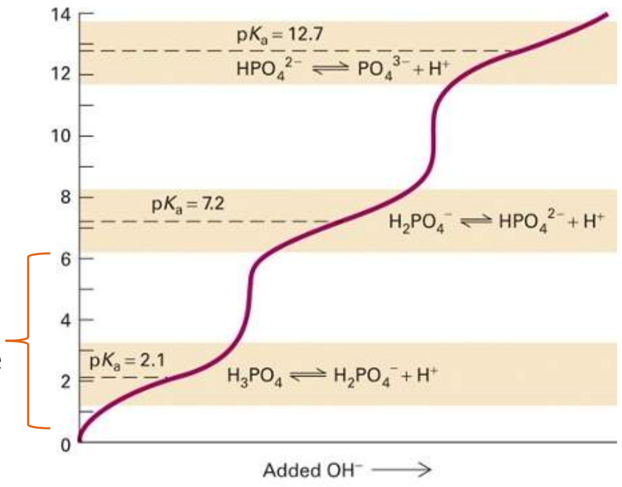
Titration curve of amino acids

Titration Curves:

- Titration Curves are produced by monitoring the pH of a given volume of a sample solution after successive **addition of acid or alkali**.
- The curves are usually plots of pH against the volume of titrant added (**acid or base**).
- Each dissociation group represent **one stage** in the titration curve.

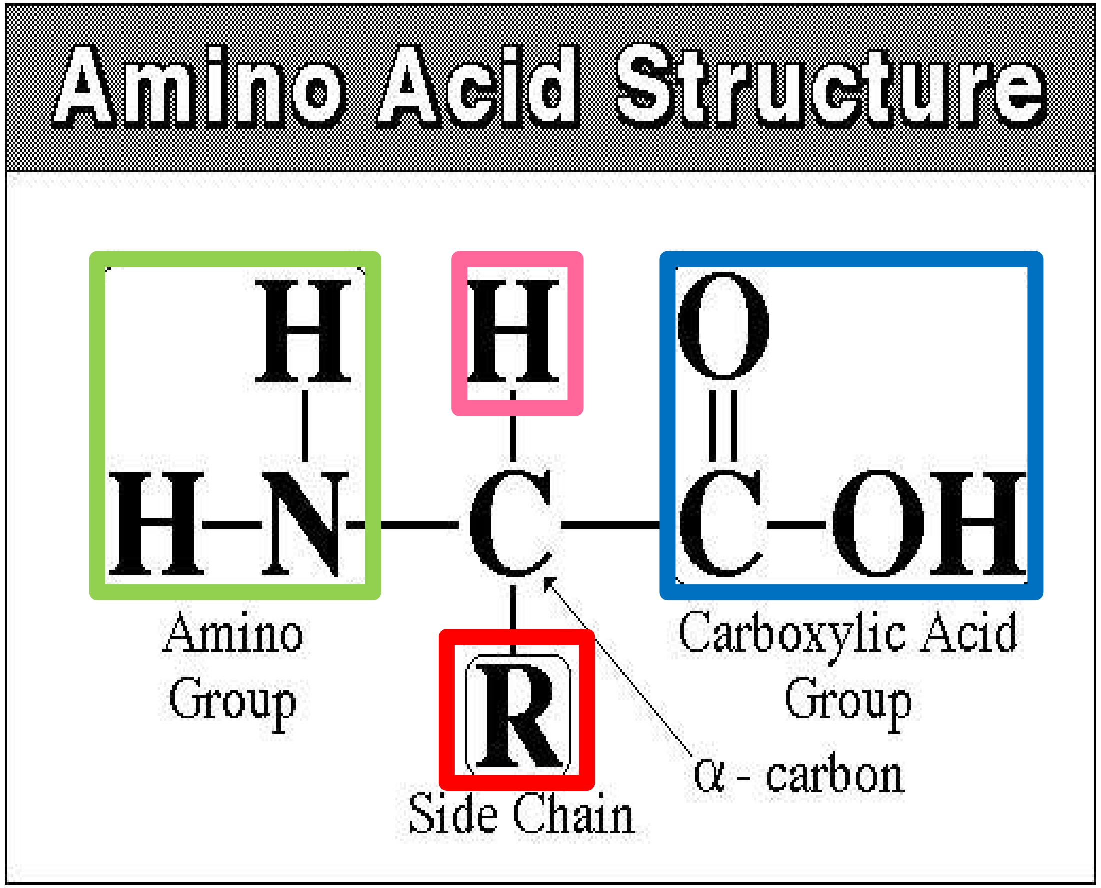
one stage



Amino acid general formula:

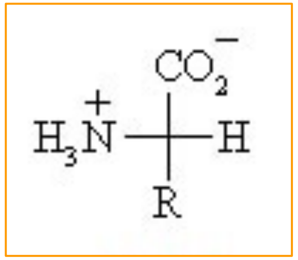
- **Amino acids consist of:**

1. A basic amino group (—NH_2)
2. An acidic carboxyl group (—COOH)
3. A hydrogen atom (—H)
4. A distinctive side chain (—R).



Titration of amino acid:

- When an amino acid is dissolved in water it exists predominantly in the isoelectric form **(Zwitterion)**



- Amino acid is an **amphoteric** compound➔ It act as either an **acid** or a **base (*based on pH*)**:
  - **Upon titration with acid** ➔ it acts as a BASE (accept a proton) → [Fully deprotonated **NH_2-CH-R-COO^-**]
  - **Upon titration with base** ➔ it acts as an ACID (donate a proton) → [Fully protonated **NH_3^+-CH-R-COOH**]

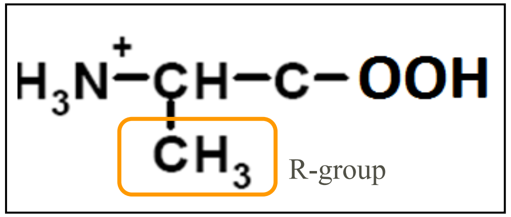
Titration of amino acid Cont.:

- Amino acids are example of **weak acid/base** which contain **more than one dissociate group.**
- **Examples:**

(1) <u>Alanine:</u>

-Contain COOH (pKa_1= 2.34) and NH_3^+ (pKa_2= 9.69) groups (it has one pI value =6.010). [Diprotic]

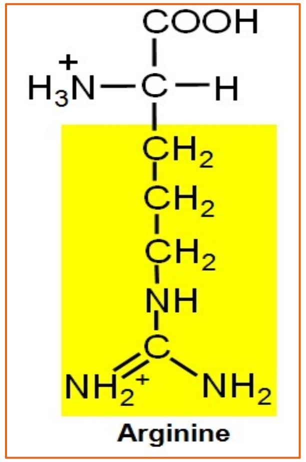
-The COOH will dissociate first then NH_3^+ dissociate later . (Because pKa_1<pKa_2)



