pH and buffers

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Objectives

1. Explain the term pH & calculate pH at different $[H^+]$

2. State the relationship between pH and the concentration of conjugate acid and base

3. Use Henderson – Hasselbalch equation to calculate pH of a given buffering system

4. Define "buffers" and explain the following terms
        a. Buffering capacity
        b. Buffering range

5. Name the buffers found in body fluid and explain their buffering action
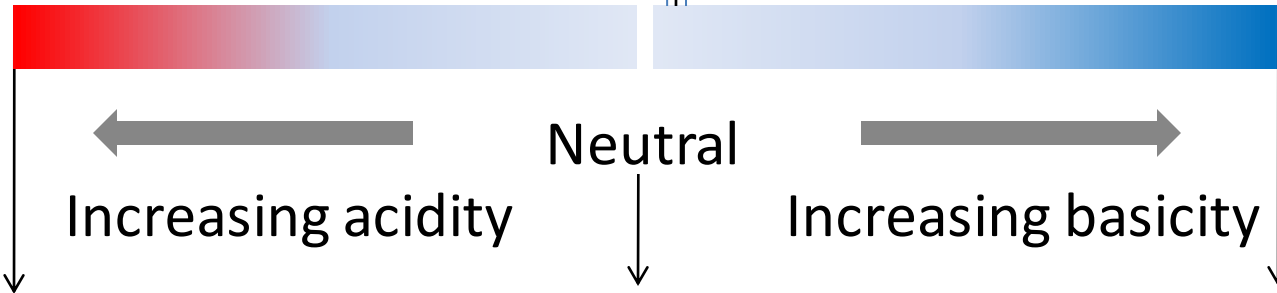
pH- a measure of H^+ concentration

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

pH = 7.40 (physiological "normal")

7.35   7.45

Reference range for blood pH

Neutral

Increasing acidity   Increasing basicity

H^+ = 1 mol/L (M)
pH = 0

H^+ = 10^{-7} mol/L (M)
pH = 7

H^+ = 10^{-14} mol/L (M)
pH = 14

pH calculations I

e.g. What is the pH of a solution whose $[H^+]$ is
3.2 x 10^{-4} mol/L ?

$pH = - log_{10} [H^+]$

$\quad = - log (3.2 \times 10^{-4})$

$\quad = - log (3.2) + -log (10^{-4})$

$\quad = - 0.5 + 4$

$\quad = 3.5$

pH calculations II

- e.g. What is the fold change of H^+ ion concentration between pH 7.35 and 7.45

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

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

$[H^+]$ at pH 7.35 $= 4.47 \times 10^{-8}$ mol/L

$[H^+]$ at pH 7.45 $= 3.55 \times 10^{-8}$ mol/L

Fold change$= 4.47/3.58 = 1.26$ (~ 25% change)

Defining acids and bases

Acids   **=**   **H^+ donors**
Bases   **=**   **H^+ acceptors**

- Strong acids / bases → **completely** dissociate

$$HCl \longrightarrow H^+ + Cl^-$$

- Weak acids / bases → **partially** dissociate

$$CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$$
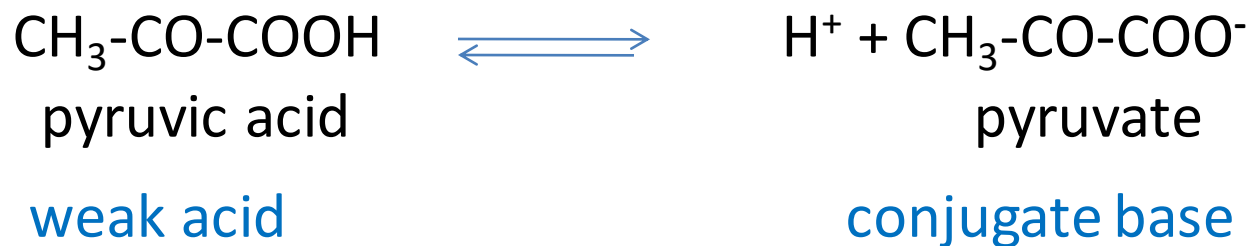
$$NH_4OH \rightleftharpoons NH_4^+ + OH^-$$

Many biologically important molecules are either weak acids or bases.

