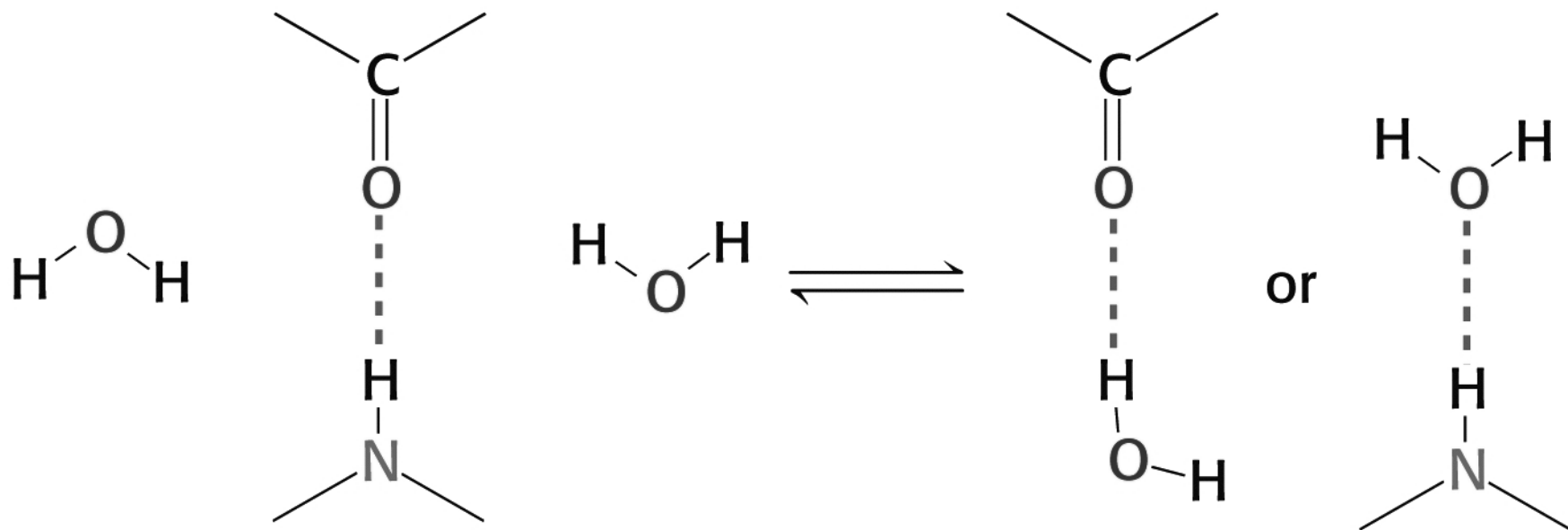
