General Phenomena: Law of mass action, dissociation of water, pH, buffers

Ionization of Water, Weak Acids and Weak Bases

- Many properties of water can be explained in terms of uncharged H_2O molecule
- Small degree of ionization of water to hydrogen ions and hydroxide ions must be taken into account
- Like all reversible reactions, the ionization of water can be described by an equilibrium constant

- Weak acids are dissolved in water, they contribute H^+ by ionizing; bases consume H^+ by being protonated

- These processes are also governed by equilibrium constants

- Total hydrogen ion concentration from all sources is experimentally measurable and is expressed as pH of the solution

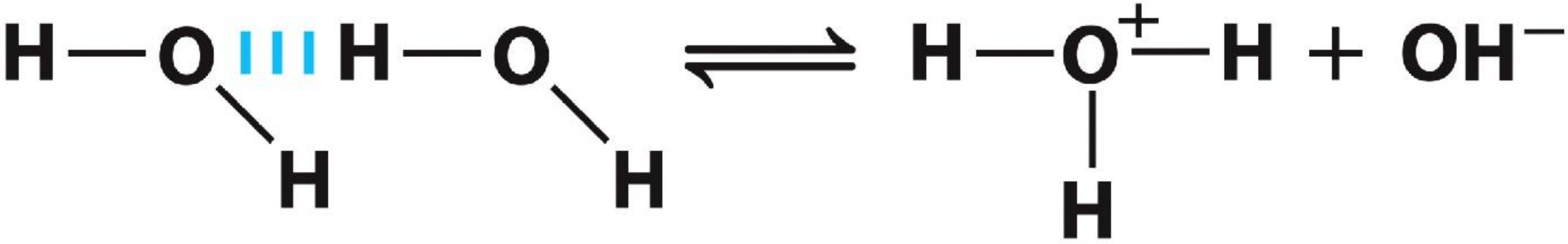
Pure Water is Slightly Ionized

- H_2O have a slight tendency to undergo reversible ionization to yield a hydrogen ion (proton) and a hydroxide ion

$$H_2O \longleftrightarrow H^+ + OH^-$$

- Dissociation product of water = H^+

- Free H^+ do not exist in solution
- H^+ ions formed are immediately hydrated to hydronium ions ($H3O^+$)
- Hydrogen binding between H_2O molecules makes the hydration of dissociating protons virtually instantaneous



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Lehninger Principles of Biochemistry, Sixth Edition

Law of mass action

- For the generalized reaction

$$A+B \longleftrightarrow C+D$$

- Rate of forward and reverse reaction is proportional to the molar concentrations of reactants A,B and products C,D respectively

Rf á [A][B], Rf=Kf[A][B]

Rr á [C][D], Rr=Kr[C][D]

At equilibrium

$$Rf = Rr$$

Or

$$Kf\,[A][B] = Kr\,[C][D]$$

Rearranging,

$$\frac{Kf}{Kr} = \frac{[C][D]}{[A][B]}$$

An equilibrium constant can be defined in terms of the concentrations of reactants (A and B) and products (C and D) at equilibrium;

$$\frac{Kf}{Kr} = Keq$$

or

Keq = $\frac{[C][D]}{[A][B]}$ (law of mass action eq.)

- **Keq**: fixed & characteristic for any given chemical reaction at a specified temperature

- **Keq**: Defines concentrations of final equilibrium mixtures, regardless of the starting amounts of reactants & products

- **Keq:** can calculate for a given reaction at a given temperature if the equilibrium concentrations of all its reactants & products are known

The Ionization of Water Is Expressed by an Equilibrium Constant

- Degree of ionization of H_2O is small
- Only about one out of 10^7 molecules in pure H_2O is ionized at any instant
- Equilibrium constant for reversible ionization of H_2O is

$$Keq = \frac{[C][D]}{[A][B]} \quad \text{(law of mass action eq.)}$$

Applying this equation to ionization of H_2O

$$Keq = \frac{[H^+][OH^-]}{[H_2O]}$$

- In pure water at 25°C, the concentration of H_2O is 55.5 M (grams of H_2O in 1L divided by its gram molecular weight; (1,000 g/L) / (18.015 g/mol)
- Accordingly, we can substitute 55.5M in the equilibrium constant expression to yield

$$Keq = \frac{[H^+][OH^-]}{55.5\ M}$$

- Which on rearranging, becomes

$$(55.5\ M)(Keq) = [H^+][OH^-] = Kw$$

- where **Kw** = the product (55.5 M)(Keq), the **ionic product of** H_2O at 25°C