Conjugate acids and bases

Deprotonated form of a weak acid- conjugate base

$$CH_3\text{-}CO\text{-}COOH \rightleftharpoons H^+ + CH_3\text{-}CO\text{-}COO^-$$

pyruvic acid   pyruvate

weak acid   conjugate base

Protonated form of a weak base – conjugate acid

$$CH_3NH_2 + H^+ \rightleftharpoons CH_3NH_3^+$$

methylamine   methylammonium

weak base   conjugate acid
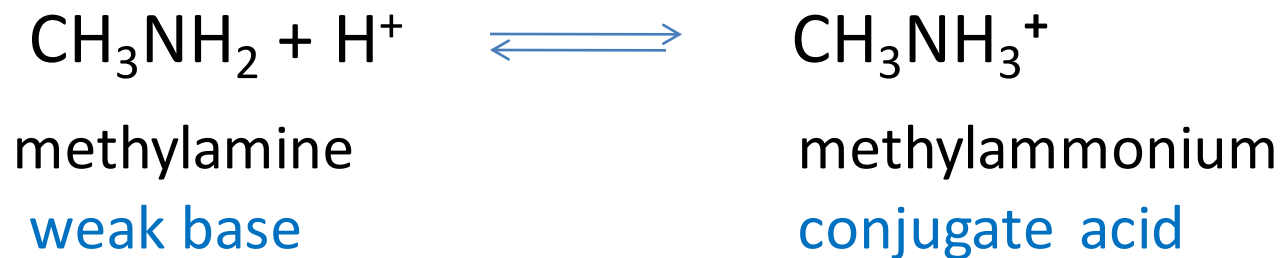
Deriving quantitative relationships

- Quantitative relationship between concentration of weak acid (HA) and its conjugate base (A^-)

$$HA \rightleftharpoons H^+ + A^-$$

Dissociation constant of acid

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

- Relative strength of acids are expressed in terms of dissociation constant

  e.g. acetic acid $K_a = 1.8 \times 10^{-5}$, formic acid $K_a = 1.8 \times 10^{-4}$

- Larger the K_a stronger the acid (formic acid > acetic acid)

Henderson – Hasselbalch equation

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

Rearranging for [H⁺]

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

Convert to log

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

Multiply by (-1)

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

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$
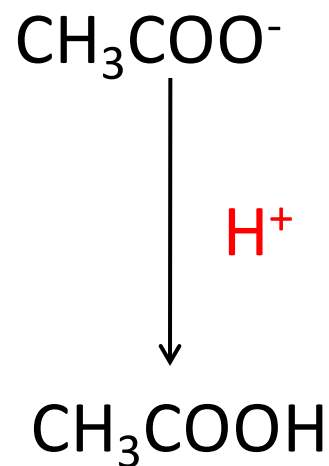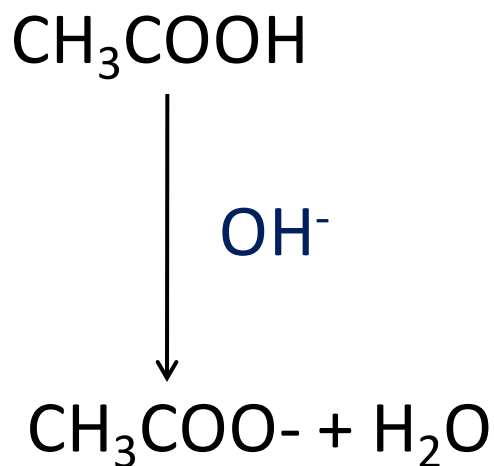
$$pK_a \, \alpha \, \frac{1}{K_a}$$