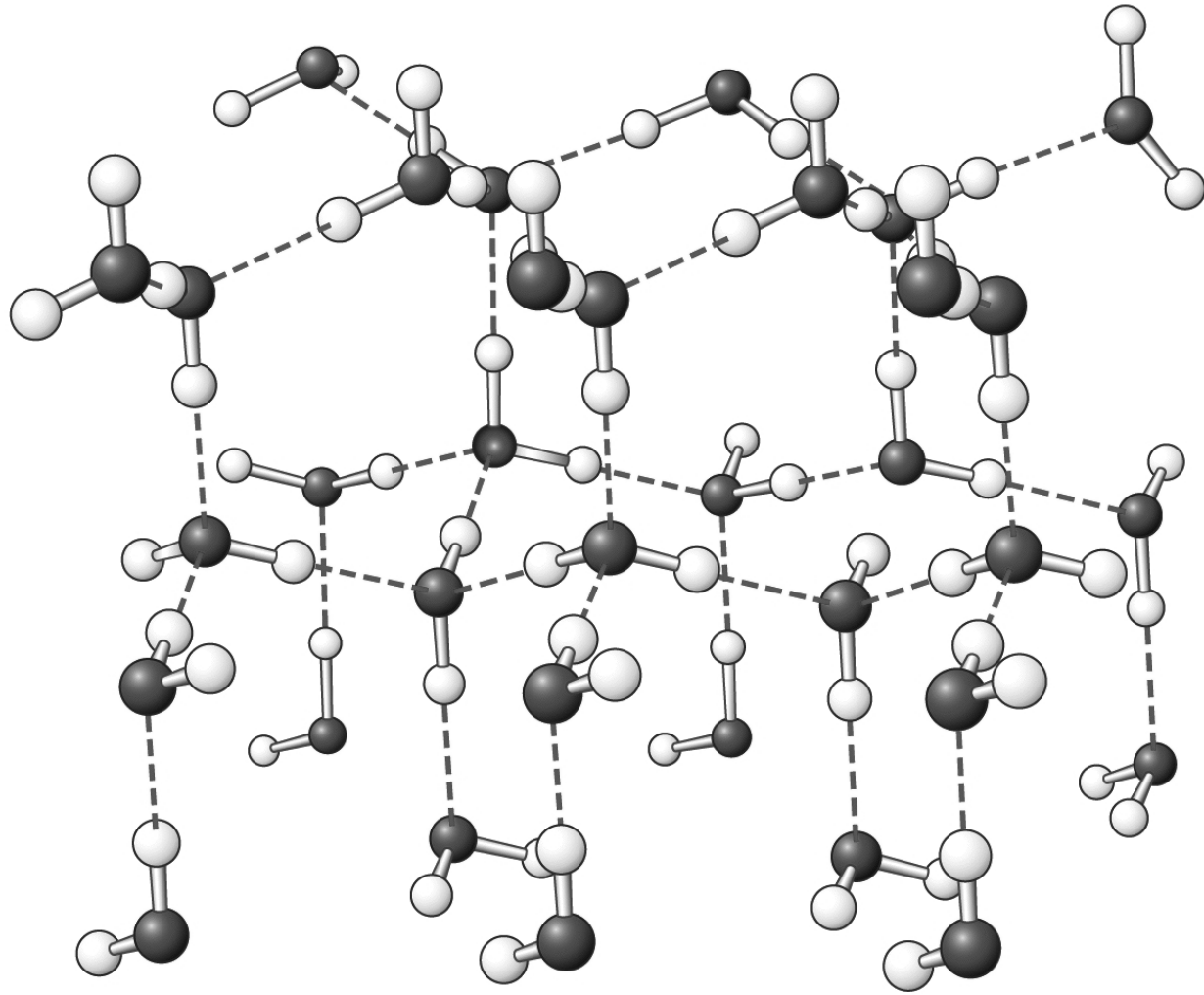


