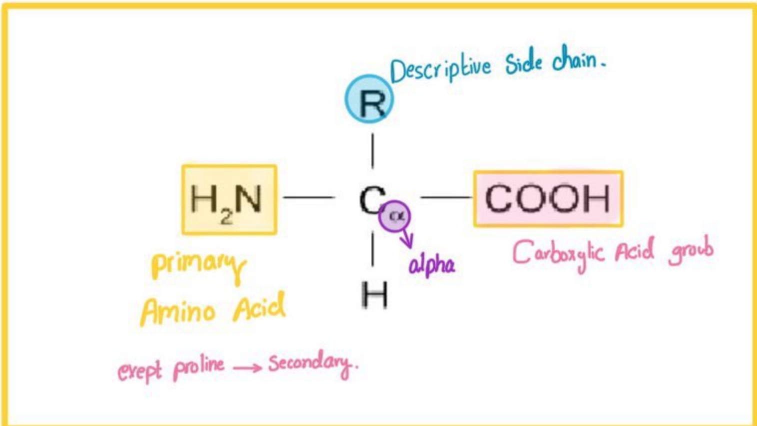
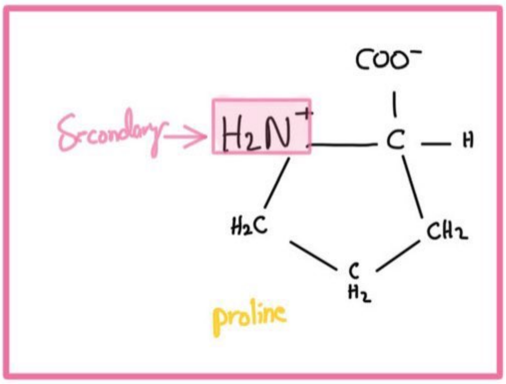
Experiment 3.

Overview

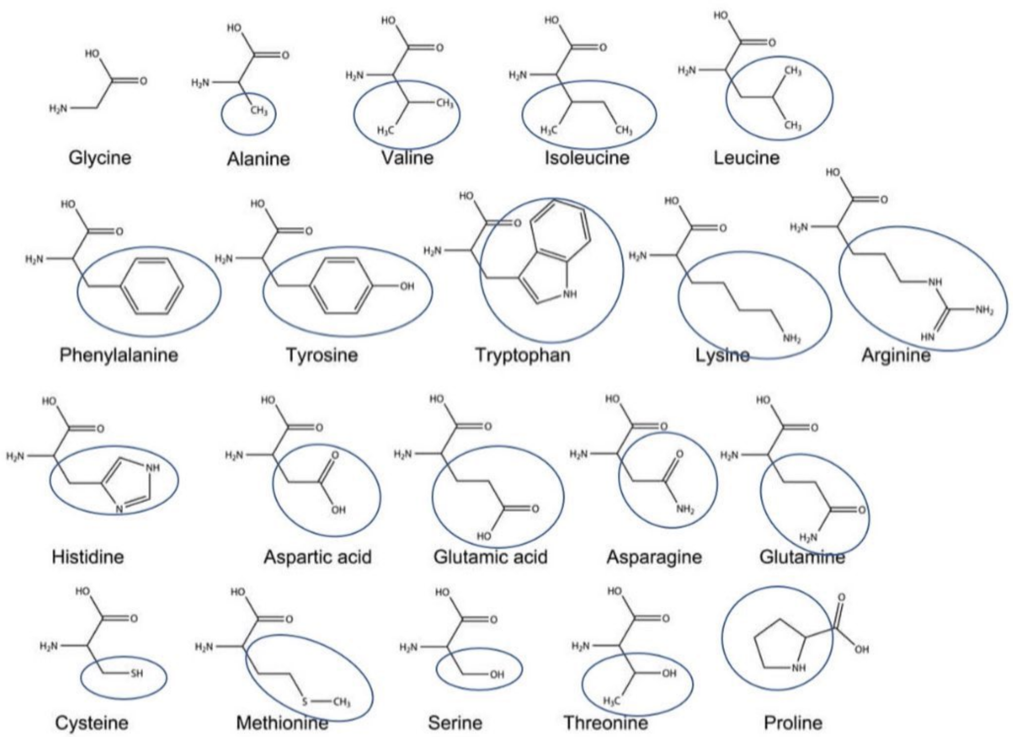
- Amino acids ⟶ basic units ⟶ peptides or enzymes
- 300 (In nature), 20 (mammalian proteins)
- Basic structure: Common