Buffers

Definition:

Solutions that resist change in pH following the addition of (small amounts) acid or base

[Solutions of weak acid & their conjugate base (or weak base & their conjugate acid) behave as buffers]

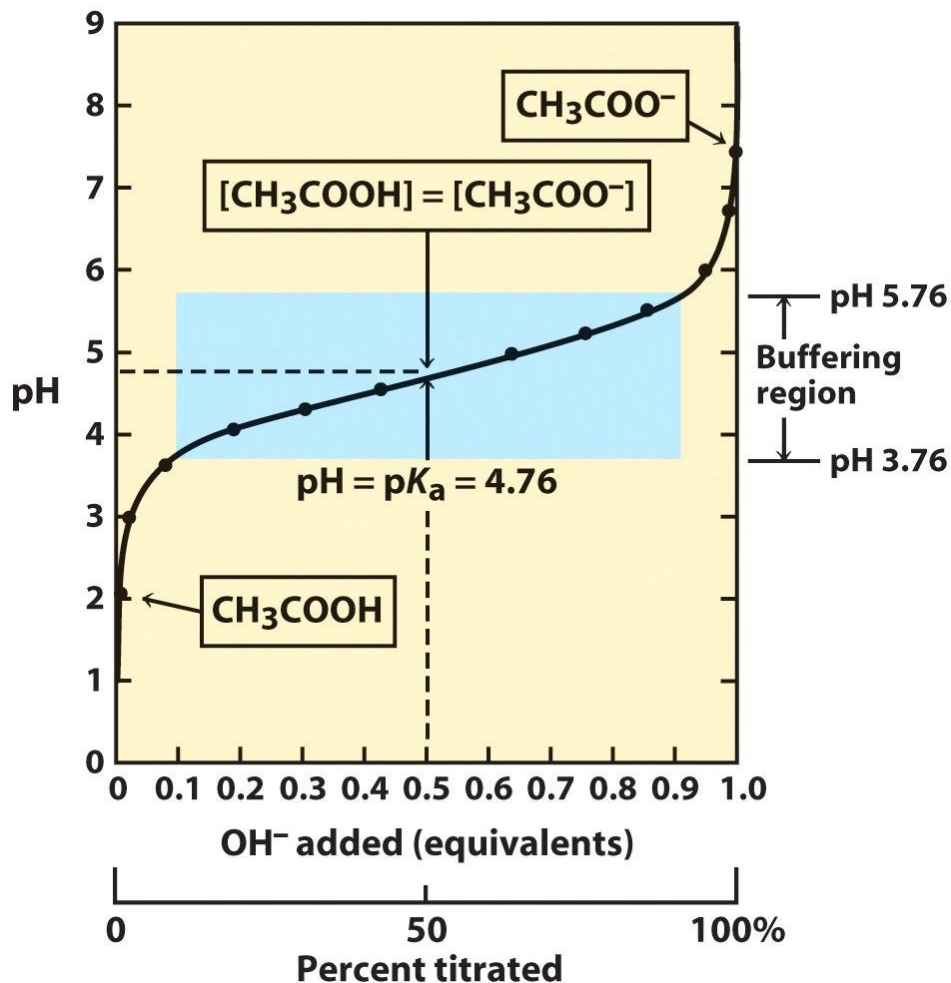CH_3COOH $\xrightarrow{\text{OH}^-}$ $CH_3COO- + H_2O$

CH_3COO^- $\xrightarrow{\text{H}^+}$ CH_3COOH

Characteristics of buffers I

- **Buffering Range**

  The pH range a buffer is most effective

  (monitor the change of pH in a weak acid with the addition of a base)

  As a general rule the buffering range is;

  $pH = pK_a \pm 1$

Characteristics of buffers II

- Buffer capacity:

The extent a buffer can resist changes in pH with the addition of a strong acid or a base

Depends on:

1. Concentration of acid and conjugate base

2. How close the pH of buffer is to the pKa of weak acid

Maximum buffering capacity occurs at a pH= pK_a

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

When acid is half neutralized [HA]=[A$^-$] , therefore; $\log_{10} 1 = 0$

$$pH = pK_a$$

pH calculations III

1. Calculate the pH of a mixture of 0.1M acetic acid & 0.2M sodium acetate. (pK_{CH_3COOH} = 4.76)

$$pH = pK_a + \log \frac{[acetate]}{[acetic \ \ acid]}$$

$$= 4.76 + \log \left[\frac{0.2}{0.1} \right]$$

$$= 4.76 + 0.30$$

$$= 5.06$$

2. Calculate the ratio of the concentrations of bicarbonate & carbonic acid required in a buffer system of pH 7.40 ($pK_{H_2CO_3}$ = 6.1)

$$pH = pK_a + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

$$\log \frac{[HCO_3^-]}{[H_2CO_3]} = pH - pK_a = 7.4 - 6.1$$

$$\frac{[HCO_3^-]}{[H_2CO_3]} = 10^{1.3} (inverse\_\log)$$

$$= 20$$

Buffers of the body fluids

- The body maintain the pH within narrow limits despite the fact that metabolic processes will constantly release or uptake protons
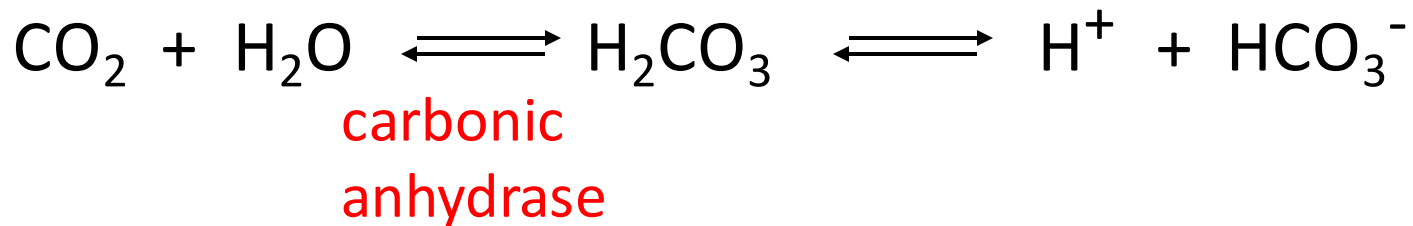
Blood	Bicarbonate	$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$
	Hemoglobin	$H^+Hb \rightleftharpoons H^+ + Hb$
	Protein	$HProt \rightleftharpoons H^+ + Prot^-$
Intracellular fluid	Protein	$HProt \rightleftharpoons H^+ + Prot^-$
	Phosphate	$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^-$
Interstitial fluid	Bicarbonate	$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$
Urine	Bicarbonate	$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$
	Phosphate	$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^-$
	Ammonia	$NH_4^+ \rightleftharpoons H^+ + NH_3$

Bicarbonate buffer system

- A major buffer system in blood

Slow reaction:
catalyzed by

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

carbonic anhydrase

By applying H-H equation; $pH = pK_{H_2CO_3} + \log \dfrac{[HCO_3^-]}{[H_2CO_3]}$

Q- How do we relate gaseous CO_2 to H_2CO_3 in the blood?

$[H_2CO_3]$ α PCO_2 therefore;

$[H_2CO_3]$ = Solubility constant of CO_2 X PCO_2

Bicarbonate buffer system ctd…

- pK$_a$ of this system is low (6.1) relative to the pH of the blood (7.4)

- The most effective range for this system = 5.1 – 7.1

- However, bicarbonate buffer system is very important for the following reasons

  - It is found in high concentration in the plasma & $[HCO_3^-]$: $[CO_2]$ = 20/1 at physiological pH of 7.4

  - Amount of CO_2 & HCO_3^- are physiologically regulated (by lungs & kidneys).