Water

- Most biochemical reactions occur in an aqueous environment.
- Water is highly polar because of its bent geometry.
- Water is highly cohesive because of intermolecular hydrogen bonding.
- Water participates in H-bonding with biomolecules.

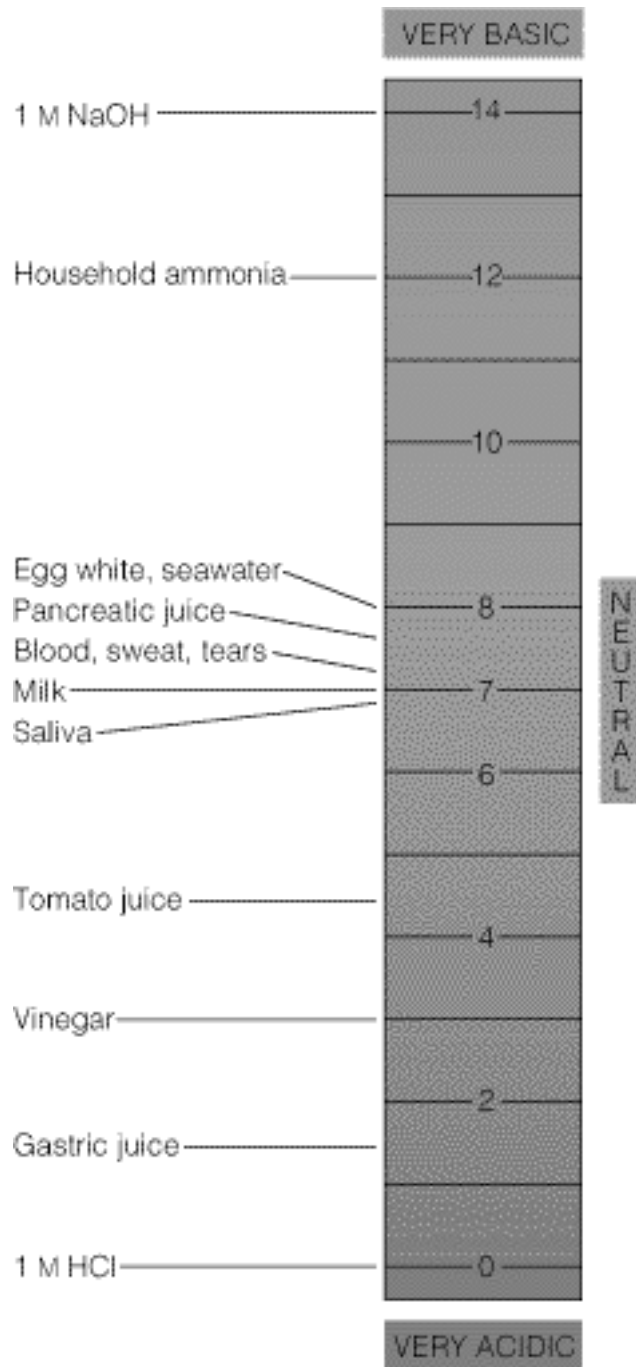




Ionization of water: $\text{H}_2\text{O} + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{OH}^-$

pH, Acids and Bases

- $\text{pH} = -\log [\text{H}^+]$
- $\text{pOH} = -\log [\text{OH}^-]$ ($[\text{H}^+]$ and $[\text{OH}^-]$ in M)
- $[\text{H}^+] \times [\text{OH}^-] = 1 \times 10^{-14} \text{ M}^2$ / $\text{pH} + \text{pOH} = 14$
- An acid is defined as a proton donor
- $\text{AH} = \text{A}^- + \text{H}^+$
- AH is the acid and A^- is its conjugate base.
- A base is defined as a proton acceptor
- $\text{B} + \text{H}_2\text{O} = \text{BH}^+ + \text{OH}^-$
- B is the base and BH^+ is its conjugate acid



The pH scale

An acidic solution is one in which $[H^+] > [OH^-]$

- In an **acidic solution**, $[H^+] > 10^{-7}$, **pH < 7**.
- A basic solution is when $[OH^-] > [H^+]$.
- In a **basic solution**, $[OH^-] > 10^{-7}$, **pOH < 7**, and **pH > 7**.
- When the pH = 7, the solution is neutral.
- Physiological pH range is 6.5 to 8.0

Weak Acids and pKa

- The strength of an acid can be determined by its dissociation constant, K_a .
- Acids that do not dissociate significantly in water are weak acids.
- The dissociation of an acid is expressed by the following reaction:
$$\text{HA} = \text{H}^+ + \text{A}^-$$
 and
the dissociation constant $K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$
- When $K_a < 1$, $[\text{HA}] > [\text{H}^+][\text{A}^-]$ and HA is not significantly dissociated. Thus, HA is a weak acid when $k_a < 1$.
- **The lesser the value of K_a , the weaker the acid.**
- Similar to pH, the value of K_a can also be represented as pKa.
- **$\text{pKa} = -\log K_a$.**
- **The larger the pKa, the weaker the acid.**
- pKa is a constant for each conjugate acid and its conjugate base pair.
- Most biological compounds are weak acids or weak bases.

