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CHAPTER 3

pH and Buffers

The compounds responsible for colour in plants are often sensitive to acids and alkalis. Blue hydrangeas grow only in acidic soils; in neutral or alkaline soils, they will revert to pink.

IONIZATION OF WATER, WEAK ACIDS AND WEAK BASES

Although many of the solvent properties of water can be explained in terms of the uncharged H_2O molecules,the small degree of ionization of water to hydrogen ions (H^+) and hydroxide ions (OH^-) must also be taken into account. *Like all reversible reactions, the ionization of water can be described by an equilibrium constant.* When weak acids or weak bases are dissolved in water, they can contribute H^+ by ionizing (if acids) or consume H^+ by being protonated (if bases). These processes are also governed by equilibrium constants. The total hydrogen ion concentration from all sources, which is experimentally measurable, is expressed as the pH of the solution.

Water molecules have a slight tendency to undergo reversible ionization to yield a hydrogen ion and a hydroxide ion :

$$H_2O \rightleftharpoons H^+ + OH^- \qquad ...(3.1)$$

This reversible ionization is crucial to the role of water in cellular functions. Henceforth, we must have a means of expressing the extent of ionization of water in quantitative terms. This is discussed below.

The position of equilibrium of any chemical reaction is given by its **equilibrium constant.** For the general reaction,

$$A + B \rightleftharpoons C + D \qquad ...(3.2)$$

an equilibrium constant can be defined in terms of concentrations of reactants (A and B) and products (C and D) present at equilibrium :

$$K_{eq} = \frac{[C]\,[D]}{[A]\,[B]} \qquad \text{...(3.3)}$$

Strictly speaking, the bracketed terms represent molar activity, rather than molar concentration.

The equilibrium constant is fixed and characteristic for any given chemical reaction at a specific temperature. It defines the composition of the final equilibrium mixture of that reaction, irrespective of the starting amounts of reactants and products. Conversely, one can calculate the equilibrium constant for a given reaction at a given temperature, if the equilibrium concentrations of all its reactants and products are known.

As mentioned, the degree of ionization of water at equilibrium (Eqn. 3.1) is small; at 25°C, only about one of every 10^7 molecules in pure water is ionized at any instant. The equilibrium constant for the reversible ionization of water (Eqn. 3.1) is :

$$K_{eq} = \frac{[H^+]\,[OH^-]}{[H_2O]} \qquad \text{...(3.4)}$$

In pure water at 25°C, the concentration of water is 55.5 M (*i.e.*, grams of H_2O in 1 litre divided by gram molecular weight or 1000/18 M = 55.5 M). This value is essentially constant in relation to the very low concentrations of H^+ and OH^-, namely 1×10^7 M. Accordingly, on substituting 55.5 M in the equilibrium constant expression (*i.e.*, Eqn. 3.4), we get :

$$K_{eq} = \frac{[H^+]\,[OH^-]}{55.5\ \text{M}} \qquad \text{...(3.5)}$$

which, on rearranging, becomes :

$$(55.5\ \text{M})\,(K_{eq}) = [\,H^+\,]\,[\,OH^-\,] = K_w \qquad \text{...(3.6)}$$

where K_w designates the product (55.5 M) (K_{eq}), the **ion product of water** at 25°C.

The value for K_{eq} is 1.8×10^{-16} M at 25°C as calculated from electrical conductivity measurements. Substituting this value for K_{eq} in Eqn. 3.6 gives :

$$\begin{aligned}
(55.5\ \text{M})\,(1.8 \times 10^{-16}\ \text{M}) &= [H^+]\,[OH^-] \\
99.9 \times 10^{-16}\ \text{M}^2 &= [H^+]\,[OH^-] \\
1.0 \times 10^{-14}\ \text{M}^2 &= [H^+]\,[OH^-] = K_w
\end{aligned}$$

Thus, the product $[H^+]\,[OH^-]$ in aqueous solutions at 25°C always equals $1 \times 10^{-14}\ \text{M}^2$. When there are exactly equal concentrations of both H^+ and OH^-, as in pure water, the solution is said to be at **neutral pH**. At this pH, the concentration of H^+ and OH^- can be calculated from the ion product of water as follows :

$$K_w = [H^+]\,[OH^-] = [H^+]^2$$

Solving for $[H^+]$ gives :

$$\begin{aligned}
[H^+] &= \sqrt{K_w} = \sqrt{1 \times 10^{-14}\text{M}^2} \\
[H^+] &= [OH^-] = 10^{-7}\ \text{M.}
\end{aligned}$$

As the ion product of water is constant, whenever the concentration of H^+ ions is greater than 1×10^{-7} M, the concentration of OH^- must become less than 1×10^{-7} M, and vice versa. When the concentration of H^+ is very high, as in a solution of hydrochloric acid, the OH^- concentration must be very low.

THE pH SCALE

The ion product of water, K_w, is the basis for the **pH scale** (Table 3–1). It is a convenient means of designating the actual concentration of H^+ (and thus of OH^-) in any aqueous solution in the range

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between 1.0 M H^+ and 1.0 M OH^-. Biochemical reactions are often defined in terms of hydrogen ion (H^+) concentrations. In 1909, Soren Sörensen, a Danish biochemist, used a logarithmic scale for expressing the H^+ concentration. This scale was called pH, where *p* stands for power and H for hydrogen ion concentration. He defined pH of a solution as the negative logarithm of the concentration (in moles/litre) of hydrogen ions. Thus,

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

The symbol *p* denotes "negative logarithm of ".

For a precisely neutral solution at 25°C, in which the concentration of hydrogen ions is 1.0×10^{-7} M, the pH can be calculated as follows :

$$pH = \log \frac{1}{1 \times 10^{-7}} = \log (1 \times 10^7)$$

$$= \log 1.0 + \log 10^7$$

$$= 0 + 7.0$$

$$= 7.0$$

In fact, the term **pH** was introduced in 1909 by Sörensen who defined it as:

> "Für die Zahl *p* schlage ich den Namen Wasserstoffionenexponent und die Schreibweise pH vor. Unter dem Wasserstoffionexponenten (pH) einer Lösung wird dann der Briggsche Logarithmus des reziproken Wertes des auf Wasserstoffionen bezagenen Normalitäts faktors de Lösung verstanden."