describe



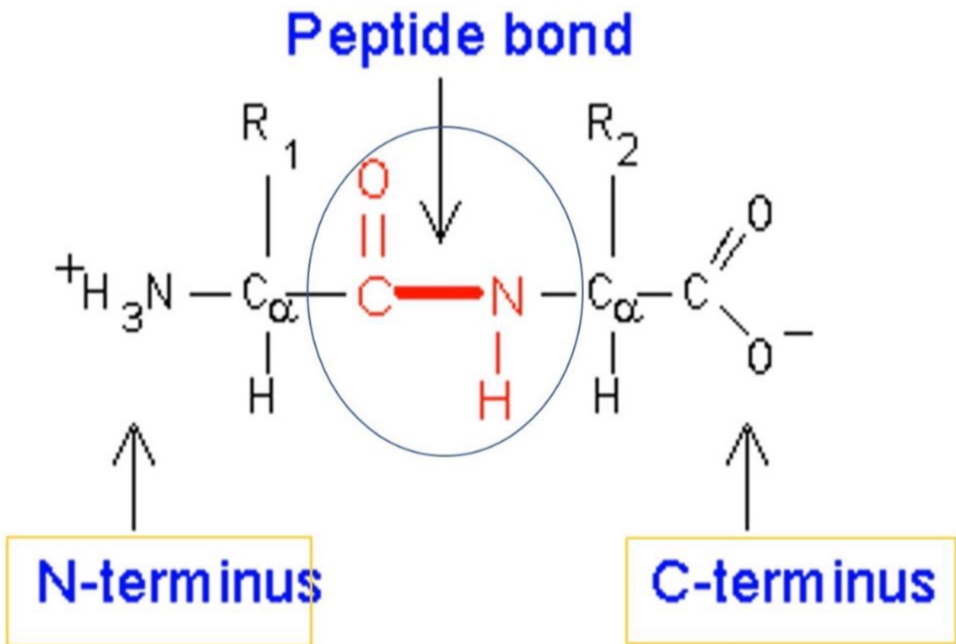


distinctive for each amino acid



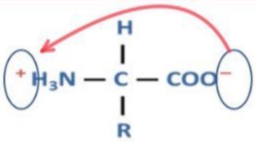
physiological pH = 7.4

between consecutive Amino Acid ← peptide linkege [positive charge ← Amino group : protonated
Negative charge ← Carboxyl group : dissociated



- Amino acid ⟶ dissolved in water ⟶ zwitterion

internal transfer of H^+ from Carboxyl group to Amino group.



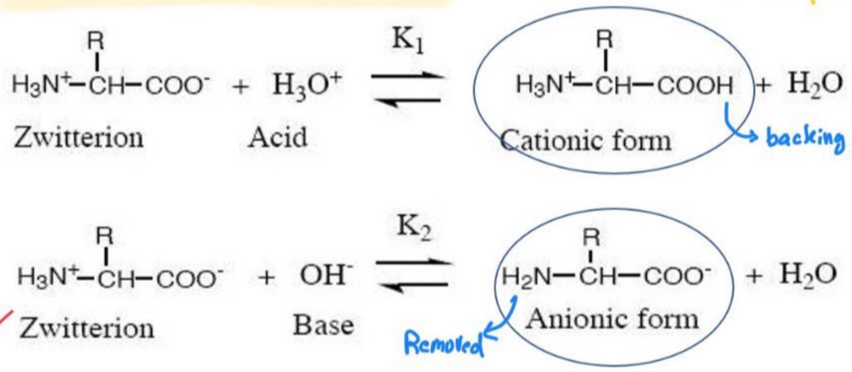
zwitterion

a form that contains both positively and negatively charged functional groups, and the net charge of the entire molecule is zero.

- Amphoteric substance ⟶ They can accept H^+ or donate H^+

حسب ال Media ممكن يتصرف كـ Acid or Base.

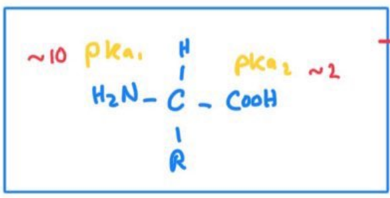
pH



- The **ionic form** of the amino acid present in an aqueous solution is dependent upon the solution's pH.

