pH and Buffers

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Many biomolecules possess weakly acidic or weakly basic groups whose ionization state can affect both biological function and stability, and many biological processes, such as oxidation–reduction reactions, produce or consume hydrogen ions. The extents of the reactions and the rates at which they proceed depend on the acidity of the reaction environment. Buffers are used to maintain a constant hydrogen ion activity.

Introductory article

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Acids and Bases

For most biochemical purposes, an acid can be defined as a substance that donates protons (hydrogen ions); a base can be defined as a substance that accepts protons. This is known as the Brønsted–Lowry concept of acids and bases. When an acid loses a proton, a base is produced; when a base accepts a proton, an acid is produced. In aqueous solution, an acid (abbreviated HA) donates a proton to water (acting as a base) producing a hydronium ion (H_3O^+) (eqn [I]).

$$HA + H_2O \rightleftharpoons H_3O^+ + A^- \qquad \textbf{[I]}$$

In the reverse direction, the acid H_3O^+ donates a proton to the base A^-. The protonated species (HA) and the deprotonated species (A^-) constitute a conjugate acid–conjugate base pair. The equilibrium constant for the reaction can be defined as in eqn [1].

$$K'_a = \frac{[H_3O^+][A^-]}{[HA][H_2O]} \qquad [1]$$

A base (e.g. A−) is a substance that accepts a proton from water to produce OH^- and a conjugate acid (eqn [II]).

$$A^- + H_2O \rightleftharpoons OH^- + HA \qquad \textbf{[II]}$$

The equilibrium constant, K_b', is given by eqn [2].

$$K'_b = \frac{[OH^-][HA]}{[A^-][H_2O]} \qquad [2]$$

Because the concentration of water remains essentially constant, the $[H_2O]$ and the K_a' (or K_b') terms can be combined into a single constant called the acid (or base) dissociation constant (eqns [3] and [4]).

$$K_a = K'_a[H_2O] = \frac{[H_3O^+][A^-]}{[HA]} \qquad [3]$$

$$K_b = K'_b[H_2O] = \frac{[OH^-][HA]}{[A^-]} \qquad [4]$$

For most purposes, it is sufficient to consider an acid as a substance that dissociates in aqueous solution to yield a proton (H^+) and a base (eqn [III]).

$$HA \rightleftharpoons H^+ + A^- \qquad \textbf{[III]}$$

Strong acids such as HCl and HNO_3 are almost completely dissociated in solution. Weak acids (which are the most common type found in cells) are only partially dissociated in solution. (Hence the double arrows in the reactions shown above.) The equilibrium between the species of eqn [III] is described by the acid dissociation constant, K_a (eqn [5]).

$$K_a = \frac{[H^+][A^-]}{[HA]} \qquad [5]$$

Equations [3] and [5] are essentially identical because H^+ and H_3O^+ are equivalent.

As shown above, K_a has units of concentration (mol L^{-1}). Strictly speaking, eqn [5] should be expressed in terms of the activities of the components, a_{H^+}, a_{A^-} and a_{HA}, rather than their concentrations. Essentially, a represents the apparent or effective concentration of a species. The activity of a species (e.g. a_{H^+}) is related to its concentration by an activity coefficient, γ (eqn [6]).

$$\gamma = a_{H^+}/[H^+] \qquad \text{or} \qquad a_{H^+} = \gamma[H^+] \qquad [6]$$

The value of γ, which represents the fraction of the actual concentration that appears to be present, depends on the ionic strength of the solution (see later). For the dilute solutions used in most biochemical studies, the use of concentrations instead of activities usually does not

introduce a large error. (In a later section we will examine operations that require attention to activities.)

Water itself is a very weak acid. It is also a very weak base (eqns [IV], [V]).

$$H_2O + H_2O \rightleftharpoons H_3O^+ + OH^- \qquad [IV]$$

$$H_2O \rightleftharpoons H^+ + OH^- \qquad [V]$$

A dissociation constant for this process analogous to eqn [5] can be defined. However, because the reaction proceeds to such a small extent, the concentration of water remains essentially constant (at about 55 mol L^{-1}). Consequently, $[H_2O]$ can be combined with the K_a to give a constant called K_w, the ion product of water (eqn [7]).

$$K_a = \frac{[OH^-][H^+]}{[H_2O]} \qquad K_w = K_a[H_2O] = [OH^-][H^+] \qquad [7]$$