The translation into English is :

> "For the sign *p* I propose the name 'hydrogen ion exponent' and the symbol pH. Then, for the hydrogen ion exponent (pH) of a solution, the negative value of the Briggsian logarithm of the related hydrogen ion normality factor is to be understood."

pH (short for "potential of hydrogen") is a symbol and denotes the relative concentration of hydrogen ions in a solution. pH values extend from O to 14; the lower the value, the higher the acidity or the more hydrogen ions the solution contains. Water at 25 °C has a concentration of H ion of 10^{-7}; the pH, therefore, is 7.

The value of 7.0 for the pH of a precisely neutral solution is not an arbitrarily chosen figure. *It is derived from the absolute value of the ion product of water at 25°C, which by convenient coincidence is a round number.*

To calculate the pH of a solution :

1. Calculate the hydrogen ion concentration, $[H^+]$.
2. Calculate the base 10 logarithm of $[H^+]$.
3. pH is the negative of the value found in step 2.

For example, for pure water at 25°C :

$$pH = -\log [H^+] = -\log 10^{-7} = -(-7) = 7.0$$

The number of hydrogen ions present in a solution is a measure of the **acidity of the solution.** All acids do not ionize completely when dissolved in water, *i.e.*, all the molecules of acid do not ionize and exist in the solution as electrically-charged particles. The hydrogen ion concentration is a measure, therefore, of the amount of *dissociated acid* rather than of the anmount of acid present. Strong acids dissociate more freely than weak acids; hydrochloric acid, for example, dissociates freely into H^+ and Cl^- whereas carbonic acid, a weak acid, dissociates much less freely into H^+ and CO_3^-. The number of free hydrogen ions is a measure of its acidity rather than an indication of the type of molecule from which the hydrogen ions originated. The **alkalinity of a solution** is dependent upon the number of hydroxyl ions present. Water is a neutral solution because each molecule contains one H^+ and one OH^-. For each molecule of water dissociated, there is one H^+ and one OH^-, each one neutralizing the other.

The pH scale was developed taking water as the standard. It is an experimental fact that only 1 mole in 5,50,000,000 moles of water ionizes into a H^+ and OH^-. This is the same proportion as 1 gram hydrogen ion in 10,000,000 litres of water. Hence, 1 litre of water contains 1/10,000,000 (or $1/10^7 = 10^{-7}$) of a gram of H^+. Later, for every day use, only the 'power' figure was used and the symbol pH placed before it.

A neutral solution, such as water, where the number of hydrogen ions is balanced by the same number of hydroxyl ions, has a pH of 7.0. The range of the pH scale is from 0 to 14. If the pH is 0, it would mean that 1 litre of water contained 1/1 = 1 gram hydrogen ion; or, at the other end of the scale, if there were no hydrogen ion present, it would be written $1/10^{14}$ or 10^{-14} or pH 14. The pH scale, thus, runs from 1 to 14; neutrality being at pH 7.0. Solutions having a pH lesser than 7 are acidic, *i.e.*, the concentration of H^+ is greater than that of OH^-. Conversely, solutions having a pH more than 7 are basic or alkaline, *i.e.*, denote an excess of OH^- over H^+ (Table 3–1). Washing soda for example, has a pH of ca 11 (Fig. 3-1).

Fig. 3-1. **pH of washing soda indicates it is basic in nature**

Table 3–1. **The pH scale**

[H^+] *(M)*	*pH*	*[OH^-]* *(M)*	*pOH**
10^0(1)	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^{-0}(1)	0

* The expression of pOH is sometimes used to describe the basicity, or OH^- concentration of a solution. pOH is defined by the expression pOH = – log [OH^-], which is analogous to the expression for pH. *Note that for all cases, pH + pOH = 14.*

Note that *the pH scale is logarithmic, not arithmetic.* Thus, when the pH of a solution decreases one unit from 5 to 4, the H^+ concentration has increased tenfold from 10^{-5} to 10^{-4} M, since decimal logarithms are used for the pH scale. Similarly, when the pH has increased three-tenth of a unit from 6.0 to 6.3, the H^+ concentration has decreased from 10^{-6} M to 5×10^{-7} M. To say that two solutions differ in pH by 1 pH unit means that one solution has ten times the H^+ concentration of the other, but it does not tell us the absolute magnitude of the difference. Figure 3–2 gives the pH of some common aqueous fluids. A coca cola drink (pH 3.0) or red wine (pH 3.7) has an H^+ concentration approximately 10,000 times greater than that of blood (pH 7.4). If we now apply the term of pH to the ion product expression for pure water, we obtain another useful expression :

$$[H^+] \times [OH^-] = 1.0 \times 10^{-14}$$

On taking logarithmic of this equation :

$$\log [H^+] + \log [OH^-] = \log (1.0 \times 10^{-14}) = -14$$

and then multiplying by –1, we get :

$$-\log [H^+] - \log [OH^-] = 14$$

If we now define $-\log [OH^-]$as pOH, a definition similar to that of pH, we have an expression relating the pH and pOH in any aqueous solution :

