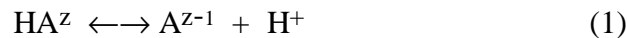


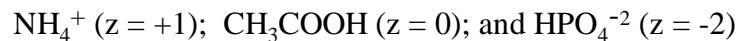
Buffer Capacity, Ionic Strength, and Tables of pK_a

The proper functioning of biological systems require control of pH, since most metabolic processes are inactivated outside a certain narrow range of concentration of hydrogen ions. A buffer is a system containing either a weak acid and its salt or a weak base and its salt, which resists changes in pH upon addition of acid or base.

All pH buffers can be thought of as weak acids in the Brønsted sense. The dissociation of a Brønsted general acid (proton donor) can be represented by the following equation:



where z is the ionic charge on HA (the acid), and A^{z-1} is the conjugate base. Examples of Brønsted acids include:



You will note that ammonium ion (NH_4^+) is considered an acid rather than ammonia (NH_3) as a base since a separate set of equations for treating bases as buffers is then unnecessary.

The dissociation of the general acid (HA^z) to its conjugate base and hydrogen ion is a reversible process. The Law of Mass Action establishes a relationship between the chemical activities of an acid and its dissociation products at equilibrium:

$$K_a = \frac{a_A a_{H^+}}{a_{HA}} \quad (2)$$

where K_a is the dissociation constant and a is the activity of the species indicated by the subscripts. The activity and concentration of a chemical species are generally not equal but converge to the same value as the solution becomes more dilute.

Activities are not convenient units to work with in the laboratory and an analogous equation expressing equilibrium conditions in terms of concentrations of the chemical species can be written:

$$K_a' = \frac{[A]}{[HA]} \cdot a_{H^+} \quad (3)$$

where $[A]$ and $[HA]$ are the molar concentrations of the species indicated and K_a' is the apparent dissociation constant. Hydrogen ions are still expressed as activity, since electrochemical systems (such as the pH meter) exist which directly measure the activity of hydrogen ion. The apparent dissociation constant K_a' (unlike K_a) is not a constant but rather is a function of the concentrations of the various species in the buffer system.

A useful relationship can be obtained from equation 3 by taking the logarithm of appropriate terms:

$$\log K_a' = \log a_{H^+} + \log \frac{[A]}{[HA]} \quad (4)$$

BY DEFINITION: $pH = -\log a_{H^+}$ and $pK_a' = -\log K_a'$

By substitution of these equivalent terms into equation 4 the Henderson-Hasselbalch equation is obtained:

$$pH = pK_a' + \log \frac{[A]}{[HA]} \quad (5)$$

Examination of this equation shows that since K_a' is approximately constant (variation with concentration is small), the pH will depend primarily on the ratio $[A]/[HA]$, but will also show a secondary dependence on the total buffer concentration, $[A]+[HA]$, (due to variation of K_a' with concentration).

BUFFER CAPACITY:

Buffer capacity represents the ability of a buffer to resist changes in pH. An instantaneous buffer capacity can be defined as the negative of the first derivative of the amount of acid added with respect to pH:

$$\text{instantaneous buffer capacity} = \frac{-dH^+}{dpH}$$

from equation 5:

$$dpH = dpK_a' + \frac{1}{2.303} d \ln \frac{[A]}{[HA]} \quad (6)$$

$$\text{HINT: } \frac{d}{dx} \log u = \frac{1}{u} \frac{du}{dx}$$

which becomes:

$$dpH = \left\{ \frac{1}{2.303} \frac{[A]}{[HA]} \right\} \left\{ \frac{d \frac{[A]}{[HA]}}{dH^+} \right\} \{dH^+\} \quad (7)$$

since K_a' is approximately constant, $dpK_a' \approx 0$.

The quantity $\frac{d}{dH^+} \frac{[A]}{[HA]}$ can be expanded to:

$$\frac{d[A]}{dH^+} - \frac{[A]}{[HA]^2} \times \frac{d[HA]}{dH^+}$$

When a mole of acid is added to a buffer it converts a mole of A into HA. Therefore:

$$\frac{d[HA]}{dH^+} = - \frac{d[A]}{dH^+} = 1$$

Substituting these quantities into equation 7 yields:

$$\text{Buffer Capacity} = \frac{-dH^+}{dpH} = 2.303 \cdot \frac{[A][HA]}{[A]+[HA]} \quad (8)$$

NOTE: that at constant buffer concentration the highest buffer capacity is observed when $[A]=[HA]$ (i.e. when $pH = pK_a'$). A second important conclusion from equation (8) is that at a fixed ratio, $[A]/[HA]$, the buffer capacity is proportional to the total buffer concentration.