The value of K_w is very close to 10^{-14} mol^2 L^{-2} at biological temperatures. Thus, in pure water $[H^+] = 10^{-7}$ mol L^{-1} and $[OH^-] = 10^{-7}$ mol L^{-1}. Note that the product $K_a \times K_b$ for any conjugate acid–conjugate base pair in aqueous solution equals K_w.

pH and the Henderson–Hasselbalch Equation

pH, defined as the negative logarithm of the hydrogen ion activity (concentration), is a shorthand way of denoting the $[H^+]$ of a solution (eqn [8]).

$$pH = -\log[H^+] = \log \frac{1}{[H^+]} \qquad [8]$$

This is equivalent to eqn [9].

$$[H^+] = 10^{-pH} \qquad [9]$$

For example, if $[H^+] = 10^{-6}$ mol L^{-1}, the pH equals 6. Pure water, where $[H^+] = 10^{-7}$ mol L^{-1}, has a pH of 7. Similarly, if $[H^+] = 5.7 \times 10^{-5}$ mol L^{-1}, the pH is 4.24, calculated as shown in eqn [10].

$$pH = \log \frac{1}{5.7 \times 10^{-5}} = \log 17544 = 4.24 \qquad [10]$$

Solutions with pH values below 7 are said to be acidic; solutions with pH values greater than 7 are basic or alkaline. Because the pH scale is logarithmic, small differences in pH correspond to a much larger difference in hydrogen ion concentration. For example, the $[H^+]$ in a fruit juice having a pH of 3.4 is 10 000 times greater than that in blood at pH 7.4.

Just as there is an equilibrium relationship between the H^+ concentration and K_a for the dissociation of a weak acid, a relationship can be derived expressing the pH of the solution in terms of pK_a (the negative logarithm of K_a) (eqn [11]).

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

$$-\log K_a = -\log \frac{[A^-][H^+]}{[HA]}$$

$$pK_a = pH - \log \frac{[A^-]}{[HA]}$$

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

or in general

$$pH = pK_a + \log \frac{[\text{conjugate base}]}{[\text{conjugate acid}]} \qquad [11]$$

Thus, $pH = pK_a$ when the solution contains equal concentrations of conjugate acid and conjugate base. One can define a similar Equation for a weak base (eqn [12]).

$$pOH = pK_b + \log \frac{[\text{conjugate acid}]}{[\text{conjugate base}]} \qquad [12]$$

For any conjugate acid–conjugate base pair we have eqn [13], and for any aqueous solution eqn [14] applies.

$$pK_a + pK_b = pK_w = 14 \qquad [13]$$

$$pH + pOH = 14 \qquad [14]$$

pH of a Solution of a Weak Acid

Dissociation of a weak monoprotic acid, HA, at an initial concentration C, yields equimolar concentrations of A^- and H^+ and an equilibrium level of HA equivalent to $C - [H^+]$. Substituting into eqn [5], we obtain eqn [15].

$$K_a = \frac{[H^+]^2}{C - [H^+]} \qquad [15]$$

The quadratic equation [15] can be solved to yield the solution in eqn [16].

$$[H^+] = \frac{\sqrt{K_a^2 + 4K_aC} - K_a}{2} \qquad [16]$$

The pH of the solution can then be calculated from eqn [8]. The deviation of eqn [16] ignores H^+ contributed by the dissociation of water. This is a reasonable simplification if the product K_aC is at least $100K_w$. If, in addition, the degree of HA dissociation is small (e.g. less than 10%), the $[H^+]$ term in the denominator of eqn [15] can be ignored. The values of $[H^+]$ and pH can then be estimated from the approximation given in eqn [17], where

pC is the negative logarithm of the initial weak acid concentration.

$$[H^+] = \sqrt{K_a C} \qquad \text{and} \qquad pH = \tfrac{1}{2}(pK_a + pC) \qquad [17]$$

For a weak base at initial concentration C, the equations are analogous to those shown above with OH^- and pOH replacing H^+ and pH, and with K_b and pK_b replacing K_a and pK_a (eqn [18]).

$$[OH^-] = \frac{\sqrt{K_b^2 + 4K_b C} - K_b}{2} \qquad [18]$$

Analogously to eqn [17], at $< 10\%$ ionization we obtain eqn [19].

$$[OH^-] = \sqrt{K_b C} \qquad \text{and} \qquad pOH = \tfrac{1}{2}(pK_b + pC) \qquad [19]$$

Degree of Dissociation

If a weak acid at initial concentration C undergoes fractional dissociation n, then at equilibrium $[HA] = (C - nC) = C(1 - n)$, $[H^+] = nC$, and $[A^-] = nC$. Substituting into eqn [5] and solving for C, we obtain eqn [20].

$$C = \frac{(1 - n)K_a}{n^2} \qquad [20]$$

Equation [20] shows that the acid will be 10% dissociated (i.e. $n = 0.1$) at $C = 90K_a$, 50% dissociated ($n = 0.5$) at $C = 2K_a$, and 90% dissociated ($n = 0.9$) at $C = 0.123K_a$. In other words, the degree of dissociation of a weak acid increases as it is diluted (an expected consequence of the Law of Mass Action).