$$pH + pOH = 14$$

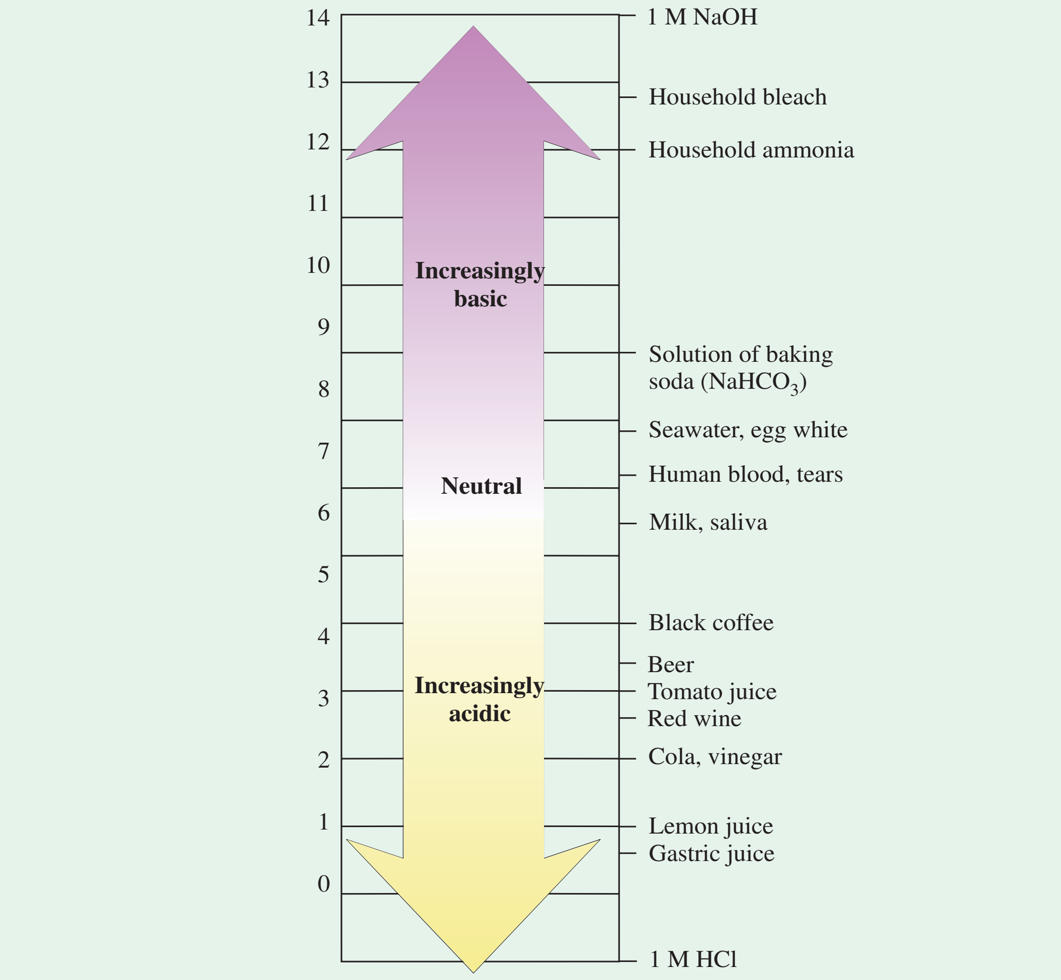

Fig. 3–2. The pH of some aqueous fluids

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Measurement of pH is one of the most important and frequently used procedures in Biochemistry (Fig. 3-3). The pH affects the structure and activity of biological macromolecules; for example, the catalytic activity of enzymes. Measurements of the pH of the blood and urine are commonly used in diagnosing disease. The pH of the blood plasma of severely diabetic people, for example, is often lower than the normal value of 7.4; this condition is called **acidosis.** In certain other disease states, the pH of the blood is higher than normal, a condition called **alkalosis.**

Fig. 3-3. Testing pH by pH meter

BRÖNSTED-LOWRY CONCEPT OF ACIDS AND BASES

J.N. Brönsted **M. Lowry**

Both famous for developing the familiar concept of acids and bases.

In aqueous systems, the addition or removal of hydrogen ions is best understood in terms of the Brönsted–Lowry concept of acids and bases, propounded in 1923. A **Brönsted–Lowry acid** is defined as a substance that can donate a proton (H^+); conversely, a **Brönsted–Lowry base** is a substance that can accept a proton. A proton donor (*i.e.*, an acid) and its corresponding proton acceptor (*i.e.*, a base) make up a **conjugate** (*coniungere*^{L} = to join together) **acid–base pair** (Table 3–2). This broad definition of acids and bases includes many substances that are not usually considered acidic or basic. In the general expressions :

$$HA \rightleftharpoons H^+ + A^-$$
$$HB^+ \rightleftharpoons H^+ + B:$$
$$HB^- \rightleftharpoons H^+ + B^{2-}$$

HA, HB^+ and HB^- represent Brönsted–Lowry acids, and the anion (A^-), neutral species ($B:$) and the negatively–charged species (B^{2-}), produced by the loss of a proton, are their respective conjugate bases. Note that each conjugate base can accept a proton to restore the corresponding acid. Because many biomolecules are capable of donating or accepting protons, they influence the H^+ concentration in biological systems. G.N. Lewis, also in 1923, proposed yet another definition of acids and bases. According to him, an acid is a compound which can accept a pair of electrons from a base. Such compounds are also called **electrophile** (electron-loving). A base is defined as a compound which can donate an electron pair to an acid. Such compounds are also called **nucleophile** (nucleus-loving).

A. Strong and Weak Acids

There are two general classes of acids — strong and weak. A **strong acid** is defined as a substance that has a greater tendency to lose its proton and therefore completely dissociates (or ionizes) in water, such as HCl and H_2SO_4. A **weak acid**, on the other hand, is a molecule that has a lesser

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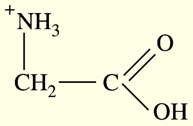
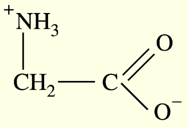
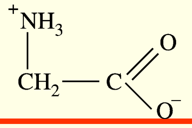
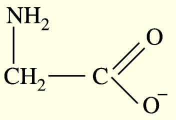
Table 3–2. Some Brönsted–Lowry acids and their conjugate bases*

Acid	*Undissociated acid (HA)*	*Conjugate base (A^-)*
Acetic Acid	CH_3COOH	CH_3COO^-
Propionic acid	CH_3CH_2COOH	$CH_3CH_2COO^-$
Pyruvic acid	$CH_3COCOOH$	CH_3COCOO^-
Phosphoric acid	H_3PO_4	$H_2PO_4^-$
Dihydrogen phosphate	$H_2PO_4^-$	HPO_4^{2-}
Monohydrogen phosphate	HPO_4^{2-}	PO_4^{3-}
Ammonium ion	NH_4^+	NH_3
Carbonic acid	H_2CO_3	HCO_3^-
Bicarbonate ion	HCO_3^-	CO_3^{2-}
Water	HOH	OH^-
Glycine		
		

* Some compounds, such as acetic acid, are **monoprotic**, as they can give up only one proton. Others are **diprotic** (carbonic acid and glycine) or **triprotic** (phosphoric acid).

tendency to lose its proton (or, in other words, displays a high affinity for its proton) and, therefore, does not readily dissociate in water, such as CH_3COOH.

B. Ionization of Weak Acids

The selective dissociation of weak acids in water is a characteristic. It is of great importance to Biochemistry because of its role in influencing the H^+ concentration of an aqueous environment. The dissociation of the weak organic compound, acetic acid, is written as :

$$CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$$

At a given temperature, the extent of ionization at equilibrium can be calculated by the following equation :

$$K_a' = [H^+]\,[CH_3\,COO^-]\,/\,[CH_3\,COOH]$$

The expression is comparable to that used to describe the dissociation of water, except that the symbol K_a' (apparent ionization constant) is substituted for K'_{eq} (equilibrium constant). The change recognizes the reaction as an ionization. The prime (′) symbol on the K_a' is used to identify the ionization constant as *apparent* (value based on the concentrations of reactants and products) rather than as a *true* value (K_a' corrected for deviation of the system from ideal behaviour).

