

Bio Currents Research Center Protocol

Correction for Analyte Buffering

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.*

There are a number of circumstances that cause the calculated flux to be smaller than measured. In the first example discussed here, extracellular buffers can collapse gradients of free analytes. This has been addressed for the collapsing of H⁺ gradients by H⁺ buffers^{3,4}. The analyte can diffuse from the surface of the cell in either its free state or bound to the buffer. ISEs only measure the free concentration of the analyte. The actual H⁺ flux from a source is the sum of the measured free H⁺ flux and the unmeasured H⁺ flux moving as H⁺ bound to buffer.

$$J_{Htotal} = J_{Hmeasured} + J_{HB}$$

Knowing the conditions under which the H⁺ flux was measured i.e. [H⁺] of medium, Ka of the buffers and concentration of buffers present, a simple relationship can be derived to determine the ratio of H⁺ diffusing as bound to buffer compared to the freely diffusing H⁺. Demarest and Morgan (1995) and Arif et al. (1995) have derived two separate sets of equations that can be simplified to the same equation given below.

$$x_i = \frac{D_B}{D_{H^+}} \cdot [B] \cdot \frac{K_a}{(K_a + [H^+])^2}$$

The correction factor, 'xi', is the ratio of the H⁺ bound buffer flux to the free H⁺ flux. Therefore

$$J_{Htotal} = J_{Hmeasured} \cdot (1 + x_i + \dots + x_n)$$

where a number of different H⁺ buffers (xi + ... + xn) could be carrying H⁺ away from the source. The correction factor is based on 3 criteria, the ratio of the diffusion coefficients of the protonated buffer to the proton, the buffer concentration and the Ka of the buffer compared to the [H⁺] of the medium. Under most conditions the first term will be relatively constant because buffer sizes and diffusion coefficients do not differ very much. The smaller bicarbonate ion, for example, (M_r 61, D = 1.2 * 10⁻⁵ cm/s) will only produce a first term of 0.13 while PIPES (M_r 302 D ~ 0.52 * 10⁻⁵ cm/s) one of the largest Good buffers, will produce a first term of 0.056, a change of only 2.3 fold. The second term shows that correction factor is directly proportional to the buffer concentration. The last term, the relationship of the Ka of the buffer to the [H⁺], could have the most significant impact on the correction factor.

In the absence of intentionally added H⁺ buffers, water and dissolved carbonates can have a significant buffering effect at neutral to alkaline pH⁴. Water, at a concentration of about 56 M with a Ka of 10⁻¹⁶ begins to impact the correction factor above pH 6. The correction factor due to water acting as a H⁺ buffer contributes to the total flux by less than 1% when the pH = 6. Bicarbonate in solution, due to atmospheric CO₂, will also have a more significant impact at neutral to alkaline pH. Bicarbonate will increase in concentration at more alkaline pH, and can add to the H⁺ flux by about 2 and 21% at pH 7 and 8 respectively.

In order to minimize the effect of buffer on H⁺ efflux a buffer with a Ka below the [H⁺] should be chosen such that most of the buffer will be in its protonated form reducing the effective buffering capacity. As in the former example, investigators can choose buffers to suit their needs, either to remove a very large H⁺ flux that is interfering with measurement of other analytes or to correct for the amount of H⁺ passing through the plasma membrane in order to accurately quantitate such a flux. See Arif et al. (1995) for a mathematical solution to multiple buffers. This correction method is theoretically applicable to other forms of analyte buffering such as for Ca²⁺ or transition metals.

Literature Cited:

3. *Demarest, J.R. and Morgan, J.L.M. (1995). Effect of pH buffers on proton secretion from gastric oxyntic cells measured with vibrating ion-selective microelectrodes. Biol. Bull. 189:219-220.*
4. *Arif, I., Newman, I.A. and Keenlyside, N. (1995). Proton flux measurement from tissues in buffered solution. Plant, Cell and Environ. 18:1319-1324.*