Noting that $C = [HA] + [A^-]$, another relationship that can be obtained from eqn [5] is that given in eqn [21], where $[HA]/C$ represents the fraction of the total acid that remains undissociated at equilibrium.

$$\frac{[HA]}{C} = \frac{[H^+]}{K_a + [H^+]} \qquad [21]$$

Equation [21] has the same form as the Henri–Michaelis–Menten equation for v/V_{max} of an enzyme-catalysed reaction.

Titration Curves

Figure 1 shows the change in pH as a 0.1 mol L^{-1} solution of a strong acid is titrated with concentrated NaOH, a strong base. The pH remains close to the original value until near the equivalence point, then it rises sharply.

In contrast, during the titration of a weak acid, the pH rises sharply at first but then becomes less sensitive to change as OH^- is added, until the equivalence point is approached. As shown in **Figure 2**, the curve is flattest around the midpoint. The solution of HA plus A^- in the pH region around pK_a acts as a buffer.

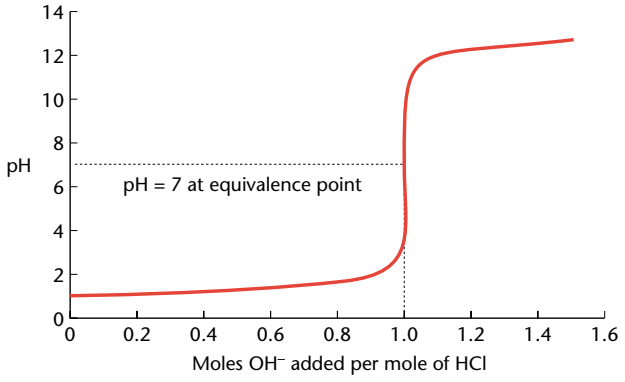


Figure 1 Titration of 0.1 mol L^{-1} HCl (a strong acid) with NaOH. The pH at the start is about 1 because HCl is nearly 100% dissociated ($[H^+] = 10^{-1}$ mol L^{-1}). The pH at any point along the curve is calculated from the concentration of HCl remaining at that point. At the equivalence point, the pH is 7 because the solution contains only NaCl, which, being a salt of a strong acid and a strong base, does not act as an acid or a base. Beyond the equivalence point, the pH depends on the concentration of excess OH^- present.

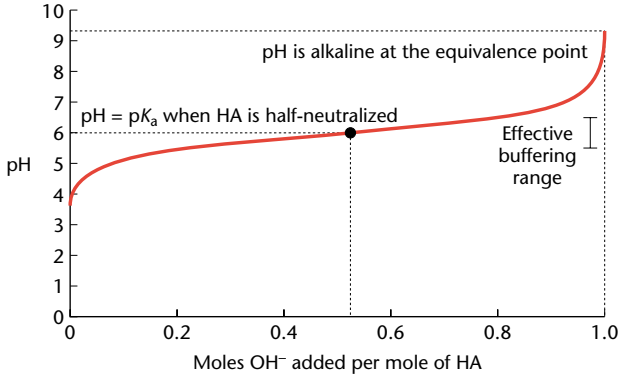


Figure 2 Titration of 0.1 mol L^{-1} HA (a weak acid, p$K_a = 6$) with NaOH. The starting pH is calculated from eqn [16] or [17]. The pH at any point along the titration curve is calculated from eqn [11]. If dilution is negligible (because concentrated or solid NaOH is used), the pH at the equivalence point is that of a 0.1 mol L^{-1} solution of A^-, a weak base (eqns [18] or [19] and [14]).