Polyprotic Acids

- Some acids are polyprotic acids; they can lose more than one proton.
- In this case, the conjugate base is also a weak acid.
- For example: Carbonic acid (H_2CO_3) can lose two protons sequentially.
- Each dissociation has a unique K_a and pK_a value.

$$K_{a_1} = [\text{H}^+][\text{HCO}_3^-] / [\text{H}_2\text{CO}_3]$$

$$K_{a_2} = [\text{H}^+][\text{CO}_3^{2-}] / [\text{HCO}_3^-]$$

Note: (The difference between a weak acid and its conjugate base differ is one hydrogen)

Some weak acids and their conjugate bases

Acid (Proton Donor)		Conjugate Base (Proton Acceptor)		pK_a	K_a (M)
HCOOH Formic acid	\rightleftharpoons	HCOO ⁻ Formate ion	+H ⁺	3.75	1.78×10^{-4}
CH ₃ COOH Acetic acid	\rightleftharpoons	CH ₃ COO ⁻ Acetate ion	+H ⁺	4.76	1.74×10^{-5}
$\begin{array}{c} \text{OH} \\ \\ \text{CH}_3\text{CH}-\text{COOH} \end{array}$ Lactic acid	\rightleftharpoons	$\begin{array}{c} \text{OH} \\ \\ \text{CH}_3\text{CH}-\text{COO}^- \end{array}$ Lactate ion	+H ⁺	3.86	1.38×10^{-4}
H ₃ PO ₄ Phosphoric acid	\rightleftharpoons	H ₂ PO ₄ ⁻ Dihydrogen phosphate ion	+H ⁺	2.14	7.24×10^{-3}
H ₂ PO ₄ ⁻ Dihydrogen phosphate ion	\rightleftharpoons	HPO ₄ ²⁻ Monohydrogen phosphate ion	+H ⁺	6.86	1.38×10^{-7}
HPO ₄ ²⁻ Monohydrogen phosphate ion	\rightleftharpoons	PO ₄ ³⁻ Phosphate ion	+H ⁺	12.4	3.98×10^{-13}
H ₂ CO ₃ Carbonic acid	\rightleftharpoons	HCO ₃ ⁻ Bicarbonate ion	+H ⁺	6.37	4.27×10^{-7}
HCO ₃ ⁻ Bicarbonate ion	\rightleftharpoons	CO ₃ ²⁻ Carbonate ion	+H ⁺	10.25	5.62×10^{-11}
C ₆ H ₅ OH Phenol	\rightleftharpoons	C ₆ H ₅ O ⁻ Phenolate ion	+H ⁺	9.89	1.29×10^{-10}
NH ₄ ⁺ Ammonium ion	\rightleftharpoons	NH ₃ Ammonia	+H ⁺	9.25	5.62×10^{-10}

■ Phosphoric acid series

■ Carbonic acid series

The Henderson-Hasselbalch equation

Dissociation of a weak acid is mathematically described by the **Henderson-Hasselbalch** equation

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

$$K_a = [H^+] \times [A^-] / [HA]$$

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

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

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

So, if CB = conjugate base and WA = weak acid, then:

$$pH = pK_a + \log \{ [CB] / [WA] \}$$

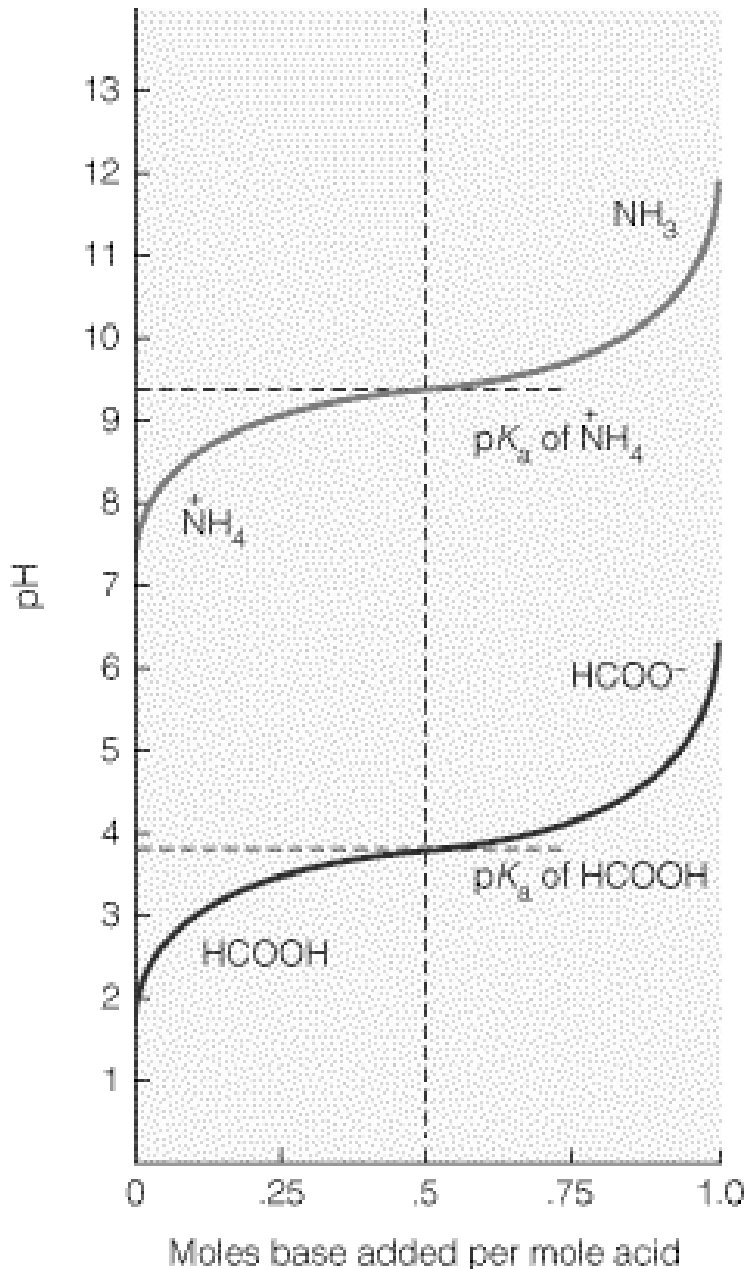
This is the Henderson-Hasselbalch equation

Note: $pH = pK_a$ when $[CB] = [WA]$

Applications of the Henderson-Hasselbalch equation

- Calculate the ratio of CB to WA, if pH is given
- Calculate the pH, if ratio of CB to WA is known
- Calculate the pH of a weak acid solution of known concentration
- Determine the pKa of a WA-CB pair
- Calculate change in pH when strong base is added to a solution of weak acid. This is represented in a titration curve
- Calculate the pI

Titration curve for weak acids



- Initially, $[\text{WA}] \gg \gg [\text{CB}]$
- When $[\text{WA}] = [\text{CB}]$, $\text{pH} = \text{pK}_a$
- The central region of the curve ($\text{pH} \pm 1$) is quite flat because:

When $[\text{CB}]/[\text{WA}] = 10$,
 $\text{pH} = \text{pK}_a + 1$;

When $[\text{CB}]/[\text{WA}] = 0.1$,
 $\text{pH} = \text{pK}_a - 1$

- Titration curve is reversible, if we start adding acid, $[\text{WA}]$ increases