* ↓↓ PH → pronated

* ↑↑ PH → depronated

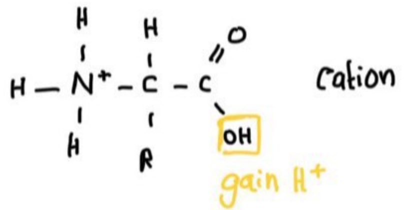


→ $pKa_1 > PH$ protonated > deprotonated (NH_3^+ > NH_2)

$pKa_2 > PH$ protonated > deprotonated ($COOH$ > COO^-)

a charge of Amino Acid depend on the pH.

* Low(pH) (high H^+) Amino acid gain a proton H^+ to form a Cation.



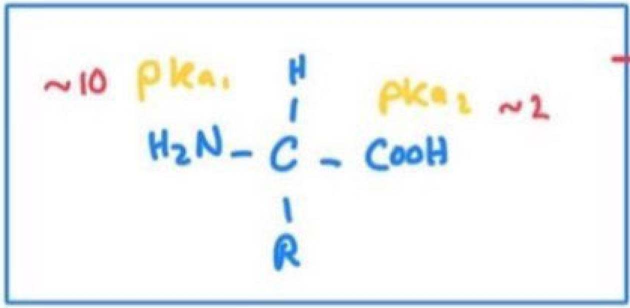
Titration curve of amino acids with strong base

- A titration curve of an amino acid is a plot of the pH of the acid **e.g., glycine** against the degree of neutralization of the acid by standard (strong) base **e.g., NaOH**

- It is useful for identification an unknown amino acids by defining:

(1) ✓the number of ionising groups
(2) ✓the pKa of the ionising group(s)
(3) ✓the buffer region(s).

Depend on the type of amino acids: diprotic or triprotic



NH_3^+ NH_2

$pKa_1 > pH$ protonated > deprotonated

$pKa_2 > pH$ protonated > deprotonated

$COOH$ > COO^-

example

* if pH = 1

pKa₁ > pH → protonated

pKa₂ > pH → protonated

NH_3^+ COOH

positive No charge

The amino Acid is positive

example

* if $pH = 6$

$pKa_1 > pH$ → protonated

$pKa_2 < pH$ → deprotonated

NH_3^+ COO^-

positive Negative

The Amino Acid is Neutral

example

* if $pH = 11$

$pKa_1 < pH$ → deprotonated

$pKa_2 < pH$ → deprotonated

NH_2 — No charge

COO^- — Negative

The Amino Acid is Negative

pH which the molecule is electrically neutral ← PI = $\frac{pKa_1 + pKa_2}{2}$

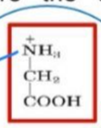
neutral aa

$\frac{9.6 + 2.3}{2} = 5.95$

Titration curve of amino acid (glycine)

↓ pH : protonated

1. At a very low pH (acidic) both groups are fully protonated where the solution predominantly contains:

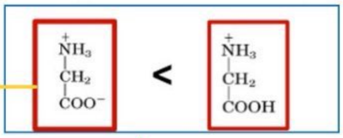


than here

first dissociated. → تنفصل

More Acidic. Lower pka.

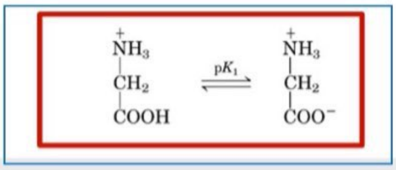
2. When the pH is raised, the –COOH group start to be deprotonated and the proportion will be:

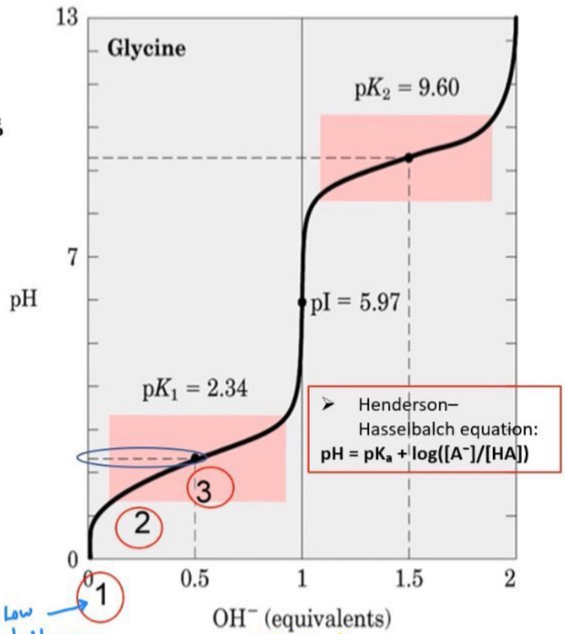


protonated > deprotonated

PKa > pH

3. pH= pKa_1, where it will act as a buffer and the solution will contain an equal amount of :