The slight amount of ionization that a 1 M solution of acetic acid undergoes ($K_a' = 1.8 \times 10^{-5}$ M at 25°C) can be readily calculated by leting $x = [H^+]$ and $[CH_3COO^-]$ and not by correcting $[CH_3\,COOH]$, which is a relatively large concentration, for the small amount of x produced.

$$x^2 = 1.8 \times 10^{-5}\ \text{M}$$

$$x = 4.2 \times 10^{-3}\ \text{M or } 0.0042\ \text{M}$$

Thus, only slightly over 0.4 per cent of a 1 M solution of acetic acid is ionized at 25°C. The pH of the solution is 2.38 (*i.e.*, – log 0.0042).

C. Titration of a Weak Acid by a Strong Base

Titration is used to determine the amount of an acid in a given solution. In this procedure (Fig. 3-4), a measured volume of the acid is titrated with a solution of a strong base (usually NaOH) of known concentration. The NaOH is added in small increments until the acid is consumed (or neutralized), as determined with an indicator dye or with a pH meter. The concentration of the acid in the original solution can be calculated from the volume and concentration of NaOH added. A plot of the pH against the amount of NaOH added (a **titration curve**) reveals the pK_a of the weak acid.

Fig. 3-4. **A pH titration in action**

Equilibrium constants for ionization reactions are more usually called *ionization or dissociation constants* and are often designated K_a. Table 3–3 lists dissociation constants of some weak acids. Stronger acids, such as formic and lactic acids, have higher dissociation constants; weaker acids, such as dihydrogen phosphate ($H_2PO_4^-$), have lower dissociation constants. Also included in the Table 3–3 are values of pK_a, which is analogous to pH and is defined by the equation :

$$pK_a = \log \frac{1}{K_a} = -\log K_a$$

The more strongly dissociated the acid, the lower is its pK_a.

A plot of the course of the titration reveals the property of Brönsted–Lowry acids and their conjugate bases that makes them useful in Biology. When acetic acid (CH_3COOH) is titrated with NaOH (Fig. 3–5), the greatest changes in pH take place at the beginning and end of the titration. The region of least change occurs at the midpoint of titration, when exactly 0.5 equivalents of base have been added. At this point, the concentration of undissociated acid (CH_3COOH or HA) is equal to that of its anion (CH_3COO^- or A^-). At these particular concentrations of HA and A^-, the pH (4.76) is equal to the pK_a' value.

Table 3–3. **Dissociation constant and pKa of some common weak acids (proton donors) at 25°C**

Acid	*Formula*	*K_a (M)*	*pK_a*
Formic acid	HCOOH	1.78×10^{-4}	3.75
Acetic acid	CH_3COOH	1.74×10^{-5}	4.76
Propionic acid	CH_3CH_2COOH	1.35×10^{-5}	4.87
Lactic acid	$CH_3CH(OH)COOH$	1.38×10^{-4}	3.86
Phosphoric acid	H_3PO_4	7.25×10^{-3}	2.14
Dihydrogen phosphate	$H_2PO_4^-$	1.38×10^{-7}	6.86
Monohydrogen phosphate	HPO_4^{2-}	3.98×10^{-13}	12.40
Carbonic acid	H_2CO_3	1.70×10^{-4}	3.77
Biocarbonate	HCO_3^-	6.31×10^{-11}	10.20
Ammonium	NH_4^+	5.62×10^{-10}	9.25

The most important point about the titration curve of a weak acid is that it shows graphically that a weak acid and its anion — a conjugate acid-base pair—can act as a buffer.

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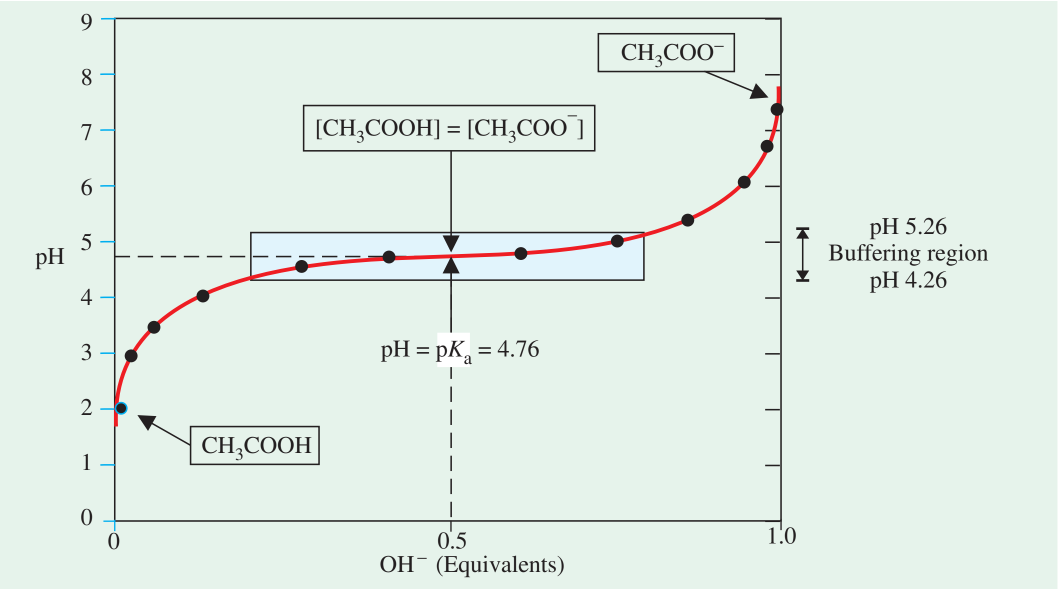

Fig. 3–5. The titration curve of acetic acid

After the addition of each increment of NaOH to the acetic acid solution, the pH of the mixture is measured. This value is plotted against the fraction of the total amount of NaOH required to neutralize the acetic acid (*i.e.*, to bring it to pH ≈ 7). The points so obtained yield the titration curve. Shown in the boxes are the predominant ionic forms at the points designated. At the midpoint of the titration, the concentration of the proton donor and proton acceptor are equal. The pH at this point is numerically equal to the pK_a of acetic acid. The screened zone is the useful region of buffering power.

