produced by depositing thin layers of gold on optical fibers are also possible.<sup>11</sup>

## Literature Cited:

3. Jung, S.-K., Trimarchi, J.R., Sanger, R.H. and Smith, P.J.S (2001) Development and application of a self-referencing glucose microsensor for the measurement of glucose consumption by pancreatic b-cells. *Anal. Chem.*, 73, 3759-3767.
4. Land, S.C., Porterfield, D.M., Sanger, R.H. and Smith, P.J.S. (1999) The self-referencing oxygen-selective microelectrode: Detection of transmembrane oxygen flux from single cells, *J. exp. Biol.* 202, 211-218.
5. Porterfield, D.M., Laskin, J.D., Jung, S.-K., Malchow, R.P., Billack, B., Smith, P.J.S., and Heck, D.E. (2001) Proteins and lipids define the diffusional field of nitric oxide. Measurement of nitric oxide fluxes from macrophages using a self-referencing electrode, *Am. J. Physiol.*, 281, L904-912.
6. Twig, G., Jung, S.-K., Messerli, M., Smith, P.J.S. and Shirihai, O (2001) Real-time detection of reactive oxygen intermediates from single microglial cells, *Biol. Bull.*, 201, 261-262.
7. Twig, G., Graf, S.A., Messerli, M.A., Smith, P.J.S., Yoo, S.H., and Shirihai, O.S. (2005), Synergistic amplification of beta-amyloid and interferon-gamma-induced microglial neurotoxic response by the senile plaque component chromogranin A, *Am. J. Physiol.*, 288, C169-C175.
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