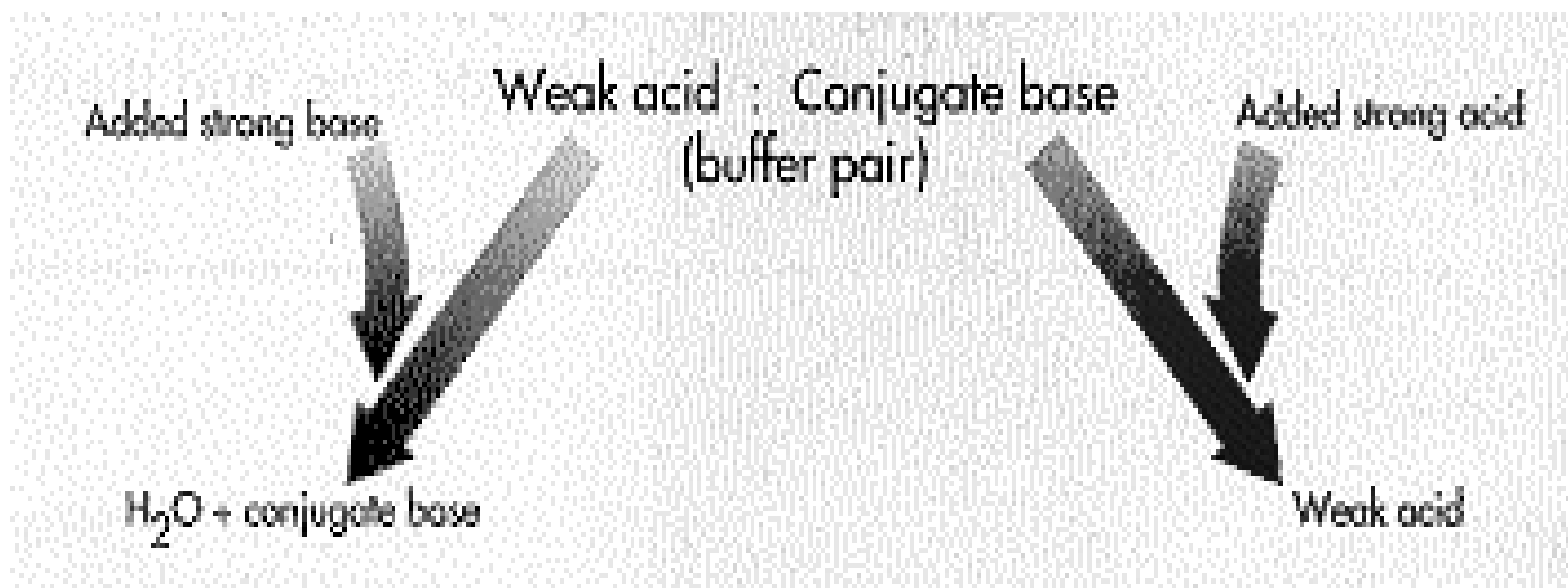
Titration of a weak acid with a strong base

- A weak acid is mostly in its conjugate acid form
- When strong base is added, it removes protons from the solution, more and more acid is in the conjugate base form, and the pH increases
- When the moles of base added equals half the total moles of acid, the weak acid and its conjugate base are in equal amounts. The ratio of CB / WA = 1 and according to the HH equation, $\text{pH} = \text{pKa} + \log(1)$ or $\text{pH} = \text{pKa}$.
- If more base is added, the conjugate base form becomes greater till the equivalence point when all of the acid is in the conjugate base form.

Buffers

- Biological systems use buffers to maintain pH.
- Definition: A buffer is a solution that resists a significant change in pH upon addition of an acid or a base.
- Chemically: A **buffer is a mixture of a weak acid and its conjugate base**
- Example: Bicarbonate buffer is a mixture of carbonic acid (the weak acid) and the bicarbonate ion (the conjugate base): $\text{H}_2\text{CO}_3 + \text{HCO}_3^-$
- All OH^- or H^+ ions added to a buffer are consumed and the overall $[\text{H}^+]$ or pH is not altered
$$\text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{H}^+ \leftrightarrow 2\text{H}_2\text{CO}_3$$
$$\text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{OH}^- \leftrightarrow 2\text{HCO}_3^- + \text{H}_2\text{O}$$
- For any weak acid / conjugate base pair, the buffering range is its $\text{pK}_a \pm 1$.