Fig. 3–6 compares the titration curves 3 weak acids with very different dissociation constants: acetic acid ($pK_a = 4.76$), dihydrogen phosphate ($pK_a = 6.86$) and ammonium ion ($pK_a = 9.25$). Although the titration curves of these acids have the same shape, they are displaced along the pH axis because these acids have different strengths. Acetic acid is the strongest and loses its proton most readily, since its K_a is highest (pK_a lowest) of the three. Acetic acid is already half dissociated at pH 4.76. $H_2PO_4^-$ loses a proton less readily, being half dissociated at pH 6.86. NH_4^+ is the weakest acid of the three and becomes half dissociated only at pH 9.25.

BUFFERS

A **buffer solution** is one that resists a change in pH on the addition of acid (H^+) or base (OH^-), more effectively than an equal volume of water. Most commonly, the buffer solution consists of a mixture of a weak Brönsted acid and its conjugate base; for example, mixtures of acetic acid and sodium acetate or of ammonium hydroxide and ammonium chloride are buffer solutions. A buffer system consists of a weak acid (the proton donor) and its conjugate base (the proton acceptor). As an example, a mixture of equal concentrations of acetic acid and acetate ion, found at the midpoint of the titration curve in Fig. 3–2, is a buffer system. The titration curve of acetic acid has a relatively flat zone extending about 0.5 pH units on either side of its midpoint pH of 4.76. In this zone, there is only a small change in pH when increments of either H^+ or OH^- are added to the system. This relatively flat zone is the buffering region of the acetic acid-acetate buffer pair. At the midpoint of the buffering region, where the concentration of the proton donor (acetic acid) exacts equals that of the proton acceptor (acetate), the buffering power of the system is maximal, *i.e.*, its pH changes least on addition of an increment of H^+ or OH^-. The pH at this point in the titration curve of acetic acid is equal to its pK_a. The pH of the acetate buffer system does change slightly when a small amount of H^+ or OH^- is

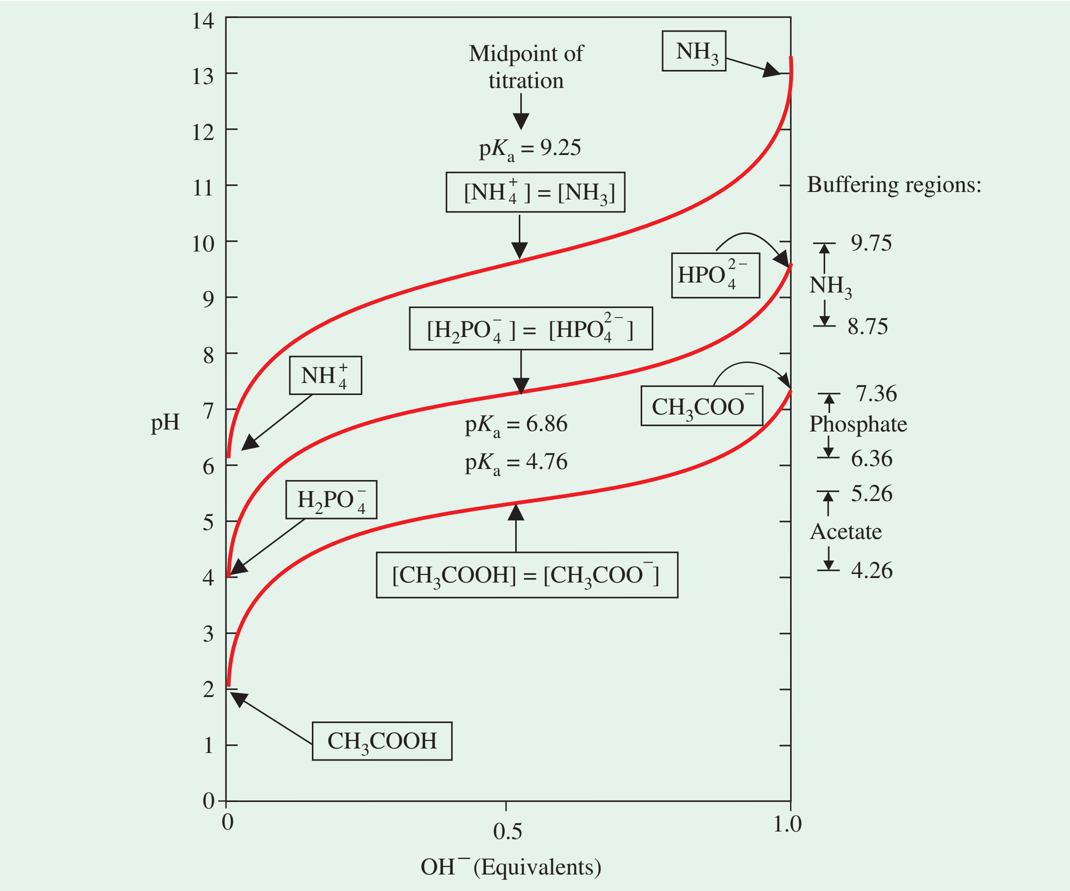

Fig. 3–6. Comparison of the titration curves of 3 weak acids, CH_3COOH, $H_2PO_4^-$ and NH_4^+

The predominant ionic forms at designated points in the titration are given in boxes. The regions of buffering capacity are indicated at the right. Conjugate acid-base pairs are effective buffers between approximately 25 and 75% neutralization of the proton-donor species.

added, but this change is very small compared with the pH change that would result if the same amount of H^+ (or OH^-) were added to pure water or to a solution of the salt of a strong acid and strong base, such as NaCl, which have no buffering power.

Each conjugate-acid-base pair has a characteristic pH zone in which it is an effective buffer (Fig. 3–3). The $H_2PO_4^-$ / HPO_4^{2-} pair has a pK_a of 6.86 and thus can serve as a buffer system near pH 6.86; the NH_4^+/NH_3 pair, with a pK_a of 9.25, can act as a buffer near pH 9.25.

Let us consider the mechanism by which a buffer solution exerts control over large pH changes, by taking example of a buffering system consisting of acetic acid and sodium acetate.

$$CH_3COOH + CH_3COONa \longrightarrow Na^+ + H^+ + 2CH_3COO^-$$

If alkali (NaOH) is added to this system, it will form salt and no free H^+ or OH^- will be available :

$$CH_3COOH + CH_3COONa + NaOH \longrightarrow 2CH_3COONa + H_2O$$

If, however, acid (HCl) is added to this system, it will also form salt and no free H^+ or OH^- will be available :

$$CH_3COOH + CH_3COONa + HCl \longrightarrow NaCl + 2CH_3COOH$$

In either cases, there is no change in H^+ concentration. The buffer acts almost as if it were ‘absorbing’ the added free hydrogen or hydroxyl ions.