Diprotic and Polyprotic Acids

A diprotic acid (H_2A) contains two dissociable acid groups. If these groups differ in acid strength by a factor of about 100 or more, the dissociation reactions can be considered as occurring in two sequential steps with the

stronger acid group producing all the H^+ of the first dissociation, i.e. H_2A dissociates to produce HA^-, which in turn dissociates to produce A^{2-} (eqn [VI]).

$$H_2A \underset{}{\overset{pK_{a1}}{\rightleftharpoons}} H^+ + HA^- \qquad [VI]$$

$$HA^- \overset{pK_{a2}}{\rightleftharpoons} H^+ + A^{2-}$$

The intermediate ion, HA^-, is the conjugate base of H^2A, but it is the conjugate acid with respect to A^{2-}. The titration curve will reveal two regions where the slope passes through a minimum as shown in **Figure 3**. If the two pK_a values differ by a factor of 2 or less, the curve will show only a single broad region of minimum slope centred around the average of the two pK_a values. Although **Figure 3** assumes that the titration starts with H_2A, the same curve (but in mirror-image) would result if A^{2-} were titrated with strong acid. One could also titrate a solution of HA^- with NaOH in the alkaline direction and with HCl in the acid direction. The titration curve of triprotic phosphoric acid (H^3PO_4) shows three regions of minimum slope occurring at pH values of about 2.1, 7.2 and 12.3.

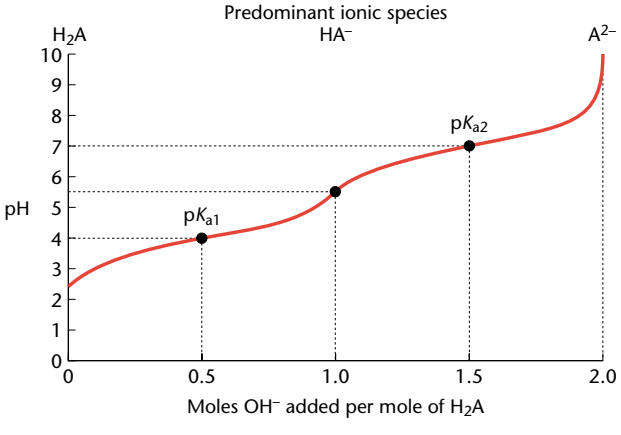
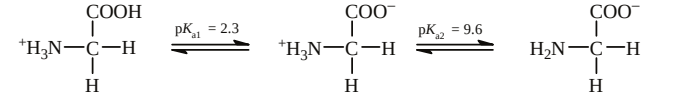


Figure 3 Titration curve of 0.1 mol L^{-1} diprotic weak acid ($pK_{a1} = 4$; $pK_{a2} = 7$). The starting pH depends almost exclusively on the dissociation of H_2A and can be calculated from eqns [16] or [17] using pK_{a1}. When half of the original H_2A has been converted to HA^-, pH = pK_{a1}. The predominant species at the first equivalence point is HA^- (~94% of the total), where the pH is the average of the two pK_a values. When half of the HA^- has been converted to A^{2-}, pH = pK_{a2}. If the volume does not change, the pH at the endpoint is that of a 0.1 mol L^{-1} solution of the base A^{2-} (eqns [18] or [19] and [14]).

Amino Acids

The common 'neutral' amino acids are essentially diprotic acids where K_{a1} and K_{a2} refer, respectively, to the dissociation of the α-carboxyl group (–COOH) and the charged α-amino group ($-NH_3^+$). The neutral species carries no net charge, but it is actually a 'zwitterion' carrying one positive and one negative charge as shown in eqn [VII] for glycine.



[VII]

Gly$^+$, e.g. glycine hydrochloride

Gly^0, i.e. isoelectric glycine; glycine 'free base'; zwitterion form

Gly$^-$, e.g. sodium glycinate

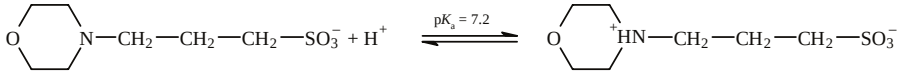
Most of the diprotic amino acids have pK_{a1} (carboxyl) values in the range of 2.0–2.5 and pK_{a2} (amino) values in the range of 9.0–9.7. The 'acidic' amino acids, aspartic acid and glutamic acid, are triprotic, each containing an additional carboxyl group with a pK_a in the region of 4. The 'basic' amino acid lysine contains an additional amino group with a pK_{a3} of 10.5. In addition to their carboxyl and amino groups, histidine contains an imidazole group ($pK_{a2} = 6.0$), while arginine contains a highly basic guanidinium group ($pK_{a3} = 12.5$). Cysteine contains a dissociable sulfhydryl group with a pK_a of 8.3. (The pK_a of its amino group is 10.8.) The pK_a value of a side chain may change somewhat when the amino acid is incorporated into a protein.

