

## pH and Buffers

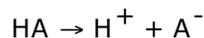
### pH

The negative logarithm of the hydrogen ion concentration, the pH is expressed as follows:

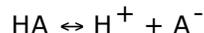
$$\text{pH} = -\log [\text{H}^+]$$

The pH scale is a measure of hydrogen ion concentration that eliminates dealing with large powers of 10 and compresses a large range of concentrations onto a more convenient scale, for aqueous solutions between 1 and 14. At a high concentration of  $\text{H}^+$  ( $10^{-1}$  M), the pH value is low,  $\text{pH} = 1$ , while at low concentration ( $10^{-12}$  M), the pH is high,  $\text{pH} = 12$ .

Hydrogen ions in solution arise from the dissociation of acids. (HA represents undissociated acid,  $\text{H}^+$  hydronium ion, and  $\text{A}^-$  conjugate base.)



Strong acids (or bases) are considered to be completely dissociated into ions in dilute solution. However, weak acids (or bases) are only partially dissociated in solution, and thus equilibrium is established between the ions and the undissociated molecules.



The equilibrium constant  $K_a$ , is defined as follows.

$$K_a = [\text{H}^+][\text{A}^-] / [\text{HA}]$$

From this expression, we can derive the **Henderson-Hasselbach equation**, commonly called the 'buffer equation', which relates the pH of solution to the  $\text{p}K_a$  of the acid and the relative concentration of the undissociated acid and the conjugate base forms.

Solving first for the hydrogen ion concentration:

$$[\text{H}^+] = K_a [\text{HA}] / [\text{A}^-]$$

Converting to the logarithmic form and multiplying by -1, we obtain:

$$-\log [\text{H}^+] = -\log K_a - \log ([\text{HA}]/[\text{A}^-])$$

Defining operator 'p = -log' we have:

$$\text{pH} = \text{p}K_a - \log ([\text{HA}]/[\text{A}^-])$$

Finally, by inverting the  $\log([\text{HA}]/[\text{A}^-])$  term, we obtain:

$$\text{pH} = \text{p}K_a + \log( [\text{A}^-] / [\text{HA}] )$$

or

$$\text{pH} = \text{p}K_a + \log( [\text{conjugate base}] / [\text{acid}] )$$

This is the **Henderson-Hasselbach equation**. It is most useful in the preparation of buffers and in understanding how the concentration of the acid and conjugate base forms of a weak acid affect the pH.

## Buffers

A buffer, by definition, resists changes in the pH of the solution. A buffer must contain the chemical species for "neutralizing" added amounts of acid or base. Generally, a buffer is a solution of a weak acid and its conjugate base or a weak base and its conjugate acid. A buffer is selected on the basis of its  $\text{p}K_a$  value and its chemical nature.

The Henderson-Hasselbach equation gives the relationship between pH,  $\text{p}K_a$ 's and the ratio of the concentration of the salt and acid forms of the buffer. As shown by this equation, when the concentration of the conjugate base and the undissociated acid are equal,  $[\text{conjugate base}] = [\text{acid}]$ , the pH of the solution equals the  $\text{p}K_a$  of the buffer. When  $[\text{conjugate base}] = 10[\text{acid}]$ , then  $\log([\text{conjugate base}] / [\text{acid}]) = \log 10 = 1$  and therefore:

$$\text{pH} = \text{p}K_a + 1$$

When  $[\text{conjugate base}] = 1/10[\text{acid}]$ , then  $\log([\text{conjugate base}] / [\text{acid}]) = \log 1/10 = -1$  and therefore:

$$\text{pH} = \text{p}K_a - 1$$

The buffers are most effective in the range  $\text{pH} = \text{p}K_a \pm 1$ . Outside that range,

the concentration of either the acid or the conjugate base is too small to effectively resist the effect of added hydrogen or hydroxide ion.

Once the desired pH range for an experiment has been decided, one can select a buffer on the basis of pK value. Since temperature affects the dissociation of some weak acids and bases the pK value for buffers is temperature dependent. Therefore, pH of buffers should always be adjusted for the temperature at which they will be used.

Following table lists several common biological buffers.

NAME	STRUCTURE	pK <sub>a</sub> at 20°C	ΔpK <sub>a</sub> (°C)
MES [2-(N-morpholino) ethanesulfonic acid]		6.15	-0.011
PIPES [piperazine-N,N'-bis (2-ethanesulfonic acid)]		6.8	-0.0085
Sodium dihydrogen phosphate	NaH <sub>2</sub> PO <sub>4</sub>	7.21	---
HEPES [N-2-hydroxyethyl piperazine-N'- 2-ethanesulfonic acid]		7.55	-0.014
TRIS [tris(hydroxymethyl) aminoethane] hydrochloride		8.3	-0.031

## Measurement of pH

Since many biochemical reactions are pH dependent, accurate measurement and control of pH is important. Measurement of pH with a pH meter is a simple process, but careful control is necessary to avoid errors.

**pH meters:** A pH meter is a potentiometer used to measure the H<sup>+</sup> concentration in solution. An electric potential is measured that depends on

the voltage difference between a reference electrode, usually calomel, and a glass electrode that is sensitive to  $H^+$  concentration. The glass membrane acts as if it is selectively permeable to  $H^+$  while other cations and anions are excluded. This permeability results in a potential across the membrane that is a linear function of the pH.

$$E = \text{const} + 2.303 (RT/F) \text{ pH}$$

The magnitude of the potential difference is measured with a voltmeter. The constant in the above equation depends on a number of factors and varies from electrode to electrode. Therefore, in the measurement of pH one must standardize the electrode and meter with solutions of known  $H^+$  concentration. The calibration circuit of the pH meter allows the operator to adjust the pH reading of the meter to the pH of the standard buffer solution, thereby eliminating the contribution of the constant from the pH reading. The measured potential is also a function of temperature as indicated in the equation. For accurate pH measurement, the temperature compensation control on the meter must be adjusted to the temperature of the solution being measured.

**pH measurement:** Before a pH meter is used, the electrodes must be rinsed with distilled water and allowed to drain (**not dried**, however). They can be blotted with a paper tissue. The meter should be standardized with two buffers, one with pH below and one with pH above the values to be measured. The temperature of the standard buffers should be measured and the temperature compensation control adjusted to the temperature of the solution. When the meter has been standardized and readings are stable, the pH of the test solution can be measured. Meters with two separate electrodes, the glass and calomel electrodes, are used if the volume of solution is adequate. A combination electrode, in which both the reference electrode and the glass electrode are incorporated into one slim tube is useful for very small volumes. Errors in pH measurement may arise in the presence of high  $Na^+$  concentration, at high ionic strength such as with ammonium sulfate solutions, and with electrodes fouled by proteins or other biopolymers. After use, electrodes should be rinsed with water and stored according to the manufacturer's directions (typically immersed in storage buffers to avoid drying).