In discussing the quantitative aspects of buffer action, we must note that two factors determine the effectiveness or *capacity* of buffer solution :

(*a*) *Molar concentration of the buffer components.* The buffer capacity is directly proportional to the concentration of the buffer components. The concentration of a buffer refers to the sum of the concentration of the weak acid and its conjugate base. Thus, a 0.1 M acetate buffer could contain 0.05 mole of acetic acid and 0.05 mole of sodium acetate in 1 litre of water. It could also contain 0.065 mole of acetic acid and 0.035 mole of sodium acetate in 1 litre of water.

(*b*) *Relative concentrations of the conjugate base and the weak acid.* Quantitatively, it should seem evident that the most effective buffer would be one with equal concentrations of acidic and basic components, since such a mixture could furnish equal quantities of acidic and basic components to react, respectively with alkali or acid. An inspection of the titration curve for acetic acid (Fig. 3–3) shows that the minimum change in pH resulting from the addition of a unit of base (or acid) occurs at the pK_a for acetic acid. At this pH, the ratio CH_3COO^- to CH_3COOH is 1. On the contrary, at values of pH far removed from the pK_a (and therefore at ratios of conjugate base to acid greatly differing from unity), the change in pH for unit of acid or alkali added is much larger.

The hydrogen ion concentration of most body fluids and secretions is on the alkaline side. Urine may be acid, and gastric juice may be very acid, but these are exceptions. Many influences tend to change this alkalinity, but the buffers present prevent marked fluctuations in hydrogen ion concentrations. The pH of blood, for instance, stays within the limits 7.3 to 7.5 in health. When these limits are exceeded, acidosis or alkalosis with alarming symptoms results, frequently with dire consequences. Table 3.4 gives some of the pH values for various human fluids.

It is quite difficult to keep a solution at constant pH if no buffer is present because of the influence of the CO_2 of the air or the alkali of the glass container or because of other influences. Consequently, buffers are frequently required. Various mixtures, consisting of definite amounts of the acid or base and its respective salt have been prepared. Since such buffer sets maintain their pH indefinitely, they are used in the indicator method of determining pH. It should be noted that buffers can be prepared not only by titration of a weak acid with OH^- (or titration of a solution of A^- with H^+), but also by addition of A^-, the salt of HA, to a solution of HA.

Table 3–4. pH values of human body fluids and secretions

Body fluid/Secretion	*pH*	*Body fluid/Secretion*	*pH*
Gastric juice (parietal secretion)	0.87	Blood	7.4
		Cerebrospinal fluid	7.4
Urine	6.0	Intestinal juice	7.7
Milk	6.6 – 6.9	Bile	7.8
Saliva	7.2	Pancreatic Juice	8.0
Aqueous humor of eye	7.2		

A. Henderson–Hasselbalch Equation

The quantitative relationship among pH, buffering action of a mixture of weak acid with its conjugate base, and the pK_a of the weak acid is given by a simple expression called **Henderson-Hasselbalch Equation.** The titration curves of acetic acid, $H_2PO_4^-$ and NH_4^+ (Fig. 3–3) have nearly identical shapes, suggesting that they all point towards a fundamental law or relationship. This is actually the case. The shape of the titration curve of any weak acid is expressed by Henderson–Hasselbalch equation. This equation is simply a useful way of restating the expression for the dissociation constant of an acid. For the dissociation of a weak acid HA into H^+ and A^-, the Henderson–Hasselbalch equation can be derived as follows :

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

1. Rearrange the K_a equation to solve for $(H^+]$:

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

2. Convert to logarithmic functions :

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

3. Make the expression negative (or multiply by –1) :

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

4. Substitute pH for – log $[H^+]$ and pK_a for –log K_a

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

5. Now, to remove the minus sign, invert the last term, *i.e.*, $-\log \frac{[HA]}{[A^-]}$ to obtain Henderson-Hasselbalch equation :

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

The equation is expressed more generally as :

$$\text{pH} = pK_a + \log \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

This equation fits the titration curve of all weak acids and enables one to deduce a number of important quantitative relationships. Henderson-Hasselbalch equation is of great predictive value in protonic equilibria as illustrated below :

A. When [A–] = [HA] or when an acid is exactly half neutralized : Under these conditions,

$$\text{pH} = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{1}{1} = pK_a + 0 = pK_a.$$

Therefore, at half neutralization, pH = pK_a. The equation, thus, shows why the pK_a of a weak acid is equal to the pH of the solution at the midpoint of its titration.

B. When the ratio [A–]/[HA] = 100 to 1 :

$$\text{pH} = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{100}{1} = pK_a + 2$$

C. When the ratio [A–]/[HA] = 1 to 10 :

$$\text{pH} = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{1}{10} = pK_a + (-1)$$

If the equation is evaluated at several ratios of $[A^-]$ / [HA] between the limits 10^3 and 10^{-3}, and the calculated pH values plotted, the result obtained describes the titration curve for a weak acid as shown in Fig. 3–7.

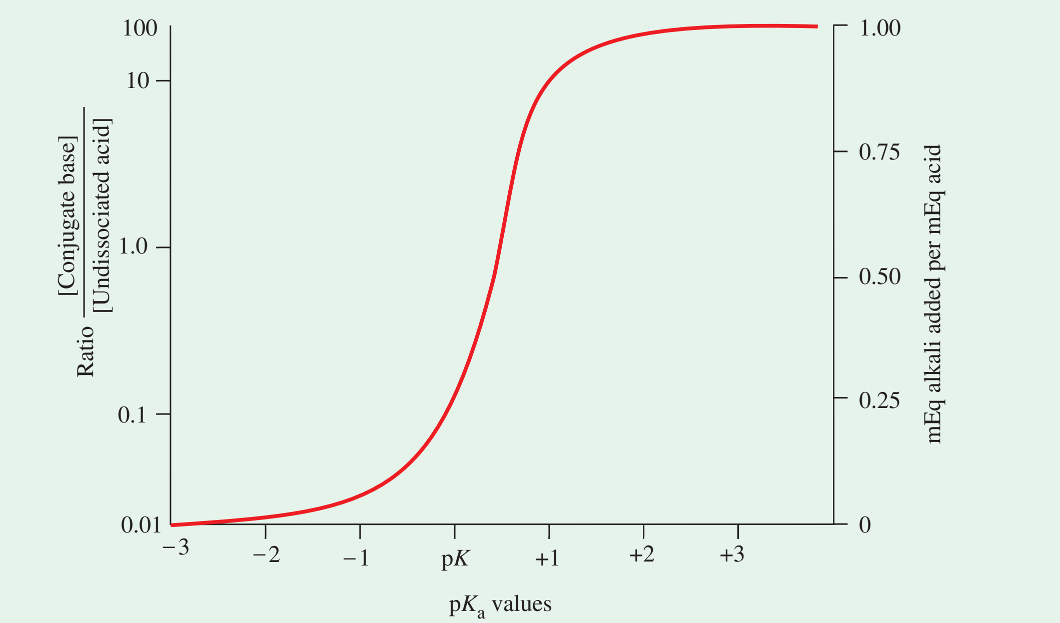

Fig. 3–7. General form of a titration curve calculated from the Henderson-Hasselbalch equation