Phosphate buffer system

- $H_2PO_4^{-} \rightleftharpoons H^+ + HPO_4^{2-}$

- pK_a = 6.8, buffer range = 5.8 – 7.8

- In the plasma, the phosphate concentration is too low for this system to be an important buffer

- But it is important intracellularly, and in the urine (Urine pH can vary from pH= 4.6 to 8)
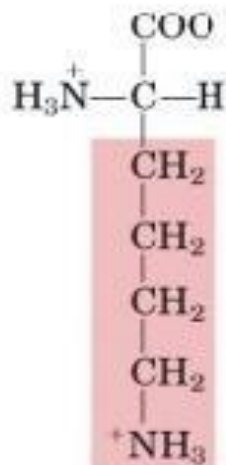
Amino acids as buffers I

- Amino acids have an amine group and a carboxylic acid group (on the alpha carbon)

- Side chains of some amino acids contain buffering groups

$pK_a = 2$

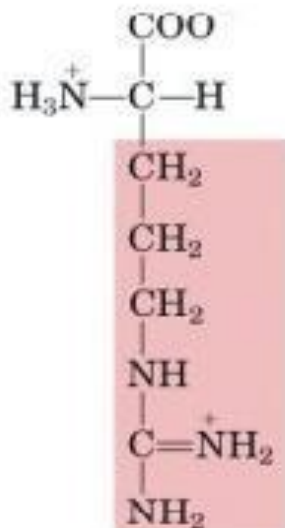$pK_a = 9\text{-}10$

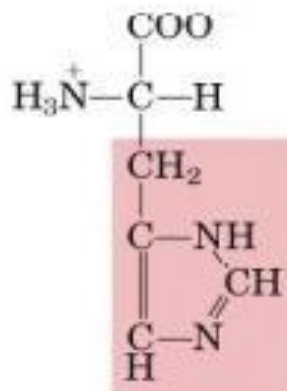Alpha (α) carbon
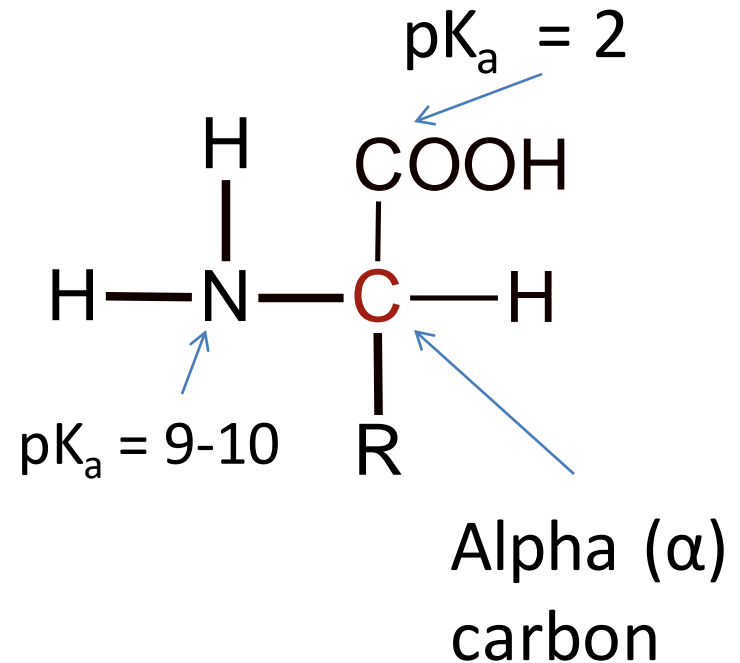
Amino acids as buffers II

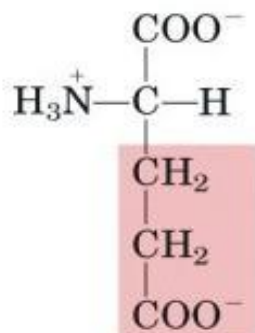Positively charged R groups

Lysine   Arginine   Histidine

Negatively charged R groups
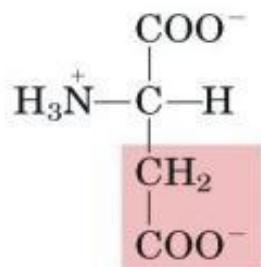
Aspartate   Glutamate