Cationic form

increase pH → deprotenation for Carboxylic group → Zwitterion ion

Still proportion for Cationic form أعلى من الـ Zwitterion

buffer region

Cationic ion = Zwitterion ion

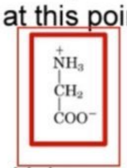
الـ Concentration متساوين

PH = pKa Henderson

Titration curve of amino acid (glycine)

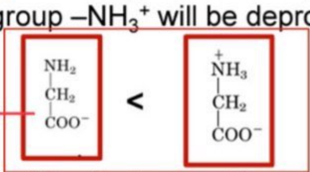
More OH⁻

4- Further increase in pH, the solution will predominantly contains zwitter ion and the pH at this point is equal to pI.

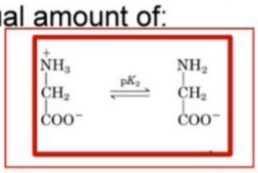


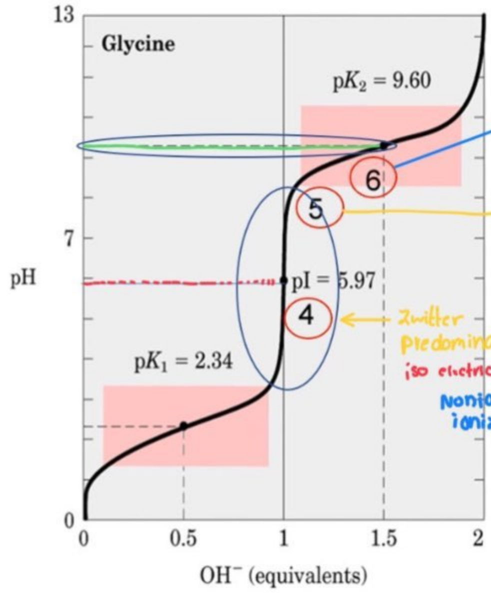
5- As the pH increases, the second group $-NH_3^+$ will be deprotonated

deprotonated < protonated
pKa > pH



6- After that, pH=pKa_2 where it will as a buffer and the solution will contain an equal amount of:



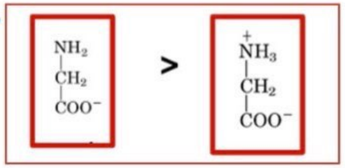


لمّا احصل على ال 2Ka لل Amino Acid

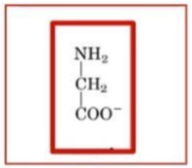
هك صار ممكن أعرف مين هو بالرجوع لل table.

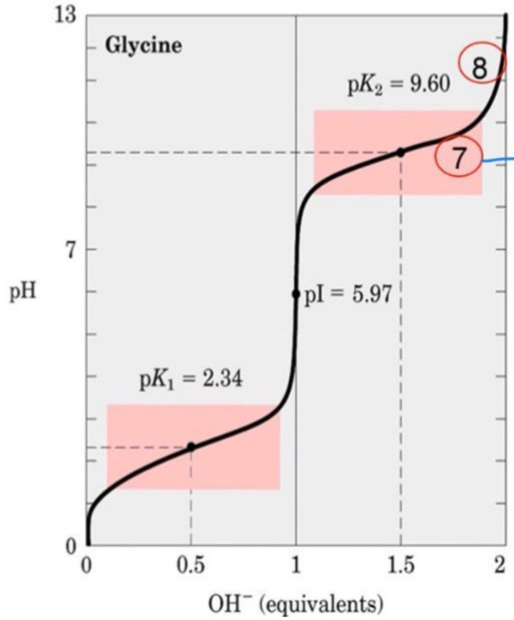
Titration curve of amino acid (glycine)