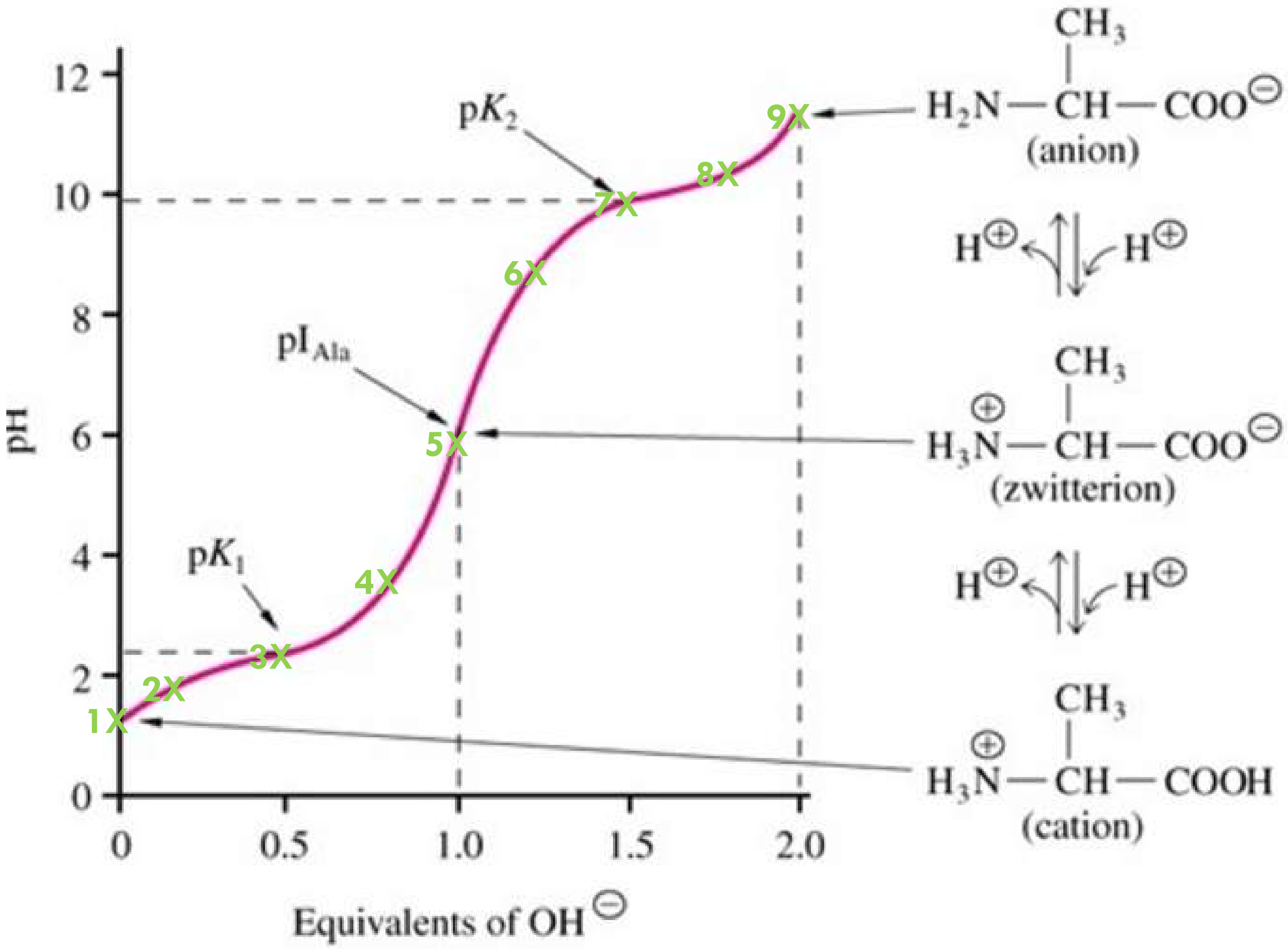
Full protonated alanine

(2) <u>Arginine:</u>

-Contain COOH (pKa_1= 2.34) , NH_3^+ (pKa_2= 9.69) groups and basic group (pKa_3=12.5) (it has one pI value=11). [Triprotic]



Titration curve of Alanine



pK_1 carboxylic acid = 2.34

pK_2 amino group = 9.69

$pI = (pK_1 + pK_2)/2$

Note that <u>before</u> pI the alanine will exist in two forms **[NH_3^+-CH-CH_3-COOH] / [NH_3^+-CH-CH_3-COO^-]**

Titration curve of alanine or glycine [diprotic]:

[1] In starting point:

- Alanine is full protonated (since we're titrating with base) so the AA will act as a weak acid