Amino acid	Charge	Side chain pK_a
Lysine	+	10.69
Arginine	+	12.48
Histidine	+	6.02
Aspartate	-	3.90
Glutamate	-	4.42

- Positively charged=basic side chain:
- Negatively charged = acidic side chain
- $RNH_2 + H^+ \rightleftharpoons RNH_3^+$
- $RCOOH \rightleftharpoons H^+ + RCOO^-$

Proteins as buffers
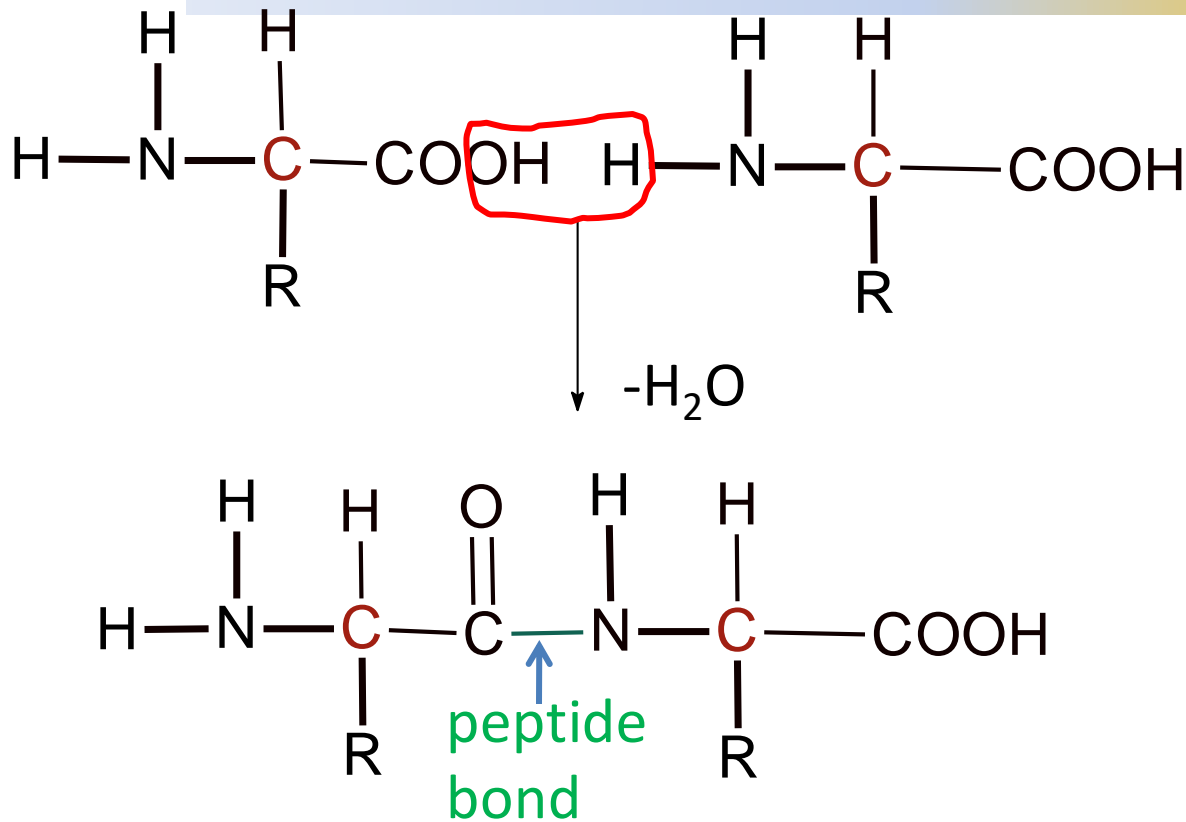
- Charged side chains of amino acids mainly contribute to the charge of a protein
- Only the N and C termini of proteins will have unconjugated amine and carboxylic ends