- **7-**



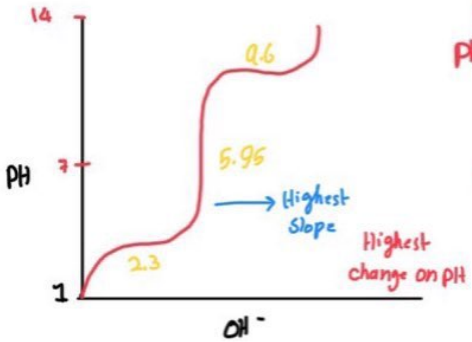
- **8-** the NH_3^+ group will dissociate and at the same time the glycine full dissociate in end point





→ unionic form
أعلى من ال
Zwitter ion

لحد مايصير
Complet dissociation
لل H⁺ من ال Amino group
predominant → unionic form



pH = 1

pKa > pH → protonated

$COOH > COO^-$

$NH_3^+ > NH_2$

$NH_3^+ - CH_2 - COOH$

pH = 6

$pKa_1 > pH$

$pKa_2 < pH$

$COO^- > COOH$

$NH_3^+ > NH_2$

$NH_3 - CH_2 - COO^-$

pH = 13

$pKa_2 < pH$

$pKa_1 < pH$

$COO^- > COOH$

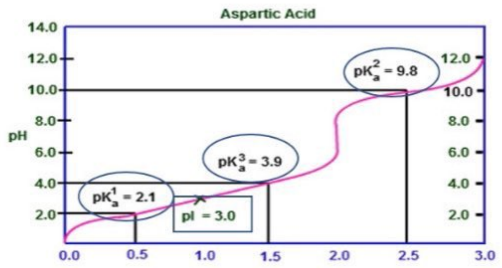
$NH_2 > NH_3^+$

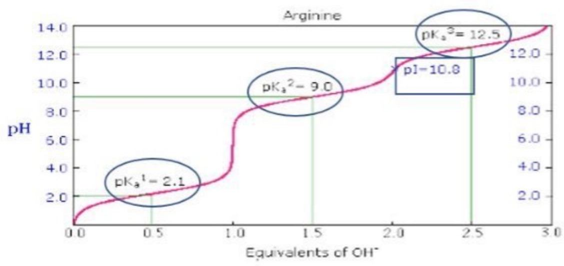
$NH_2 - CH_2 - COO^-$

More Complex → dissociated H^+ on 3 steps

Titration curve of triprotic amino acids

- Titration curves of triprotic a.a. are more complex with three stages→ They have 3 pKa values.
- If additional acidic or basic groups are present as side-chain functions, **the pI is the average of the pK_a's of the two most similar acids (value).**
- In the case of aspartic acid, the similar acids are the alpha-carboxyl group (pK_a = 2.1) and the side-chain carboxyl group (pK_a = 3.9), so pI = (2.1 + 3.9)/2 = 3.0.
- For arginine, the similar pKa`s values are the pKa for guanidinium group on the side-chain (pK_a = 12.5) and pKa for alpha-ammonium group (pK_a = 9.0), so the calculated pI = (12.5 + 9.0)/2 = 10.75.





Practical part

Procedure:

1. Record the code letter for your unknown solution.

2. Place 10ml of your 0.1M amino acid sample into a 400 mL beaker.

3. Clamp the pH electrode to the ring stand with the tip submerged in the solution in the 400 mL beaker. You may have to add 50-100 mL of distilled H2O to make sure the electrode tip is under to solution.

Add 5 N HCl until pH is between 1.7 –1.9.

4. Record the initial pH of your solution and the initial reading of your burette (which doesn't have to be 0.0 mL but it helps).

5. One person should be collecting burette volume readings while the partner watches the change in pH of your solution. Record burette reading for each change in pH in your notebook. You do not have to take a reading for each pH unit shown, but try not to skip more than two units.

6. Initially, the volume of KOH needed to cause a change in pH will be fairly large (over 1 mL).

7. As the titration continues, the volume of KOH solution needed to cause pH changes will become less and less. Near the equivalence point, a single drop may change the pH dramatically.

8. As the volume of KOH needed to change the pH decreases, it is STRONGLY recommended that you proceed drop by drop. You may increase the amount added each time as the pH changes become less (after passing the equivalence point). Continue this procedure until you reach pH 13.0.