- **Keq = 1.8 × 10^{-16} M** at 25°C (determined by electrical-conductivity measurements)

- **Kw** = $[H^+][OH^-]$ = **($55.5\ M$)($1.8 \times 10^{-16}\ M$) = $1.0 \times 10^{-14}\ M^2$**

- Thus the product $[H^+][OH^-]$ in aqueous solutions at 25°C always equals $1.0 \times 10^{-14}\ M^2$

- When there are exactly equal concentrations of both H^+ and OH^-, as in pure H_2O, 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 H_2O as follows:

$$Kw = [H^+][OH^-] = [H^+]^2$$

Solving for $[H^+]$ gives

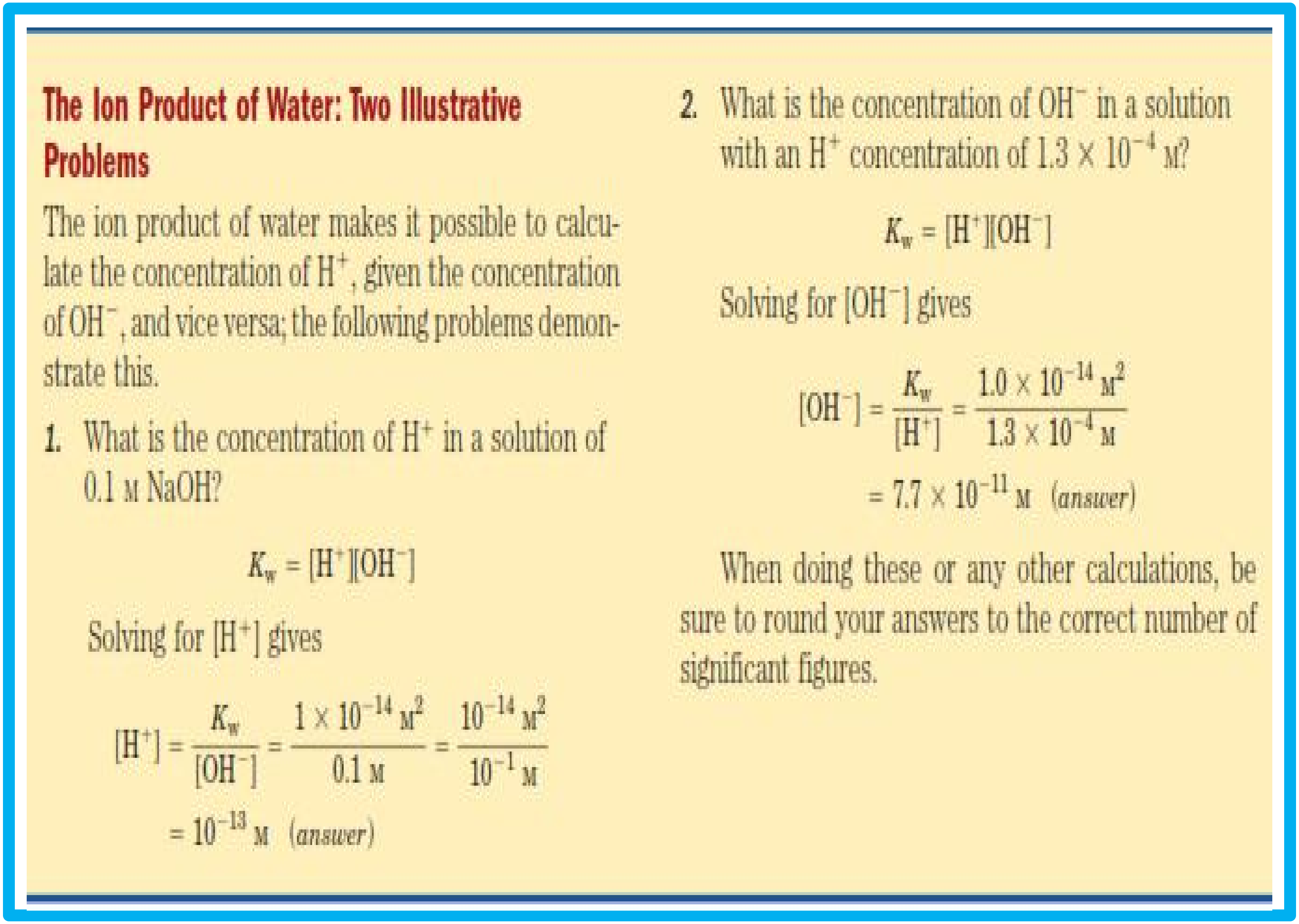
$$\sqrt{[H^+]^2} = \sqrt{Kw} = \sqrt{1.0} \times 10^{-14}\ M^2$$