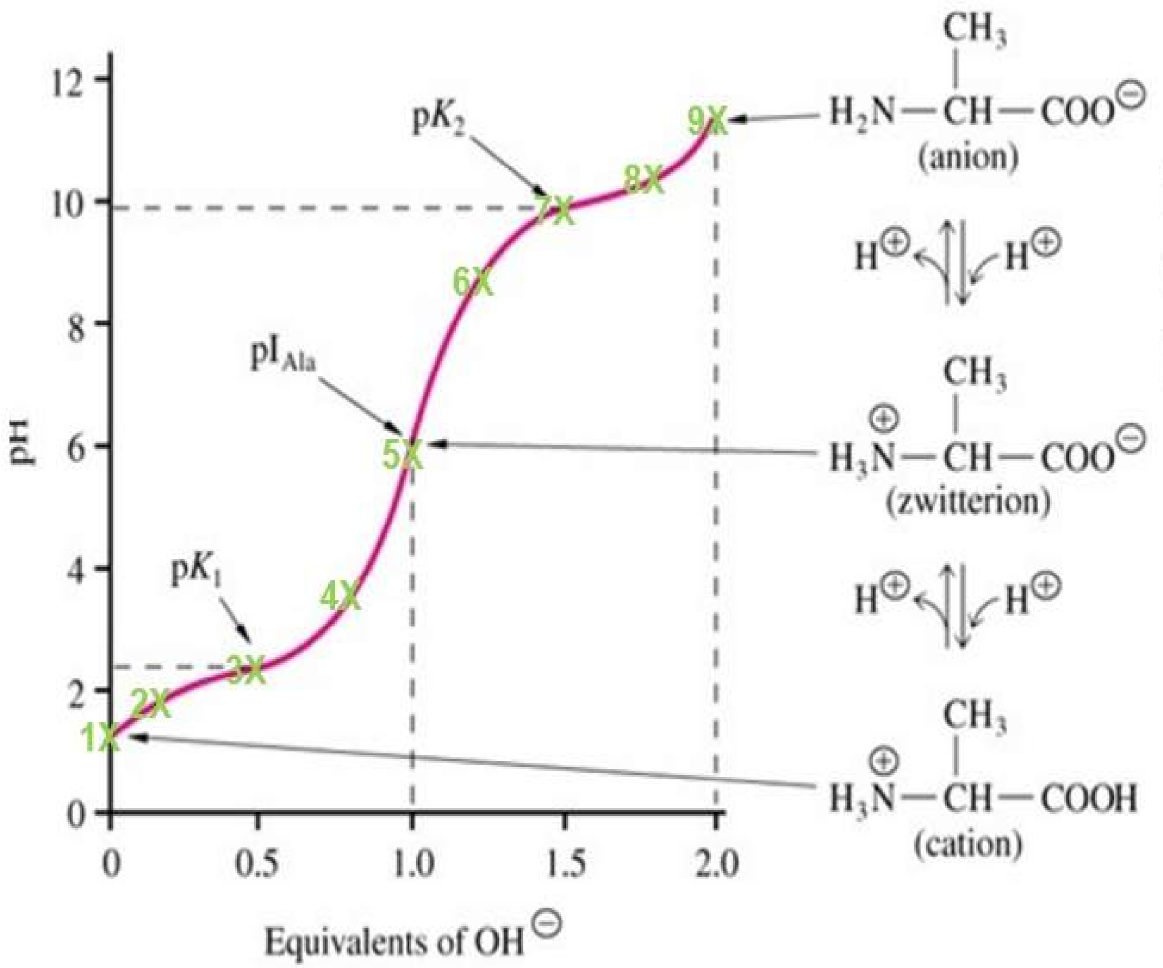
[NH_3^+-CH-CH_3-COOH].

[2] COOH will <u>dissociate</u> first:

- [NH_3^+-CH-CH_3-COOH] > [NH_3+-CH-CH3-COO^-]
- pH < pKa_1.

[3] In this point the component of alanine act as buffer:

- [NH_3^+-CH-CH_3-COOH] = [NH_3^+-CH-CH_3-COO^-].
- pH = pKa_1



Note that **after** pI the alanine will exist in two forms **[NH_3^+-CH-CH_3-COO^-] / [NH_2-CH-CH_3-COO^-]**

Titration curve of alanine or glycine [diprotic]:

[4] In this point:

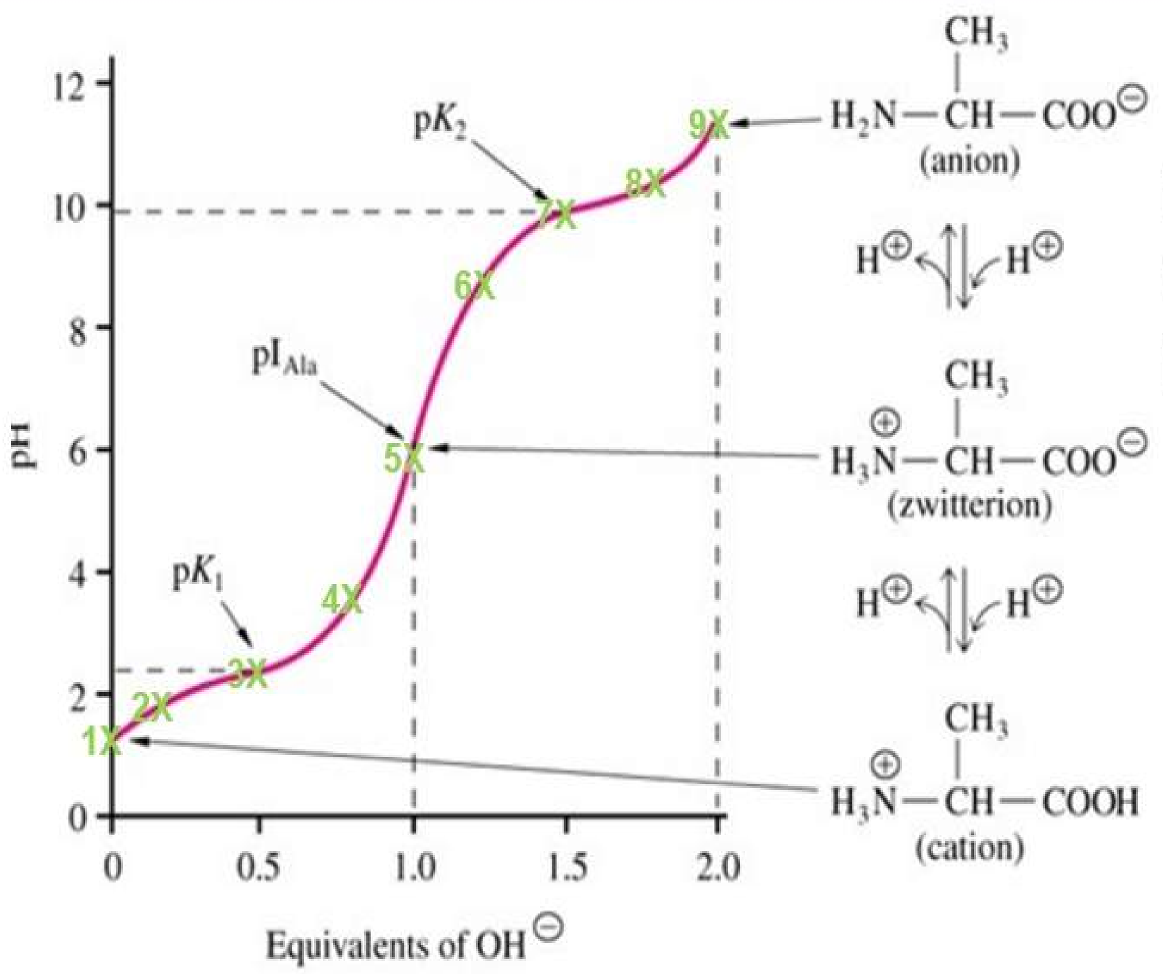
- [NH_3^+-CH-CH_3-COOH] < [NH_3^+-CH-CH_3-COO^-].
- pH > pKa_1.

[5] Isoelectric point:

- The COOH is full dissociate to COO^-.
- **[NH_3^+-CH-CH_3-COO^-]**
- Con. of -ve charge = Con. of +ve charge.
- The amino acid present as Zwetter ion (neutral form) .
- Remember that: pI (isoelectric point) is the pH value at which the net charge of amino acid equal to zero.
- pI = (pKa_1 + pKa_2) /2 = (2.32+9.96)/2= 6.01

[6] The NH_3^+ start dissociate:

- [NH_3^+-CH-CH_3-COO^-] > [NH_2-CH-CH_3-COO^-].
- pH < pKa_2



Titration curve of alanine or glycine [diprotic]:

[7] **In this point the component of alanine act as buffer:**

- $[NH_3^+\text{-CH-CH}_3\text{-COO}^-] = [NH_2\text{-CH-CH}_3\text{-COO}^-]$.
- $pH = pKa_2$.