Amino acids containing acidic or basic side chains introduce positive and negative charges throughout the polypeptide chain of a protein. In a soluble protein, most of the ionizable side chains can equilibrate with the aqueous buffer. Consequently, the pH of the medium will influence the charges carried by the protein. For example, a cysteine-free protein containing 31 total glutamate plus aspartate residues, 12 histidine residues, and 25 total lysine plus arginine residues will have a net charge of about –6 at pH 8. (The glutamate and aspartate side chains will be almost completely in the negatively-charged form; the lysine and arginine side chains will be almost completely in the positively-charged form; and the histidine residues will be almost completely in the neutral form.) At pH 8, this protein will migrate in an electric field toward the positive electrode and (at low ionic strength) will bind well to an anion-exchange resin. However, at pH 6.0, about 50% of the histidine side chains will be in the positively-charged

form at any time while the gluatamate, aspartate, lysine, and arginine side chains will remain nearly completely charged. Consequently, at pH 6 the protein will carry no net charge. This pH is called the isoelectric pH or isoelectric point of the protein, pI. In general, proteins display the lowest solubility at their pI.

Buffers

A buffer is something that resists change. A pH buffer is a substance or mixture of substances that resists a change in pH upon addition or utilization of H^+ or OH^-. As shown in Figures **2** and **3**, a mixture of a weak acid and its conjugate base will buffer a solution in the region of its pK_a. Natural buffers used by cells to maintain a near-constant intracellular pH include phosphate, bicarbonate and proteins. Biochemists use a variety of natural and synthetic compounds to produce buffers for laboratory experiments. Many of the synthetic buffers currently available commercially were introduced by N. E. Good in 1966. These 'Good buffers' are mainly *N*-substituted sulfonic acids. Mops (4-morpholinepropanesulfonic acid) [VIII] is an example. Unlike phosphate, pyrophosphate, and diprotic and triprotic carboxylate buffers, the Good buffers have low or negligible metal-binding ability. This makes them quite suitable for studying enzyme-catalysed reactions in which the free metal ion (e.g. Mg^{2+}) concentration must be carefully controlled.



[VIII]

$Mops^-$
(conjugate base,
e.g. sodium salt)

$Mops^0$
(conjugate acid, i.e.
'free acid', zwitterion form)

How do Buffers Work?

A buffer resists changes in pH because the interconversion of the conjugate acid and the conjugate base is an equilibrium reaction with H^+ as one component. As required by the Law of Mass Action, the HA dissociation reaction shifts in one direction or the other upon addition or removal of H^+. Thus, if H^+ is added to (or produced in) a buffer, a portion of that additional H^+ reacts with the conjugate base of the buffer to form the conjugate acid. Essentially, some of the extra H^+ is neutralized. Similarly, if H^+ is consumed in a reaction, the conjugate acid of the buffer dissociates to replenish a portion of that H^+. This process cannot maintain the pH absolutely constant because a change in the equilibrium ratio of $[A^-]/[HA]$ requires a compensatory change in $[H^+]$ in order for equilibrium conditions to be satisfied. This can be shown in a simple mathematical way. Suppose 2 mmol L^{-1} H^+ was produced in a reaction mixture containing 20 mmol L^{-1} Mops buffer, pH 7.2. Because the initial pH equals the pK_a, $[Mops^-]$ must equal $[Mops^0]$, i.e. both species must have been present initially at 10 mmol L^{-1}. (Buffer concentrations are given in terms of the total of all buffer species present.) If the 2 mmol L^{-1} H^+ were completely absorbed by the buffer, the final concentrations would be $[Mops^-] = (10 - 2)\,\text{mmol}\,L^{-1} = 8\,\text{mmol}\,L^{-1}$ and $[Mops^0] = (10 + 2)\,\text{mmol}\,L^{-1} = 12\,\text{mmol}\,L^{-1}$. In order for the pH to remain at 7.2, eqn [22] must hold.

$$7.2 = 7.2 + \log \frac{[\text{Mops}^-]}{[\text{Mops}^0]} \qquad [22]$$

But this cannot be true when $[Mops^-] \neq [Mops^0]$. The actual final pH can be calculated from eqn [23].

$$\text{pH} = 7.2 + \log \frac{[8]}{[12]} = 7.0 \qquad [23]$$

(Note that the pH would have dropped to 2.7 (i.e. $-\log(2 \times 10^{-3})$) if the buffer were not present.) The calculation of the final pH assumed that the entire 2 mmol L^{-1} H^+ was consumed by the buffer. This is an excellent approximation as long as the buffer concentration is greater than that of the H^+ added or used.