$$[H^+] = [OH^-] = 10^{-7}\ M$$

- Kw is constant, whenever $[H^+]$ is greater than 1.0×10^{-7} M , $[OH^-]$ must be less than 1.0×10^{-7} M, and vice versa

- When $[H^+]$ high, (e.g. HCl), $[OH^-]$ must be very low.

- From the ion product of H_2O, we can calculate $[H^+]$ if we know $[OH^-]$ and vice versa.



The Ion Product of Water: Two Illustrative Problems

The ion product of water makes it possible to calculate the concentration of H^+, given the concentration of OH^-, and vice versa; the following problems demonstrate this.

1. What is the concentration of H^+ in a solution of 0.1 M NaOH?

$$K_w = [H^+][OH^-]$$

Solving for $[H^+]$ gives

$$[H^+] = \frac{K_w}{[OH^-]} = \frac{1 \times 10^{-14}\ \text{M}^2}{0.1\ \text{M}} = \frac{10^{-14}\ \text{M}^2}{10^{-1}\ \text{M}}$$
$$= 10^{-13}\ \text{M} \quad (\textit{answer})$$

2. What is the concentration of OH^- in a solution with an H^+ concentration of 1.3×10^{-4} M?

$$K_w = [H^+][OH^-]$$

Solving for $[OH^-]$ gives

$$[OH^-] = \frac{K_w}{[H^+]} = \frac{1.0 \times 10^{-14}\ \text{M}^2}{1.3 \times 10^{-4}\ \text{M}}$$
$$= 7.7 \times 10^{-11}\ \text{M} \quad (\textit{answer})$$

When doing these or any other calculations, be sure to round your answers to the correct number of significant figures.

The pH Scale Designates the H^+ and OH^- Concentrations

- Kw - Basis for the pH scale

- Convenient means of designating the H^+ (and thus of OH^-) in any aqueous solution

- Term pH is defined by the expression

$$pH = \log 1/ [H^+]$$

OR

$$pH = - \log [H^+]$$

TABLE 2–6 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 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 in all cases, pH + pOH = 14.

Table 2-6
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- Neutral solution at 25 °C, in which the concentration of H^+ is 1.0×10^{-7} M,

$$pH = - \log [H^+]$$

$$pH = - \log \ 1.0 \times 10^{-7}$$

$$= \ \ - \log (1.0 \times 10^{-7})$$

$$= - (\log 1.0 + \log 10^{-7)}$$

$$= - [0 + (-7 \log 10)]$$

$$= - [0 + (-7\times1)]$$

$$= - [0+(-7)]$$

$$= - (-7)$$

$$pH = 7$$

- pH scale - **Logarithmic**, not **arithmetic**

- When 2 solutions differ in pH by 1 pH unit, it means that one solution has ten times the H^+ concentration of the other, but it does not tell the absolute magnitude of the difference

- A cola drink (pH 3.0) has an H^+ concentration approximately 10,000 times that of blood (pH 7.4)
- pH can be **approximately** measured by various indicator dyes, including litmus, phenol red & phenolphthalein
- **Accurate** measurement of pH - pH meter

- Measurement of pH - most important & frequently used procedures in biochemistry
- pH affects structure & activity of biological macromolecules
- Catalytic activity of enzymes strongly dependent on pH