Mechanism by which Buffers Operate



Example:



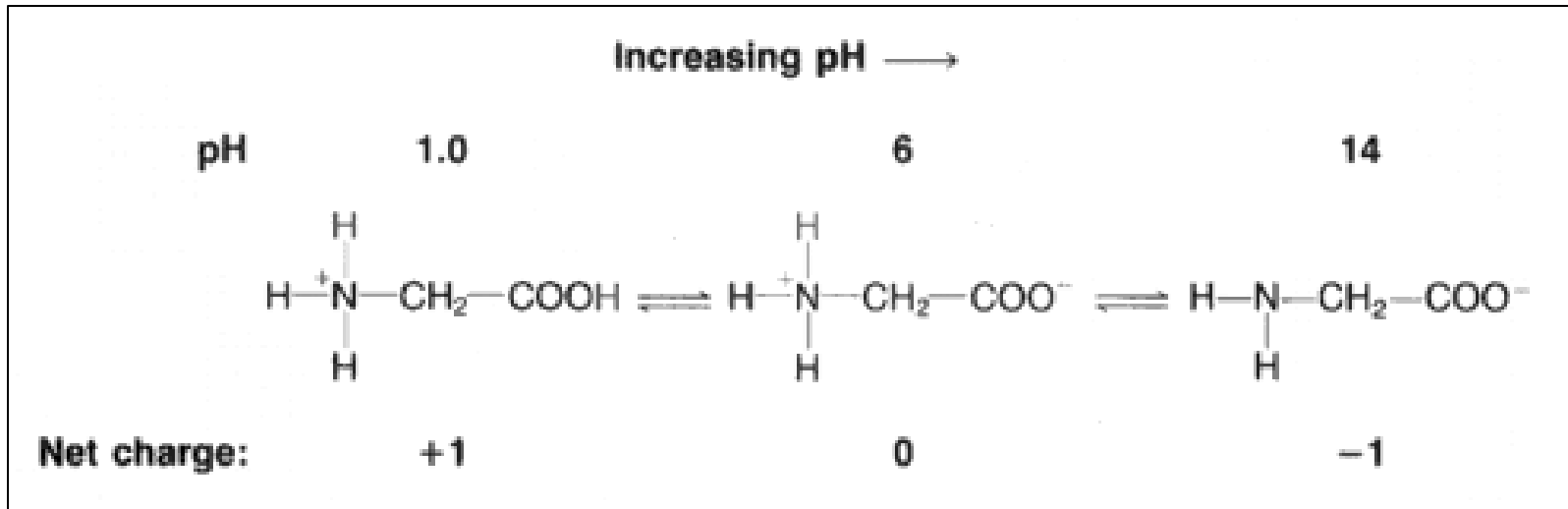
Ampholytes

- A molecule containing ionizing groups with both acidic and basic pKa values is called an ampholyte.
- The ionic form of each group in the compound is dependent on the pH of the solution.
- If the pH of solution is greater than the pKa, the group is in the conjugate base form (deprotonated).
- If the pH of solution is less than the pKa, the group is in the conjugate acid form (protonated).