Buffer Capacity

The ability of a buffer to resist a change in pH can be described in terms of its buffer capacity. Theoretical or instantaneous buffer capacity, β, is the reciprocal of the slope of the titration curve at any point, i.e. $d[OH^-]/dpH$, which is equivalent to $d[A^-]/dpH$ or $d[H^+]/dpH$. A little calculus leads to eqns [24], where $[A^-]$ and [HA] are the initial concentrations of conjugate base and conjugate acid, respectively, and C is the total buffer concentration ($[A^-] + [HA]$).

$$\beta = \frac{2.303[A^-][HA]}{[A^-] + [HA]} \quad \text{or} \quad \beta = \frac{2.303\,K_a[H^+]C}{(K_a + [H])^2} \qquad [24]$$

The maximum instantaneous buffer capacity occurs where $[H^+] = K_a$ (i.e. where $pH = pK_a$). At this point,

$\beta_{max} = 0.576C$. Because β is the reciprocal slope at a point, its value is the same in both the acidic and basic directions. A more useful index is the practical buffer capacity. Practical buffer capacity in the acid direction, BC_a, is the concentration of H^+ that must be added to decrease the pH by one unit. Similarly, practical buffer capacity in the basic direction, BC_b, is the concentration of OH^- that must be added (or of H^+ that must be consumed) to increase the pH by one unit. Practical buffer capacities can be calculated from eqns [25].

$$BC_a = \frac{9\,[HA][A^-]}{10\,[HA] + [A^-]}$$

$$\text{and } BC_b = \frac{9\,[HA][A^-]}{10\,[A^-] + [HA]} \qquad [25]$$

The choice of which buffer to use should take into account the likely direction of any pH change that might occur during the process being studied, because BC_a and BC_b can be quite different. As a general rule, though, a buffer should be used within ± 0.5 pH unit of its pK_a.

The Bicarbonate/CO_2 Blood Buffer

Carbon dioxide reacts to a slight extent with water to produce H_2CO_3, a weak diprotic acid that ionizes to form H^+ and bicarbonate (HCO_3^-). This system is one of the two major blood buffers. (The other is haemoglobin.) The buffer can be considered to be composed of dissolved CO_2 (as the conjugate acid) and HCO_3^- (as the conjugate base). The apparent pK_a is 6.1. (The dissociation of HCO_3^- to form H^+ and CO_3^{2-} has a pK_a of about 12 and is not important in controlling the pH of blood and cells. The pH of blood is about 7.4. So how can a buffer with a pK_a of 6.1 be effective at pH 7.4? Actually, the CO_2/HCO_3^- system has a rather high buffer capacity because it is an open system in which the concentration of the conjugate acid is maintained relatively constant. That is, excess H^+ reacts with HCO_3^- to produce $CO_2 + H_2O$ and the excess CO_2 is exhaled by the lungs. To illustrate the difference between a typical closed system and the open CO_2/HCO_3^- system, consider the effect of a reaction that produces 5 mmol L^{-1} H^+ in a 25.2 mmol L^{-1} CO_2/HCO_3^- buffer originally at pH 7.4. (The concentration of HCO_3^- in blood plasma is about 25 mmol L^{-1}.) At the start, equations [26] apply.

$$pH = pK_a + \log\frac{[HCO_3^-]}{[CO_2]} \qquad 7.4 = 6.1 + \log\frac{[HCO_3^-]}{[CO_2]},$$

$$1.3 = \log\frac{[HCO_3^-]}{[CO_2]} \qquad \frac{[HCO_3^-]}{[CO_2]} = \frac{20}{1} \qquad [26]$$

Therefore, equations [27] and [28] hold.

$$[HCO_3^-] = \frac{20}{21}(25.2\,\text{mmol}\,L^{-1}) = 24\,\text{mmol}\,L^{-1} \qquad [27]$$

$$[CO_2] = \frac{1}{21}(25.2\,\text{mmol}\,L^{-1}) = 1.2\,\text{mmol}\,L^{-1} \qquad [28]$$

If the system were closed, the final pH would be given by eqn [29].

$$pH = 6.1 + \log\frac{[24-5]}{[1.2+5]} = 6.6 \qquad [29]$$

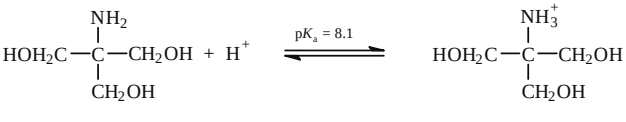
Clearly there is very little buffer capacity in a closed system, However, in the open system, the 5 mmol L^{-1} excess CO_2 produced is exhaled, leaving the conjugate acid concentration at 1.2 mmol L^{-1}. Thus, even though the concentration of conjugate base has decreased, the final pH will be given by eqn [30].

$$pH = 6.1 + \log\frac{[24-5]}{[1.2]} = 7.3 \qquad [30]$$

Oxidative metabolism replenishes the lost HCO_3^-.