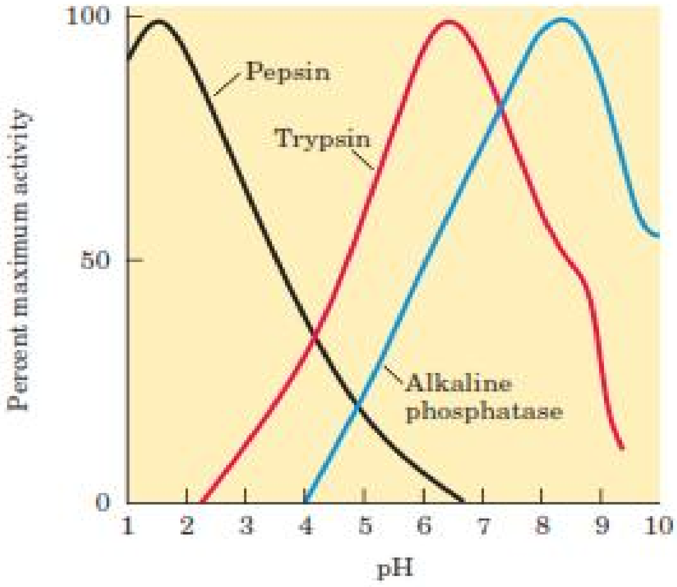


FIGURE 2-21 **The pH optima of some enzymes.** Pepsin is a digestive enzyme secreted into gastric juice; trypsin, a digestive enzyme that acts in the small intestine; alkaline phosphatase of bone tissue, a hydrolytic enzyme thought to aid in bone mineralization.

- Enzymes – optimum pH
- Measurement of the pH of blood & urine commonly used in medical diagnosis
- Normal pH of blood plasma - 7.4
- **Acidosis:** pH of blood plasma below 7.4
- **Alkalosis:** pH of blood plasma higher than 7.4

1. Calculate pH? When $[H^+]$ =0.0067M, 1.34 X 10^{-8} M

   (hint: pH = log 1/ $[H^+]$ or pH = - log $[H^+]$)

2. Calculate $[H^+]$? When pH = 5.5, 6.2, 8.9

   (hint: $[H^+]$= 1/antilog pH)

Weak Acids and Bases Have Characteristic Dissociation Constants

- Strong acids, strong bases
- Of more interest to biochemists is the behavior of weak acids and bases
- Those not completely ionized when dissolved in water
- Common in biological systems & play important roles in metabolism & its regulation

- Proton donor & its corresponding proton acceptor make-up a conjugate acid-base pair

- Acetic acid (CH_3COOH), a proton donor & the acetate ion (CH_3COO^-), the corresponding proton acceptor, constitute a conjugate acid-base pair, related by the reversible reaction

$$CH3COOH \longleftrightarrow H^+ + CH3COO^-$$

- Each acid has a characteristic tendency to lose its protons in an aqueous solution

- Stronger the acid, the greater its tendency to lose its protons
- Tendency of any acid (HA) to lose a proton and form its conjugate base (A-) is defined by an equilibrium constant (Keq) for the reversible reaction

$$HA \longleftrightarrow H^{+} + A^{-}$$

Which is

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

- Keq usually called ionization or dissociation constants, often designated Ka

$$[H^+][A^-] = Ka.\ [HA],$$

first solve for $[H^+]$:

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

then take the negative logarithm of both sides:

$$-\log [H^+] = -\log Ka + (-\log \frac{[HA])}{[A^-]}$$

Substitute pH for - log [H+] and pka for - log Ka:

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

Now invert - log [HA]/[A-], which involves changing its sign, to obtain the Henderson-Hasselbalch equation:

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

Henderson-Hasselbalch equation

Stated more generally as,

$$\mathbf{pH = pka + \log \frac{[base]}{[acid]}}$$

- It is possible to calculate
  - **pka**, given pH and molar ratios of proton donor and acceptor
  - **pH**, given pka and molar ratios of proton donor and acceptor
  - **molar ratios of proton donor and acceptor**, given pH and pka

BOX 2–3 WORKING IN BIOCHEMISTRY

Solving Problems Using the Henderson-Hasselbalch Equation

1. Calculate the pK_a of lactic acid, given that when the concentration of lactic acid is 0.010 M and the concentration of lactate is 0.087 M, the pH is 4.80.