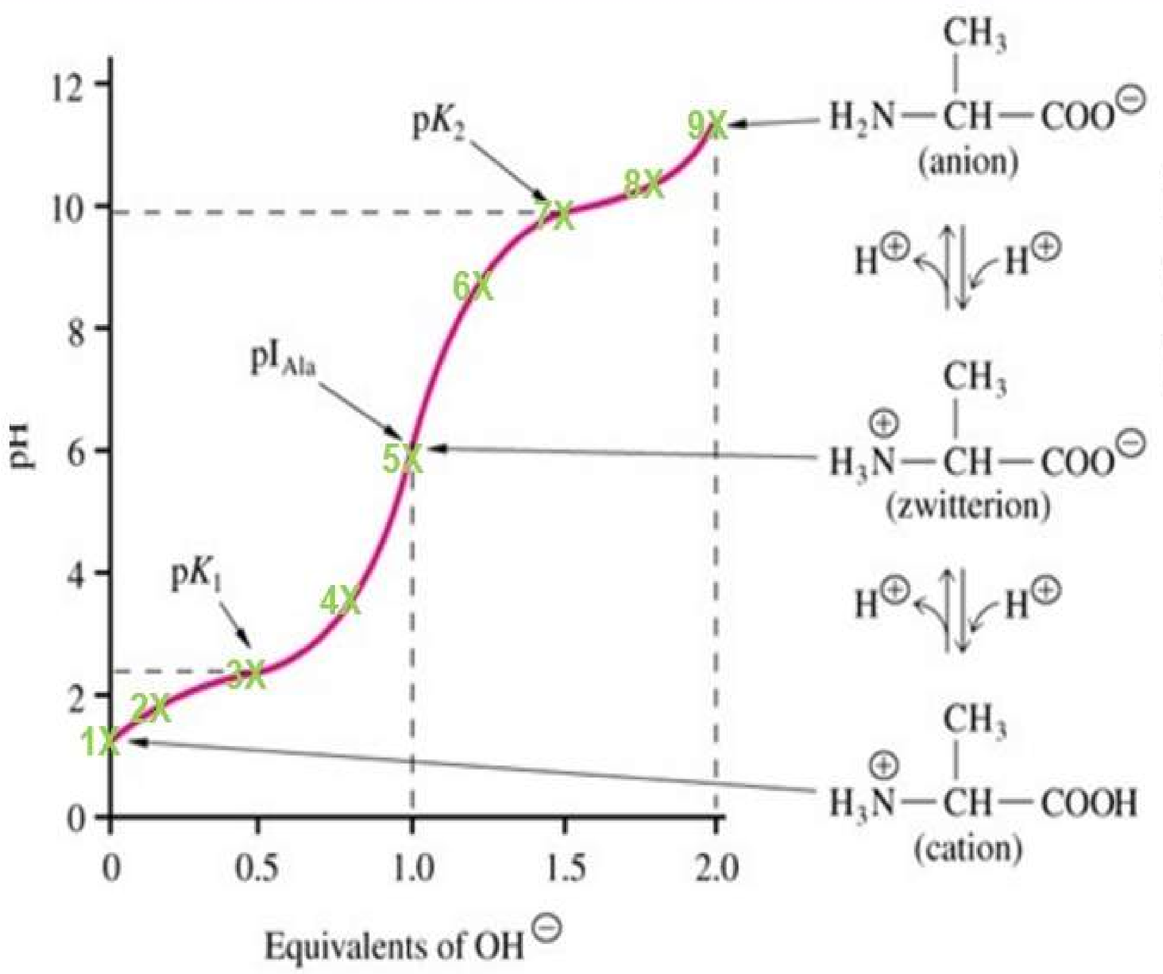
[8] **In this point:**

- $[NH_3^+\text{-CH-CH}_3\text{-COO}^-] < [NH_2\text{-CH-CH}_3\text{-COO}^-]$.
- $pH > pKa_2$