Preparation of Buffers

Usually, there are several ways to prepare a buffer at a desired pH. The method of choice depends on the materials available and any considerations about other ions that may or should not be present. One of the most commonly used buffers for studying enzyme-catalysed reactions in the laboratory is the synthetic compound tris(hydroxymethyl)aminomethane (abbreviated to 'Tris' or THAM). Tris is generally purchased as the conjugate base ($Tris^0$, sometimes called the 'free base') and the appropriate portion is converted to the conjugate acid, $Tris^+$, by titration with a strong acid. The Tris species are shown in [IX].



$Tris^0$ (conjugate base, i.e. 'free base') $Tris^+$ (conjugate acid, e.g. Tris hydrochloride) [IX]

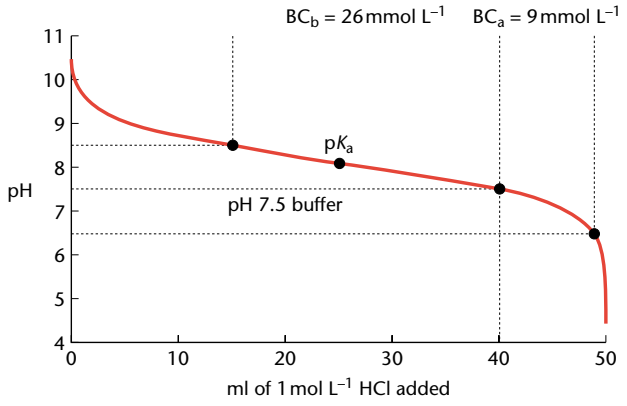


Figure 4 Titration of 0.05 mol L^{-1} Tris (pK_a = 8.1) with 1 mol L^{-1} HCl. The volume at the start is assumed to be 1.0 litre. Note that the pH 7.5 buffer has a rather poor practical buffer capacity in the acid direction.

Consider the preparation of one litre of 0.05 mol L^{-1} Tris-HCl buffer at pH 7.5 from solid Tris^0 (the conjugate base) and a stock solution of 1 mol L^{-1} HCl. We would start by dissolving 0.05 mol of Tris^0 in a volume of water somewhat less than 1 litre. Concentrated HCl would then be added until the pH was 7.5 (In practice, the pH would be monitored with a glass electrode and a pH meter.) Additional water would then be added to bring the volume to exactly one litre. The titration curve for Tris is shown in **Figure 4**.

The volume of HCl required to prepare the buffer can be estimated from the fraction of the Tris^0 that must be converted to the conjugate acid form (eqns [31] to [33]).

$$\mathrm{pH} = \mathrm{p}K_a + \log\frac{[\mathrm{Tris}^0]}{[\mathrm{Tris}^+]}$$

$$7.5 = 8.1 + \log\frac{[\mathrm{Tris}^0]}{[\mathrm{Tris}^+]} \qquad [31]$$

$$-0.6 = \log\frac{[\mathrm{Tris}^0]}{[\mathrm{Tris}^+]} \quad \text{or}$$

$$0.6 = \log\frac{[\mathrm{Tris}^+]}{[\mathrm{Tris}^0]} \qquad [32]$$

$$\frac{[\mathrm{Tris}^+]}{[\mathrm{Tris}^0]} = \frac{3.98}{1}$$

$$\therefore\ [\mathrm{Tris}^+] = \frac{3.98}{4.98}(0.05\ \mathrm{mol\ L^{-1}}) = 0.04\ \mathrm{mol\ L^{-1}} \qquad [33]$$

Thus 80% of the original 0.05 mol of Tris^0 must be converted to Tris$^+$. This requires the addition of 0.04 mol of HCl, or 40 ml of the 1 mol L^{-1} stock solution. One could also start with 0.05 mol of Tris hydrochloride and convert 0.01 mol to the conjugate base by the addition of 0.01 mol of NaOH (provided that the presence of Na$^+$ in the buffer is not undesirable). Perhaps the most convenient procedure is to mix 0.05 mol L^{-1} solutions of Tris^0 and Tris hydrochloride to the desired pH. An advantage of this method is that overtitration with one component can be reversed easily by adding more of the other component; the buffer concentration will remain 0.05 mol L^{-1}. If, instead of HCl, the conjugate acid of Mops (pK_a = 7.2) or Mes (4-morpholineethanesulfonic acid; pK_a = 6.2) is used to titrate the Tris^0, buffers with high capacities over a broader pH range in the acid direction can be prepared.