$$\mathrm{pH} = \mathrm{p}K_a + \log \frac{[\text{lactate}]}{[\text{lactic acid}]}$$

$$\mathrm{p}K_a = \mathrm{pH} - \log \frac{[\text{lactate}]}{[\text{lactic acid}]}$$

$$= 4.80 - \log \frac{0.087}{0.010} = 4.80 - \log 8.7$$

$$= 4.80 - 0.94 = 3.9 \quad (\textit{answer})$$

2. Calculate the pH of a mixture of 0.10 M acetic acid and 0.20 M sodium acetate. The pK_a of acetic acid is 4.76.

$$\mathrm{pH} = \mathrm{p}K_a + \log \frac{[\text{acetate}]}{[\text{acetic acid}]}$$

$$= 4.76 + \log \frac{0.20}{0.10} = 4.76 + 0.30$$

$$= 5.1 \quad (\textit{answer})$$

3. Calculate the ratio of the concentrations of acetate and acetic acid required in a buffer system of pH 5.30.

$$\mathrm{pH} = \mathrm{p}K_a + \log \frac{[\text{acetate}]}{[\text{acetic acid}]}$$

$$\log \frac{[\text{acetate}]}{[\text{acetic acid}]} = \mathrm{pH} - \mathrm{p}K_a$$

$$= 5.30 - 4.76 = 0.54$$

$$\frac{[\text{acetate}]}{[\text{acetic acid}]} = \text{antilog } 0.54 = 3.5 \quad (\textit{answer})$$

To see the effect of pH on the degree of ionization of a weak acid, see the Living Graph for Equation 2–9.

Buffers - Mixtures of Acids and Their Conjugate Bases

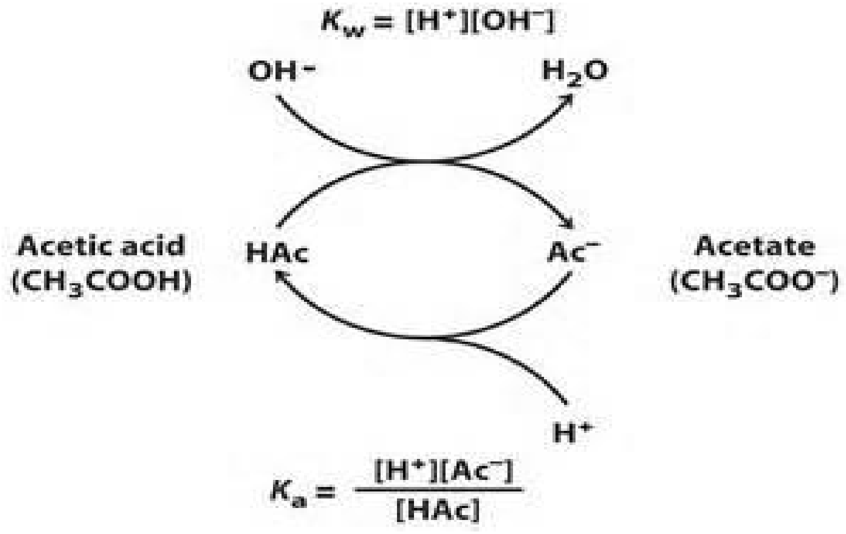
- Buffers - aqueous systems that tend to resist changes in pH when small amount of acid (H^+) or base (OH^-) are added.

- Buffer system consist of A weak acid (the proton donor) and its strong conjugate base (the proton acceptor)

Buffering Against pH Changes In Biological Systems

- Almost every biological process - pH dependent
- A small change in pH produces a large change in the rate of the process

- Cells & organisms maintain a specific & constant cytosolic pH near pH 7
- Multicellular organisms - the pH of extracellular fluids is also tightly regulated
- Constancy of pH is achieved primarily by biological buffers: mixtures of weak acids & their conjugate bases