BIOLOGICAL BUFFER SYSTEMS

Almost every biological process is pH-dependent; a small change in pH produces a large change in the rate of the process. This is true not only for the many reactions in which the H^+ ion is a direct participant, but also for those in which there is no apparent role for H^+ ions. The enzymes and many of the molecules on which they act, contain ionizable groups with characteristic pK_a values. The protonated amino ($-NH_3^+$) and carboxylic groups of amino acids and the phosphate groups of nucleotides, for example, function as weak acids; their ionic state depends upon the pH of the solution in which they are dissolved.

Cells and organisms maintain a specific and constant cytosolic pH, keeping biomolecules in their optimal ionic state, usually near pH 7. In multicelled organisms, the pH of the extracellular fluids (blood, for example) is also tightly regulated. Constancy of pH is achieved primarily by **biological buffers** : mixtures of weak acids and their conjugate bases. Table 3–5 lists some important buffering systems of body fluids which help maintaining pH. A certain amount of many of these is usually present in the body and cellular fluids, and so the maintenance of a constant pH depends on a complex system.

Table 3–5. Body fluids and their principal buffers

Body fluids	*Principal buffers*
Extracellular fluids	Biocarbonate buffer Protein buffer
Intracellular fluids	Phosphate buffer Protein
Erythrocytes	Hemoglobin buffer

A description of various buffering systems follows :

1. The Phosphate Buffer System

This system, which acts in the cytoplasm of all cells, consists of $H_2PO_4^-$ as proton donor and HPO_4^{2-} as proton acceptor :

$$H_2\,PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$$

The phosphate buffer system works exactly like the acetate buffer system, except for the pH range in which it functions. The phosphate buffer system is maximally effective at a pH close to its pK_a of 6.86 (see Table and Fig.), and thus tends to resist pH changes in the range between 6.4 and 7.4. It is, therefore, effective in providing buffering power in intracellular fluids.

Since the concentration of phosphate buffer in the blood plasma is about 8% of that of the bicarbonate buffer, its buffering capacity is much lower than bicarbonate in the plasma. The concentration of phosphate buffer is much higher in intracellular fluid than in extracellular fluids. The pH of intracellular fluids (6.0 – 6.9) is nearer to the pK_a of the phosphate buffer. Therefore, the buffering capacity of the phosphate buffer is highly elevated inside the cells and the phosphate is also effective in the urine inside the renal distal tubules and collecting ducts.

In case the ratio of $[HPO_4^{2-}]$ / $[HPO_4^-]$ tends to be changed by the formation of more $H_2PO_4^-$, there occurs the renal elimination of $H_2PO_4^-$ for which the ratio ultimately remains unaltered.

2. The Bicarbonate Buffer System

This is the main extracellular buffer system which (also) provides a means for the necessary removal of the CO_2 produced by tissue metabolism. The bicarbonate buffer system is the main buffer in blood plasma and consists of carbonic acid as proton donor and bicarbonate as proton acceptor :

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

This system has an equilibrium constant

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3]}$$

and functions as a buffer in the same way as other conjugate acid-base pairs. It is unique, however, in that one of its components, carbonic acid, is formed from dissolved (*d*) carbon dioxide and water, according to the reversible reaction :

$$CO_2(d) + H_2O \rightleftharpoons H_2CO_3$$

which has an equilibrium constant given by the expression :

$$K_2 = \frac{[H_2CO_3]}{[CO_2(d)][H_2O]}$$

Carbon dioxide is a gas under natural conditions, and the concentration of dissolved CO_2 is the result of equilibration with CO_2 of the gas phase (*g*) :

$$CO_2\,(g) \rightleftharpoons CO_2(d)$$

This process has an equilibrium constant given by :

$$K_3 = \frac{[CO_2\,(d)]}{[CO_2(g)]}$$

The pH of a bicarbonate buffer system depends on the concentration of H_2CO_3 and HCO_3^-, the proton donor and acceptor components. The concentration of H_2CO_3, in turn, depends on the concentration of dissolved CO_2, which, in turn, depends on the concentration or partial pressure of CO_2 in the gas phase.

With respect to the bicarbonate system, a $[HCO_3^-]$ / $[H_2CO_3]$ ratio of 20 to 1 is required for the pH of blood plasma to remain 7.40. The concentration of dissolved CO_2 is included in the $[H_2CO_3]$ value, *i.e.*,

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$$[H_2CO_3] \; = \; [H_2CO_3] + [CO_2 \text{ (dissolved)}]$$

If there is a change in the ratio in favour of H_2CO_3, **acidosis** results. This change can result from a decrease in $[HCO_3^-]$ or from an increase in $[H_2CO_3]$. Most common forms of acidosis are metabolic or respiratory. *Metabolic acidosis* is caused by a decrease in $[HCO_3^-]$ and occurs, for example, in uncontrolled diabetes with ketosis or as a result of starvation. *Respiratory acidosis* is brought about when there is an obstruction to respiration (euphysema, asthma or pneumonia) or depression of respiration (toxic doses of morphine or other respiratory depressants). If acidosis is not treated promptly, the patient may go into a comma.

Alkalosis results when $[HCO_3^-]$ becomes favoured in the bicarbonate/carbonic acid ratio. *Metabolic alkalosis* occurs when the HCO_3^- fraction increases with little or no concomitant change in H_2CO_3. Severe vomiting (loss of H^+ as HCl) or ingestion of excessive amounts of sodium bicarbonate (bicarbonate of soda) can produce this condition. *Respiratory alkalosis* is induced by hyperventilation because an excessive removal of CO_2 from the blood results in a decrease in $[H_2CO_3]$. Hyperventilation can result in anxiety, hysteria, prolonged hot baths or lack of O_2 at high altitudes. Alkalosis can produce convulsive seizures in children and tetany, hysteria, prolonged hot baths or lack of O_2 as high altitudes. Alkalosis can produce convulsive seizures in children and tetany in adults (characterized by sharp flexion of the wrist and ankle joints, muscle twitchings, and cramps).