Buffering system in the RBC - Hb

- Hemoglobin (Hb) can act as a buffer due to the presence of Histidine residues

- 32 histidines / Hb (present in large amounts)

←imidazole group

pK_a=6.0 to 6.5

Hb + H$^+$ ⇌ **H$^+$Hb**
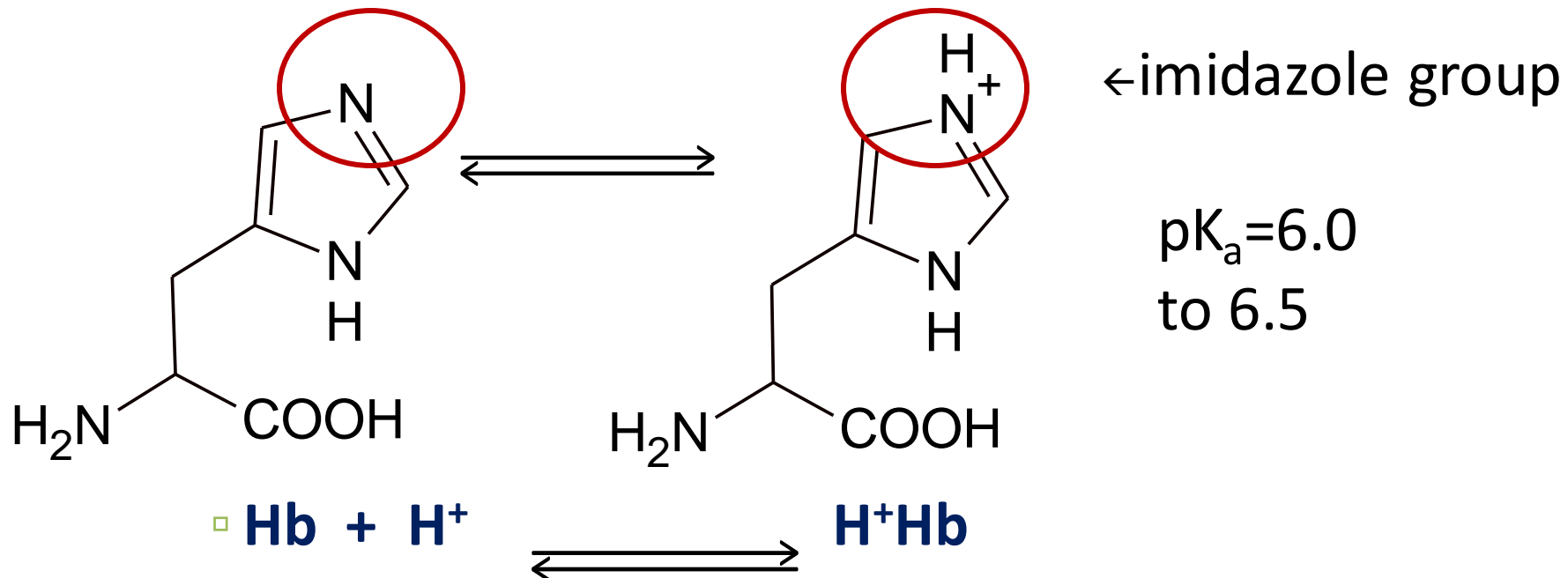
Acidosis & Alkalosis

- When arterial pH
  - » falls below 7.35 → acidosis
  - » rises above 7.45 → alkalosis

2 types;
1) Metabolic
   - a decrease in HCO_3^- → metabolic acidosis
   - an increase in HCO_3^- → metabolic alkalosis

2) Respiratory
   - a rise in arterial PCO_2 → respiratory acidosis
   - decline in PCO_2 → respiratory alkalosis

Thank you !!

Questions?