[9] **End point:**

- The alanine is full dissociated.
- $[NH_2\text{-CH-CH}_3\text{-COO}^-]$ (weak base form)
- pOH= (pkb+p[A-])/2

➔ $pKb = pKw - pKa_2$



Calculating the pH at different point of the titration curve :

The pH calculated by different way :

[1] at starting point :

$$pH = (pka + p[HA])/2$$

[2] At any point within the curve (before or in or after middle titration):

$$pH = pka + \log([A-]/[HA])$$

[3] At end point:

$$pOH = (pKb + P[A-])/2$$

$$pH = pKw - pOH$$

$$pKb = pKw - pKa2$$

Remember !!
At start of titration with acid and base together, assume that amino acid is in at its **isoelectric form [NH_3^+-CH-CH_3-COO^-]**

Example:

- **Determine the pH value of 10 ml of 0.1M alanine solution, titrated with 0.1M NaOH/HCl after the addition of 4 ml of 0.1M NaOH and 1 ml of 0.1M HCl, COOH (pKa1= 2.34) NH_3^+ (pKa2= 9.69)**

[1] pH after the addition of 4 ml of 0.1M NaOH:

HA + NaOH → A + H_2O
So, **NaOH + NH_3^+ → NH_2 + H_2O**

Mole of HA (NH_3^+) [original] – mole of A^- (NaOH) [added] = mole of HA (NH_3^+) remaining.

-No. of NaOH [A^-] mole = 0.1 X 0.004 L = 0.0004 mole
-No. of HA mole originally = 0.1 X 0.01 L = 0.001 mole
-No. of HA mole remaining = 0.001 - 0.0004 = 0.0006 mole

So,
pH= pKa_2 + log[A-]/[HA]
pH = 9.69 + log [0.0004]/[0.0006]
pH = 9.52 (pH<pKa_2)

→ [NH_3^+-CH-CH_3-COO^-] > [NH_2-CH-CH_3-COO^-].

[2] pH after the addition of 1 ml of 0.1M HCl:

A^- + HCl → HA
So, **HCl + COO^- → COOH**

Mole of A^- (COO^-) [original] – mole of HA (HCl) [added] = mole of A^- (COO^-) remaining.

-No. of HCl [HA] mole = 0.1 X 0.001 L = 0.0001 mole
-No. of A^- mole originally = 0.1 X 0.01 L = 0.001 mole
-No. of A^- mole remaining = 0.001 - 0.0001 = 0.0009 mole

So,
pH= pKa_1 + log[A-]/[HA]
pH = 2.34 + log [0.0009]/[0.0001]
pH = 3.29 (pH > pKa_1)

→ [NH_3^+-CH-CH_3-COOH] < [NH_3^+-CH-CH_3-COO^-].

Practical Part

Objectives:

- To study titration curves of amino acid
- To use this curve to estimate the pKa values of the ionizable groups of the amino acid
- To determine pI
- To determine the buffering region
- To understand the acid base behaviour of an amino acid

Method:

- Add 10 ml of **0.1M alanine** solution to a beaker
- Titrate it with **0.1M NaOH** (dropwise) then mix properly
- Recording the pH after each **0.5 ml** NaOH added until you reach pH=11
- Repeat the procedure with **0.1 M HCl**, and stop the titration when you reach pH=2.17

ml of 0.1M NaOH	pH	ml of 0.1M HCl	pH
0		0	
0.5		0.5	
1		1	
1.5		1.5	
2		2	
2.5		2.5	
3		3	
3.5		3.5	
4 … etc		4 … etc	

Results:

- Record the titration table and plot a curve of pH versus ml of titrant added.
- Calculate the pH of the alanine solution after the addition of 0 ml, 5 ml, of 0.1M NaOH, and calculate the pH after the addition of 0.5 ml, 2 ml of HCl.
- Compare the calculated pH values with those obtained from the curve.
- Determine the pKa of ionizable groups of amino acids from the curve.
- Determine the pI value from your result the curve