Ionic forms of Glycine

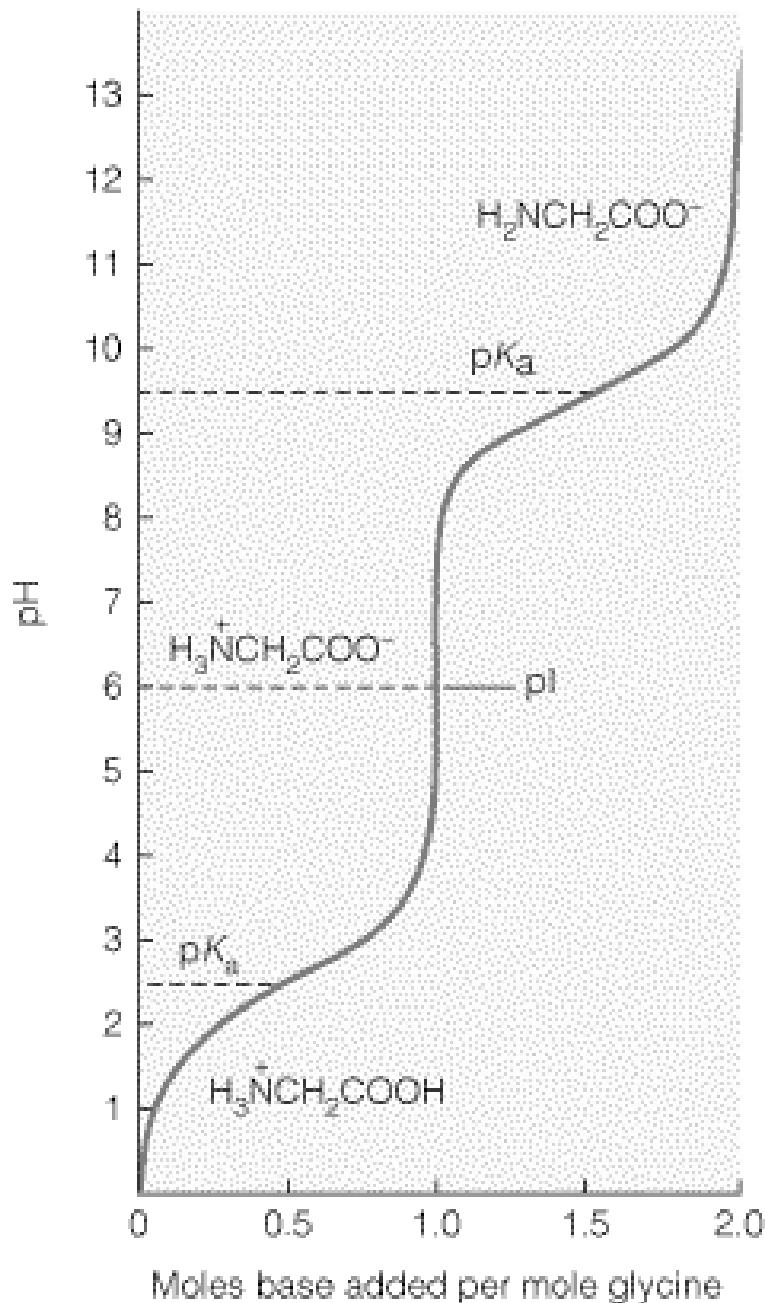
- Glycine is $\text{H}_2\text{N}-\text{CH}_2-\text{COOH}$.
- pKa of carboxylate group is 2.3 ; pKa of amino group is 9.6

(Note: glycine can serve as a buffer in 2 different buffer ranges).



- The ionic form with a net charge of zero is called a zwitterion
- The isoelectric point (pI) is the pH at which the net charge on the ampholyte is zero (or equal number of + and - charged ions).

Titration of ampholyte glycine



Carboxylate and amino groups lose their protons successively.

The first mole equivalent of added base converts the carboxylate to its conjugate base; next, the amino group gets deprotonated.

Note the steep jump in pH around the pI.

Calculation of pI for Glycine

- Use the Henderson-Hasselbalch equation to calculate the pI.
- At isoelectric point, $\text{pH} = \text{pI}$
- $$\text{pI} = \text{pK}_{\text{COOH}} + \log \frac{[\text{H}_3\text{N}^+\text{CH}_2\text{COO}^-]}{[\text{H}_3\text{N}^+\text{CH}_2\text{COOH}]}$$
- $$\text{pI} = \text{pK}_{\text{NH}_3^+} + \log \frac{[\text{H}_2\text{NCH}_2\text{COO}^-]}{[\text{H}_3\text{N}^+\text{CH}_2\text{COO}^-]}$$
- Adding up:
$$2\text{pI} = \text{pK}_{\text{COOH}} + \text{pK}_{\text{NH}_3^+} + \log \frac{[\text{H}_2\text{NCH}_2\text{COO}^-]}{[\text{H}_3\text{N}^+\text{CH}_2\text{COOH}]}$$
- When $\text{pH} = \text{pI}$, $[\text{H}_2\text{NCH}_2\text{COO}^-] = [\text{H}_3\text{N}^+\text{CH}_2\text{COOH}]$
- $2\text{pI} = \text{pK}_{\text{COOH}} + \text{pK}_{\text{NH}_3^+}$ or $\text{pI} = \{\text{pK}_{\text{COOH}} + \text{pK}_{\text{NH}_3^+}\}/2$