The pH of blood is maintained at 7.4 when the buffer ratio $[HCO_3^-]/[H_2CO_3]$ becomes 20. If the bicarbonate neutralizes any acid or base, there may be the change of buffer ratio and the blood pH value. But the buffer ratio remains by the respiratory elimination of H_2CO_3 as CO_2 or the urinary elimination of HCO_3^-.

Since cells contain much lower amounts of HCO_3^-, the importance of bicarbonate buffer inside the cell is negligible.

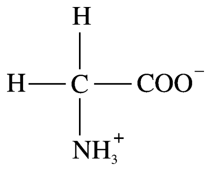
3. The Protein Buffer Systems

The protein buffers are very important in the plasma and the intracellular fluids but their concentration is very low in cerebrospinal fluid, lymph and interstitial fluids. The proteins exist as anions serving as conjugate bases (Pr^-) at the blood pH 7.4 and form conjugate acids (HPr) accepting H^+. They have the capacity to buffer some H_2CO_3 in the blood.

$$H_2CO_3 + Pr^- \rightleftharpoons HCO_3^- + HPr$$

4. The amino acids buffer system

This system also operates in humans. Amino acids contain in their molecule both an acidic (– COOH) and a basic (– NH_2) group. They can be visualized as existing in the form of a neutral zwitterion in which a hydrogen atom can pass between the carboxyl and amino groups. The glycine may, thus, be represented as :



$$H-\overset{\displaystyle H}{\underset{\displaystyle NH_3^+}{C}}-COO^-$$

By the addition or subtraction of a hydrogen ion to or from the zwitterion, either the cation or anion form will be produced :

$$\underset{\text{Cation form}}{^+H_3N-CH_2-COOH} \xleftarrow{+H^+} \underset{\text{Zwitterion}}{^+H_3N-CH_2-COO^-} \rightleftharpoons \underset{\text{Anion form}}{H_2N-CH_2-COO^- + H^+}$$

Thus, when OH^- ions are added to the solution of amino acid, they take up H^+ from it to form water, and the anion is produced. If H^+ ions are added, they are taken up by the zwitterion to produce the cation form. In practice, if NaOH is added, the salt $H_2N-CH_2-COONa$ would be formed

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and the addition of HCl would result in the formation of amino acid hydrochloride, $ClH{—}H_3N{—}CH_2{—}COOH$, but these substances would ionize in solution to some extent to form their corresponding ions. Hemoglobin and plasma proteins act as buffers in a similar way.

Amino acids differ in the degree to which they will produce the cation or anion form. In other words, a solution of an amino acid is not neutral but is either predominantly acidic or basic, depending on which form is present in greater quantity. For this reason, different amino acids may be used as buffers for different pH values, and a mixture of them possesses a wide buffer range.

5. The Hemoglobin Buffer Systems

These buffer systems are involved in buffering CO_2 inside erythrocytes. The buffering capacity of hemoglobin depends on its oxygenation and deoxygenation. Inside the erythrocytes, CO_2 combines with H_2O to form carbonic acid (H_2CO_3) under the action of carbonic anhydrase. At the blood pH 7.4, H_2CO_3 dissociates into H^+ and HCO_3^- and needs immediate buffering. Oxyhemoglobin (HbO_2^-), on the other side, loses O_2 to form deoxyhemoglobin (Hb^-) which remains undissociated (HHb) by accepting H^+ from the ionization of H_2CO_3. Thus, Hb^- buffers H_2CO_3 in erythrocytes :

$$HbO_2^- \rightleftharpoons Hb^- + O_2$$

$$Hb^- + H_2CO_3 \rightleftharpoons HHb + HCO_3^-$$

Some of the HCO_3^- diffuse out into the plasma to maintain the balance between intracellular and plasma bicarbonates. This causes influx of some Cl^- into erythrocytes along the electrical gradient produced by the HCO_3^- outflow (*chloride shift*).

$HHbO_2$, produced in lungs by oxygenation of HHb, immediately ionizes into H^+ and HbO_2^-. The released hydrogen ions (H^+) are buffered by HCO_3^- inside erythrocyte to form H_2CO_3 which is dissociated into H_2O and CO_2 by carbonic anhydrase. CO_2 diffuses out of erythrocytes and escapes in the alveolar air. Some HCO_3^- return from the plasma to erythrocytes in exchange of Cl^- and are changed to CO_2.

$$HHb + O_2 \rightleftharpoons HHbO_2 \rightleftharpoons HbO_2^- + H^+$$

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2$$

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Contents

PROBLEMS

1. What is the pH of each of the following solutions ?

(*a*) 0.35 M hydrochloric acid

(*b*) 0.35 M acetic acid

(*c*) 0.035 M acetic acid

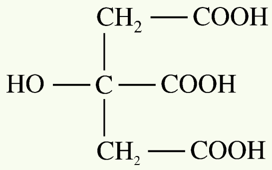
2. What is the pH of the following buffer mixtures ?

(*a*) 1 M acetic acid plus 0.5 M sodium acetate

(*b*) 0.3 M phosphoric acid plus 0.8 M KH_2PO_4

3. You need to make a buffer whose pH is 7.0, and you can choose from the weak acids shown in Table 3–3. Briefly explain your choice.

4. It is possible to make a buffer that functions well near pH 7, using citric acid, which contains only carboxylate groups. Explain.



$$\mathrm{HO-\overset{\displaystyle CH_2-COOH}{\underset{\displaystyle CH_2-COOH}{\overset{|}{\underset{|}{C}}}}-COOH}$$

Citric acid

5. If a weak acid is 91% neutralized at pH 5.7, what is the p*K*′ of the acid ?

6. Metabolic alkalosis is a condition in which the blood pH is higher than its normal value of 7.40 and can be caused, among other reasons, by an increase in $[HCO_3^-]$. If a patient has the following blood values, pH = 7.45, $[CO_2]$ = 1.25 mM, what is the $[HCO_3^-]$? The p*K*′ for the bicarbonate/carbonic acid system is 6.1. How does the calculated value of bicarbonate compare with the normal value ?

7. Why does pure water have no taste or colour ?