- Acids & their anions (as base)
- Protein$^-$/Protein.H
- Hb$^-$/Hb.H

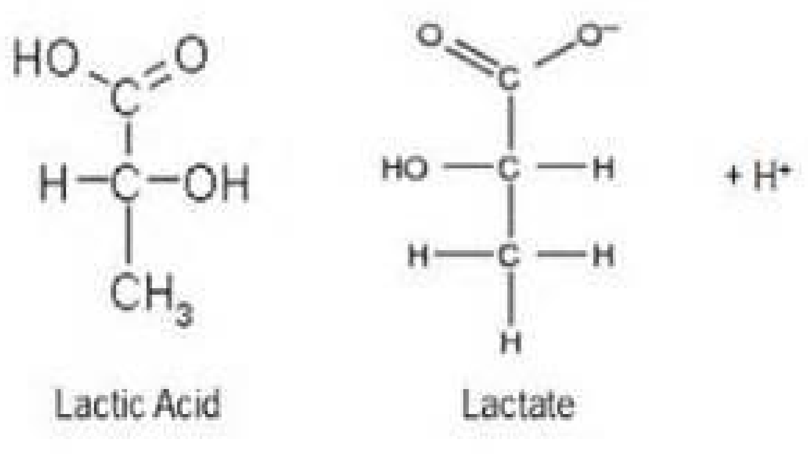
MAJOR BIOLOGICAL BUFFERS

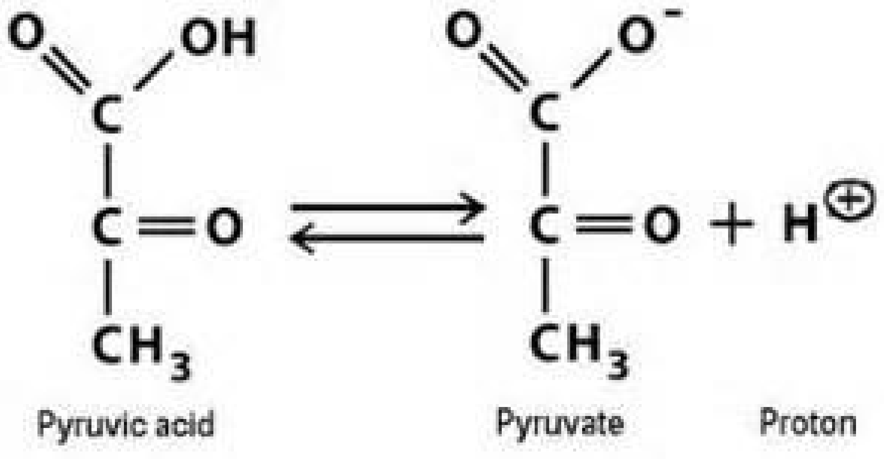
- **Extracellular Fluids:**

  1. H_2CO_3/HCO_3^- 2. Protein. H/Protein$^-$

- **Intracellular Fluids:**

  1. $H_2PO_4^-/HPO_4^-$ 2. Protein. H/Protein$^-$





Application of HH equation

- Preparation of buffer solutions

- **Question:** Calculate quantity of acid & base required to prepare 0.1 M, 100 mL potassium phosphate buffer having pH 7.4. pKa is 7.2

Potassium phosphate buffer= **KH_2PO_4/K_2HPO_4**

Acid = KH_2PO_4 mol.wt=39+2+31+16x4=136

Base = K_2HPO_4 mol.wt=39x2+1+31+16x4=174

Step 1: calculate molar ratio

Applying HH equation;

$$\text{pH} = \text{pka} + \log [\text{Base}]/[\text{Acid}]$$

$$7.4 = 7.2 + \log [\text{Base}]/[\text{Acid}]$$

$$7.4 - 7.2 = \log [\text{Base}]/[\text{Acid}]$$

$$0.2 = \log [\text{Base}]/[\text{Acid}]$$

Taking Antilog on both sides;

$$\text{Antilog } 0.2 = \text{Antilog} \times \log [\text{Base}]/[\text{Acid}]$$

$$1.58 = [\text{Base}]/[\text{Acid}]$$

Ratio [Base] : [Acid]

1.58:1

Step 2: convert molar ratio into molarity

Molarity of Base = 1.58

Molarity of Acid = 1

Total molarity of buffer = 1.58+1=2.58

But required molarity in question: 0.1M

Molarity of Base (out of required molarity:0.1M)=1.58/2.58X0.1

= **0.061M**

Molarity of Acid (out of required molarity:0.1M)=1/2.58X0.1

= **0.038M**

Step 3: convert molarity into moles

Molarity = No. of moles/litre of solution

OR

No. of moles=molarity x volume (litre)

No. of moles of Base = 0.061 x 0.1* **= 0.0061**

No. of moles of Acid = 0.038 x 0.1* **= 0.0038**

*** 100 mL = 0.1L**

Step 4: convert moles into quantity (g)

No. of moles = weight (g)/mol.wt

weight (g) = No. of moles x mol.wt

Quantity (g) of Base = No. of moles of Base x mol.wt
= 0.0061 x 174 = **1.06 g**

Quantity (g) of Acid = No. of moles of Acid x mol.wt
= 0.0038 x 136 = **0.51 g**