IONIC STRENGTH:

Generally, it is more important to specify the ionic strength rather than the concentration of a buffer. Ionic strength is defined by:

$$I = \frac{1}{2} \sum_i z_i^2 [x_i] \quad (9)$$

where z_i is the charge on the ion i present at a molar concentration $[X_i]$.

Points to remember in calculating ionic strength:

1. Uncharged species do not contribute to ionic strength.
2. If a solution contains more than one type of salt or buffering species, the ionic strength contributions of each salt must be summed.
3. The calculation of ionic strengths in solutions containing multiply charged ions can be simplified by recognizing that each class of salts has a corresponding integer ratio of ionic strength to molarity (I/M): **This integer relationship holds only if one of the ions has a charge of +1 or -1.**

$$\begin{aligned} \text{for NaCH}_3\text{COO (a 1:1 salt)} \quad \underline{I} &= \frac{1}{2} (1^2[\text{Na}^+] + 1^2[\text{CH}_3\text{COO}^-]) \\ &= \frac{1}{2} (1 [1 \underline{M}] + 1 [1 \underline{M}]) = 1 \underline{M} \end{aligned}$$

$$\text{thus } \underline{I}/\underline{M} = 1$$

$$\begin{aligned} \text{for Na}_2\text{HPO}_4 \text{ (a 2:1 salt)} \quad \underline{I} &= \frac{1}{2} (1^2[\text{Na}^+] + 2^2[\text{HPO}_4^-]) \\ &= \frac{1}{2} (1 [2 \underline{M}] + 4 [\underline{M}]) = 3 \underline{M} \end{aligned}$$

$$\text{thus } \underline{I}/\underline{M} = 3$$

$$\begin{aligned} \text{for Na}_3\text{PO}_4 \text{ (a 3:1 salt)} \quad \underline{I} &= \frac{1}{2} (1^2[\text{Na}^+] + 3^2[\text{PO}_4^-]) \\ &= \frac{1}{2} (1 [3\underline{M}] + 9 [\underline{M}]) = 6 \underline{M} \end{aligned}$$

$$\text{thus } \underline{I}/\underline{M} = 6$$

Theory and experimental results indicate that \underline{I} is a more valid measure, than concentration, of the effects ionic species have on other components of a solution. Ionic species interact with one another and behavior of ionic solutions (even at great dilution) is far from ideal.

IONIC STRENGTH and pK_a' :

Activity and activity coefficients When a solute dissolves in water or another solvent, the crystal structure is destroyed and there is generally an accompanying temperature change. The solute may go into solution as molecules, ion pairs, or as ions. The solute molecules or ions become solvated by interacting with solvent. Such interaction between solute and solvent or between different molecules/ions of solute results in nonideal behavior (i.e. the effective concentration of the solute species is often quite different from its real, known concentration). The effective concentration is called the activity of the species and may be less than, equal to, or greater than the molar or formal concentration of the species. The activity of a species can be related to its concentration by the expression:

$$a_i = \gamma_i C_i \quad (10)$$

where a_i is the activity of substance i , γ_i is the activity coefficient of substance i , and C_i is the molar or formal concentration.

Equation 10 holds for solute species which do not dissociate. If the solute dissociates, however, the expression for activity is more complex. Generally speaking, the activity coefficients for non-ionic solutes are approximately 1 except in concentrated solution. In the case of ionic substances, however, the activity coefficient approaches 1 only in very dilute solution. The differences between concentration and activity arise because of ionic interaction. For electrolytes activity coefficients may easily be as small as 0.1 and deviations from ideal behavior can be significant at concentrations as low as 0.01 \underline{M} .

