Oroboros O2k-Protocols

Mitochondrial Physiology Network 08.16(09):1-4 (2022)
Version 09: 2022-03-01 ©2003-2022 Oroboros
Updates: http://wiki.oroboros.at/index.php/MiPNet08.16_pH-Calibration

pH measurement and temperature dependence of pH

Gnaiger E

Oroboros Instruments
High-Resolution Respirometry
Schoepfstrasse 18, 6020 Innsbruck, Austria
Email: instruments@oroboros.at
www.oroboros.at

Contents

1. Background

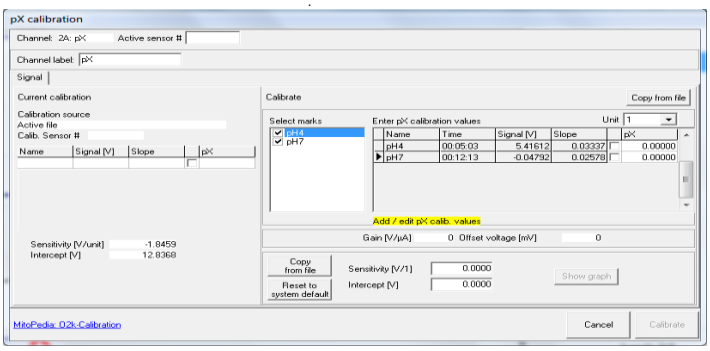
pH of blood and intracellular compartments is tightly regulated. Blood pH at 37 °C is 7.4. However, when temperature is lowered, the pH of blood and of intracellular buffer systems increases, which has been reported as early as 1927 but was ignored for the following 30 years. The phenomenon of a change in blood pH with temperature of about –0.016 pH units/°C was rediscovered by comparative physiologists in studies of “cold blooded” vertebrates (turtles, fish), and soon it was recognized that the same pH/temperature relation applies to “warm blooded” mammals (rat, human). The physicochemical basis of pH/temperature relations and the consequences for acid-base balance and protein function were primarily analyzed by Rahn and Reeves (1979).

Besides the importance for physiological and biochemical systems, the temperature dependence of pH of buffer systems has experimental significance for the measurement of pH at different temperatures, and for the choice of buffer systems when designing experiments at various temperatures.

2. pH measurement

2.1. Instrument manual

Standard instrument manuals give an excellent introduction into all practical aspects of pH measurement in the laboratory. While it should go without saying that the manual must be studied carefully as a basis of measurement, several important aspects are frequently ignored. This may cause inaccuracies and shortens the electrode lifetime unnecessarily.



In DatLab, the two-point calibration calculations are preformed automatically (further information pX calibration - DatLab).

The principle of the two-point calibration is illustrated in Figure 1.

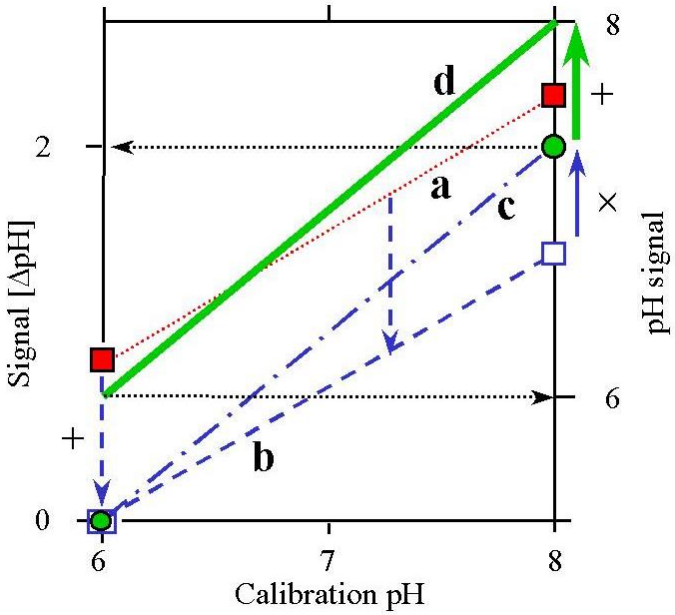


Figure 1. Two-point calibration of a pH electrode system at pH 6.00 and 8.00. (**a**) Signal before calibration (dotted line); (**b**) Calibration at pH 6 at electronic zero (0 mV), using the zero adjustment (+; additive as shown by the downwards arrows of equal length); (**c**) calibration of gain setting (slope) at pH 8, observing the ΔpH of 2.0 units (x; multiplicative or proportional, as shown by the constant position of the 0 mV signal); (**d**) The full line of correspondence is obtained by shifting the entire curve to the correct absolute pH value (+, additive).

2.2. Some specific aspects

1. The following notes do not replace the instrument manual but are a short list of operation instructions which are frequently neglected.
2. Open the electrolyte reservoir of the reference electrode for pressure equilibration during measurement. Close the electrolyte inlet on the glass shaft by the rubber cap only for storage.
3. If possible, use bracketing calibrations in a range closely matching the experimental pH values.
4. Each glass electrode/reference electrode has an electrical zero point. For a two-point calibration, the slope (gain) of the pH electrode system should first be calibrated between electrical zero

and the difference between the two buffer systems (Figure 1; c); second the absolute pH value should be set by adjustment of the additive zero suppression (Figure 1; d).

5. Distinguish three different types of the pH/temperature dependence:
   - Slope of the pH electrode system.
   - pH value of the calibration buffers.
   - pH value of the experimental buffer systems.