Effect of Temperature

Temperature affects the degree of dissociation of weak acids and bases. The phenomenon is described by the van't Hoff equation (eqn [34]).

$$\log\frac{K_{a@T_2}}{K_{a@T_1}} = \mathrm{p}K_{a@T_1} - \mathrm{p}K_{a@T_2} = \frac{\Delta H_{ion}(T_2 - T_1)}{2.303RT_2T_1} \qquad [34]$$

where ΔH_{ion} is the enthalpy of ionization. If the ΔH_{ion} is close to zero (as it is for phosphate, pyrophosphate and carboxylic acids), the pK_a and hence the pH of a buffer will be relatively insensitive to a change in temperature. However, for many buffers ΔH_{ion} is in the range + 25 to + 54 kJ mol^{-1}. As a result, the pH of the buffer will change appreciably with changing temperature. For example, a 50 mmol L^{-1} Tris buffer prepared to have a pH of 8.1 at 25°C will have a pH of 8.68 at 5°C and a pH of 7.80 at 37°C (ΔpK_a per degree is about − 0.03; Tris$^+$ becomes a stronger acid as T increases). Tables listing the pK_a values of various buffers at different temperatures or the ΔpK_a per degree of temperature change are available. Clearly, the most reliable procedure is to adjust the pH of a buffer to the desired value at the temperature at which it will be used.

Effect of Ionic Strength

Ionic strength, I, is a measure of the concentration of charged particles in solution. The value of I can be calculated from eqn [35].

$$I = \tfrac{1}{2}\sum M_i Z_i^2 \qquad [35]$$

Here M_i = the molarity of the ion and Z_i = the net charge (regardless of sign). Charged buffer components contribute to the ionic strength of a solution and, in turn, ionic strength affects the activity coefficients of buffer components. (In general, the higher the value of I, the lower the value of the activity coefficient.) Ionic strength may influence biological activity. Consequently, variations in I should be considered when preparing a buffer. For example, if one wished to prepare a series of buffers to measure the activity of an enzyme at different pH values, it

would be advisable to adjust all the buffers to the same ionic strength (so that the observed effects are a result of pH differences alone, not differences in I as well as pH). This can be accomplished by calculating I for each buffer and then adding an innocuous salt (e.g. NaCl) to bring all of the buffers to the same final I value. If the enzyme is sensitive to high ionic strength, I can be minimized by using a buffer in which one of the components is uncharged, e.g. 50 mmol L^{-1} potassium Pipes (1,4-piperazinediethanesulfonic acid) at pH 6.8 (where the only charged species are 25 mmol L^{-1} $Pipes^{-}$ and 25 mmol L^{-1} K^{+}) instead of 50 mmol L^{-1} potassium phosphate, which contains approximately 25 mmol L^{-1} HPO_4^{2-}, 25 mmol L^{-1} $H_2PO_4^{-}$ and 75 mmol L^{-1} K^{+}.

Because activity coefficients are highly sensitive to ionic strength, buffers often display a change in pH upon dilution. For example, when a phosphate buffer is diluted with water, the activity coefficient of HPO_4^{2-} (the activity coefficient, γ, of the more highly-charged species) increases to a greater extent than does the activity coefficient of $H_2PO_4^{-}$, producing a rise in pH. In contrast, dilution of a Tris-HCl buffer results in a decrease in pH (because the activity coefficient of $Tris^{+}$ increases while the activity coefficient of $Tris^{0}$ remains essentially constant). One way to account for ionic strength effects is to define apparent pK_a values for different total ionic strengths. For example, the apparent pK_{a2} of phosphate is 6.8 in a 0.1 mol L^{-1} buffer, compared to 7.2 at infinite dilution.

At high dilutions of buffer, another factor – a change in the degree of dissociation of the conjugate acid – may also affect the pH. Clearly, to avoid problems, a concentrated stock buffer should be prepared so that it has the desired pH after dilution and at the temperature of use.

Further Reading

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