The Debye-Hückel theory postulates that all deviations from ideal behavior by ionic solutions arise from electrostatic interactions between ions. For example, a positive ion in solution will attract and therefore "see" more negative ions than positive ions. Each ion is thought of as surrounded by an ion atmosphere of opposite charge. When approximations to very dilute solutions are made the Debye-Hückel Limiting Law is obtained:

$$-\ln \gamma_i = \frac{e^3 Z_i^2}{(\epsilon k T)^{3/2}} \left(\frac{2 \pi N \underline{I}}{1000} \right)^{1/2}$$

where:

γ_i = activity coefficient of i

e = electronic charge

Z_i = number of charges on i

ϵ = dielectric constant of medium

k = Boltzman constant = 1.3805×10^{-12} erg deg⁻¹

T = Kelvin temperature

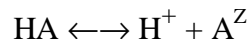
N = Avagadro's number = 6.022×10^{23}

\underline{I} = ionic strength of solution

Substituting values for the constants and converting logarithms to base 10 gives the Debye-Hückel limiting law at 25°C:

$$-\log \gamma_i = 0.509 Z_i^2 \sqrt{\underline{I}}$$

However, because we cannot study ions of a single charge only the mean activity coefficient can be directly measured. Thus for the reaction:



$$\log \gamma_{\pm} = 0.509 \sqrt{\underline{I}} \left(\frac{Z_{\text{H}^+}^2 + Z_{\text{A}^Z}^2}{2} \right)^{1/2} \text{ at } 25^\circ \text{C}$$

The Debye-Hückel equation simply states that the activity coefficient of any ion depends on the ionic strength of the solution. This equation also predicts that K_a' (apparent dissociation constant) will also depend on ionic strength \underline{I} , but not on the nature of the salts contributing to \underline{I} . Thus, $\text{p}K_a'$ will also depend on ionic strength. It is common practice to use $\text{p}K_a$ values when calculating pH and buffer problems instead of $\text{p}K_a'$ values, appropriate to the buffer concentration. The $\text{p}K_a'$ values are known, as a function of buffer concentration, for only a few common buffers, but it is possible to calculate approximate $\text{p}K_a'$ from $\text{p}K_a$ values for a given ionic strength. Use of $\text{p}K_a'$ allows more accurate prediction of buffer pH. The following table gives values for ($\text{p}K_a' - \text{p}K_a$) as a function of ionic strength \underline{I} , and temperature for various values of Z (charge on HA) as defined in equation 1.

Values of (pK_a' - pK_a)

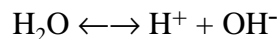
I	Temp.	z = +1	z = 0	z = -1	z = -2
0.01M	0 °C	0.04	-0.04	-0.13	-0.21
	20 °C	0.04	-0.04	-0.13	-0.22
	37 °C	0.05	-0.05	-0.14	-0.23
0.05M	0 °C	0.08	-0.08	-0.24	-0.40
	20 °C	0.08	-0.08	-0.25	-0.42
	37 °C	0.09	-0.09	-0.26	-0.43
0.10M	0 °C	0.10	-0.10	-0.31	-0.51
	20 °C	0.11	-0.11	-0.32	-0.53
	37 °C	0.11	-0.11	-0.33	-0.55

Example: What is the pK_a' for acetic acid at 37°C in a 0.1 I buffer? The pK_a at 37°C is 4.77. At 37°C and 0.1 I and for z = 0 the correction factor, (pK_a' - pK_a), is 0.11. pK_a' = pK_a + (pK_a' - pK_a) = 4.77 + (0.11) = 4.66.

The Debye-Hückel prediction that K_a' depends on ionic strength I, but not on the nature of the salts contributing to I, has been verified experimentally at low ionic strengths. For example, the K_a' of acetic acid is the same function of I (for I ≤ 0.2) regardless of whether the solution contains NaCl, LiCl or BaCl₂. For I ≥ 0.2, K_a' depends on the nature of the salt added.

Solvent Corrections for buffer calculations:

The solvent of interest in most biochemical systems is water, which itself is a weak acid (Brønsted sense a proton donor), and dissociates:



We already have defined

$$K_w' = [\text{H}^+] [\text{OH}^-]$$

At 25°C the numerical value of K_w' is approximately 10⁻¹⁴. For the purposes of buffer calculations pK_w' is assumed to be 14. Normally, water is ignored in setting up the equations to calculate pH of a buffer. However, when the pH is extreme (outside the usual pH range of 3 to 11) or the buffer is sufficiently dilute, the H⁺ and OH⁻ contributed by the dissociation of H₂O have a significant effect on the buffer equilibrium, and their effects on the pH must be taken into account. If neither H⁺ nor OH⁻ appear explicitly in the dissociation reactions of buffers, a reaction of the

buffer species with water should be written to show, for the purposes of calculation, the origin of the H^+ and OH^- ions.