3. Temperature dependence of pH

3.1. Calibration buffers

Calibration buffers are designed to have a small temperature dependence of their pH value. The pH values at each temperature are given in manuals. The change in pH over the temperature range of 0 to 40 °C is typically <0.1 units (acid buffers), but may be as large as 1 pH unit for buffers with high pH.

3.2. Water

The pH of pure water is strongly temperature dependent, which is a consequence of the temperature dependence of the dissociation constant of water, K_w (Table 1):

$$pK_w = pH + pOH$$

The dissociation constant of water, as expressed by pK_w, is 14 at 25 °C, and ranges from 14.94 at 0 °C to 13.53 at 40 °C. By definition, water is neutral when the activities of OH^- and H^+ are identical, or pH = pOH. At neutrality, 2 pN = pK_w, or pH = 0.5 pK_w. Therefore, at 25 °C neutrality is obtained at pH 7, but neutrality shifts to a lower pH at higher temperature. Neutrality is obtained at pH 7.47 at 0 °C and at pH 6.77 at 40 °C, when the OH^-:H^+ ratio remains 1.0.

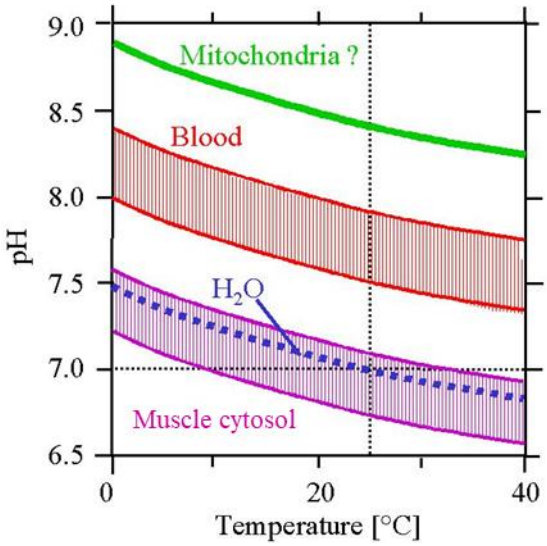
Table 1. Temperature dependence of p*K* and pH in water.

Temperature [°C]	pK_w	pH (neutral)
0	14.94	7.47
25	14.00	7.00
40	13.53	6.77

3.3. Blood and intracellular buffers

pH of blood and intracellular buffers has a strong pH dependence, typically –0.015 to –0.020 pH units/°C, remarkably similar to that of water, with the effect to maintain acid-base balance by pH adjustment when temperature is shifted.

The basis of the pH dependence of physiological buffers is the dominance of the α-imidazole group of histidine and histidyl groups in the cellular and extracellular buffering capacity. The acid dissociation constant pK_a of the histidine α-imidazole group



in the range of 6.0 to 7.3 makes it an efficient buffer at physiological pH. The enthalpy of neutralization of the α-imidazole group and of water is –30 to –34 kJ·mol^{-1} H$^+$ at 0 to 40 °C (Gnaiger 1980; von Stockar et al 1993), directly related to the temperature dependence of pK_a. In contrast, the enthalpy of neutralization of phosphate buffer is low (–4.2 and –3.6 kJ·mol^{-1} H$^+$ at 25 and 37 °C; Gnaiger 1980), with the effect of a nearly constant dissociation constant and constant pH at varying temperature.

Figure 2. The relationship of water, blood, muscle cytosol and mitochondria to body temperature in ectothermic animals. Modified after Somero (1981).

Dipeptides (carnosine) and proteins are the most important physiological buffers, yielding 50 to 100 mM of imidazole moieties (Somero 1981). At 0-40 °C, the temperature coefficient of pH averages –0.0176 pH units/°C in blood and cells. **α-stat regulation** (maintaining the dissociation of imidazole constant over the change in temperature) is important for maintenance of enzyme function and must be clearly distinguished from **pH-regulation**.

The –SH group of cystein (free amino acid or bound in glutathione) has a similar pK_a (8.4 or 8.7) and enthalpy of neutralization as the histinide α-imidazole group. Glutathione at intracellular concentrations up to 10 mM, therefore, contributes to α-stat pH-regulation.

4. References

Gnaiger E (1980) Das kalorische Äquivalent des ATP-Umsatzes im aeroben und anoxischen Metabolismus. Thermochim Acta 40: 195-223. - »Bioblast link«

Reeves RB (1977) The interaction of body temperature and acid-base balance in ectothermic vertebrates. Ann Rev Physiol 39: 559-586.

Reeves RB, Rahn H (1979). Patterns in vertebrate acid-base regulation, evolution of respiratory processes: a comparative approach. Wood SC, Lenfant C (eds) New York, Marcel Dekker, Inc.: 225-252.

Somero GN (1981) pH-temperature interactions on proteins: principles of optimal pH and buffer system design. Marine Biol Lett 2: 163-178.

Von Stockar U, Gustafsson L, Larsson C, Marison I, Tissot P, Gnaiger E (1993) Thermodynamic considerations in constructing energy balances for cellular growth. Biochim Biophys Acta 1183: 221-240. - »Bioblast link«

Full version – *go Bioblast*
» http://wiki.oroboros.at/index.php/MiPNet08.16_pH-Calibration

O2k-Manual: » https://wiki.oroboros.at/index.php/O2k-Manual
» http://wiki.oroboros.at/index.php/O2k-pH_ISE-Module