When titrations are carried out to the pH extremes, a significant amount of titrant is consumed in changing the pH of the solvent (H_2O). In these instances corrections are made by titrating an equal volume of water alone (solvent blank correction).

pK_a FOR BUFFERS

Buffer	z	0 °C	20 °C	37 °C
Oxalic acid	0	----	1.27 (25 °C)	----
EDTA	0	----	1.7	----
Maleic acid	0	----	1.92 (25 °C)	----
Aspartic acid	+1	2.13	2.02	1.95
Phosphoric acid	0	2.06	2.13	2.21
Asparagine	+1	----	2.1 (25 °C)	----
Glycine	+1	2.44	2.36	2.33
Pyruvic acid	0	----	2.49 (25 °C)	2.42
EDTA	-1	----	2.6	----
Pyrophosphoric acid	-1	----	2.64 (25 °C)	----
Malonic acid	0	----	2.86 (25 °C)	----
Tartaric acid	0	3.12	3.04	3.02
Citric acid	0	3.22	3.14	3.11
β-alanine	+1	3.65	3.57	3.52
Formic acid	0	3.79	3.75	3.76
Lactic acid	0	3.89	3.86	3.87
Aspartic acid	0	4.01	3.92	3.88
γ-Aminobutyric	+1	4.09	4.04	4.03
Succinic acid	0	4.28	4.22	4.19
Oxalic acid	-1	4.20	4.25	4.32
Tartaric acid	-1	4.43	4.37	4.37
ε-Aminocaproic acid	+1	4.42	4.38	4.38
Acetic acid	0	4.78	4.76	4.77
Citric acid	-1	4.84	4.77	4.75
Propionic acid	0	4.90	4.87	4.89
Pyridine	+1	----	5.17 (25 °C)	----
Succinic acid	-1	5.68	5.64	5.65
Malonic acid	-1	5.67	5.68	5.74
MES	0	6.4	6.15	6.0
Maleic acid	-1	----	6.23 (25 °C)	----
Cacodylic acid	0	6.24	6.24	----
EDTA	-2	----	6.3	----
Carbonic acid	0	6.58	6.38	6.30
Citric acid	-2	6.39	6.39	6.43
BIS-TRIS	+1	----	6.46 (25 °C)	----
Pyrophosphoric acid	-2	----	6.76 (25 °C)	----
ACES	0	7.3	6.9	6.6
Imidazole	+1	----	6.9 (25 °C)	----

pK_a FOR BUFFERS

Buffer	z	0 °C	20 °C	37 °C
MOPS	0	7.4	7.2	7.0
Phosphoric acid	-1	7.31	7.22	7.18
2:4:6-Collidine	+1	----	7.4 (23 °C)	7.32
TES	0	8.0	7.55	7.2
N-Ethylmorpholine	+1	----	7.65 (25 °C)	----
Triethanolamine	+1	8.29	7.86	7.53
5,5-Diethylbarbituric acid	0	8.40	8.06	7.82
Tricine	0	8.6	8.15	7.8
Glycinamide	+1	8.8	8.2	7.7
Tris	+1	8.85	8.21	7.75
Bicine	0	8.7	8.4	8.3
Morpholine	+1	----	8.6 (25 °C)	----
Asparagine	0	----	8.8 (25 °C)	----
Diethanolamine	+1	9.55	9.01	8.59
Boric acid	0	9.50	9.28	9.15
Ammonia	+1	10.08	9.40	8.89
Pyrophosphoric acid	-3	----	9.42 (25 °C)	----
Ethanolamine	+1	10.31	9.65	9.15
Glycine	0	10.50	9.91	9.48
Aspartic acid	-1	10.63	10.12	9.75
β-Alanine	0	11.00	10.38	9.91
Carbonic acid	-1	10.63	10.38	10.24
EDTA	-3	----	10.6	----
γ-Aminobutyric	0	11.37	10.71	10.21
Triethylamine	+1	11.18	10.78	10.40
Ethylamine	+1	11.31	10.79	10.35
ε-Aminocaproic acid	0	11.71	10.98	10.42
Piperidine	+1	11.96	11.28	10.75

Abbreviations: MES, 2-(N-morpholino) ethanesulfonic acid; MOPS, 2-(N-morpholino) propanesulfonic acid; BIS-TRIS, bis (2-hydroxyethyl) imino-tris (hydroxymethyl) methane; ACES, N-(2-acetamido)-2-aminoethanesulfonic acid; TES, N-tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid; Tricine, N-tris (hydroxymethyl) methylglycine; Tris, 2-hydroxymethyl-2-amino-1,3-propanediol; Bicine, N, N-bis (2-hydroxyethyl) glycine.