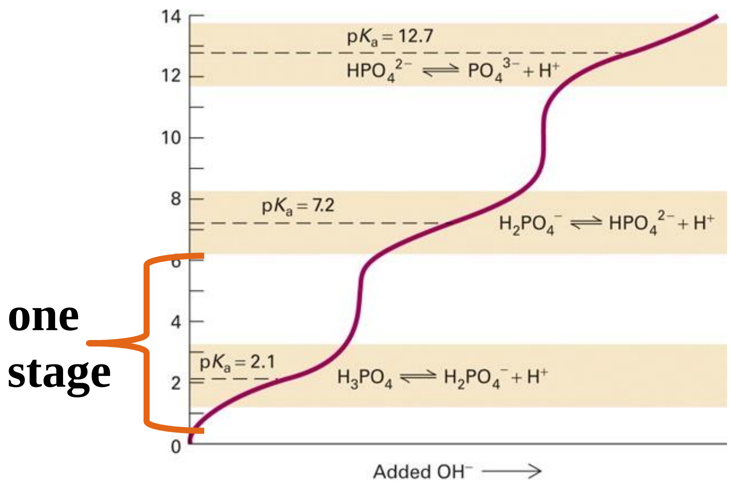
Titration curve of amino acids

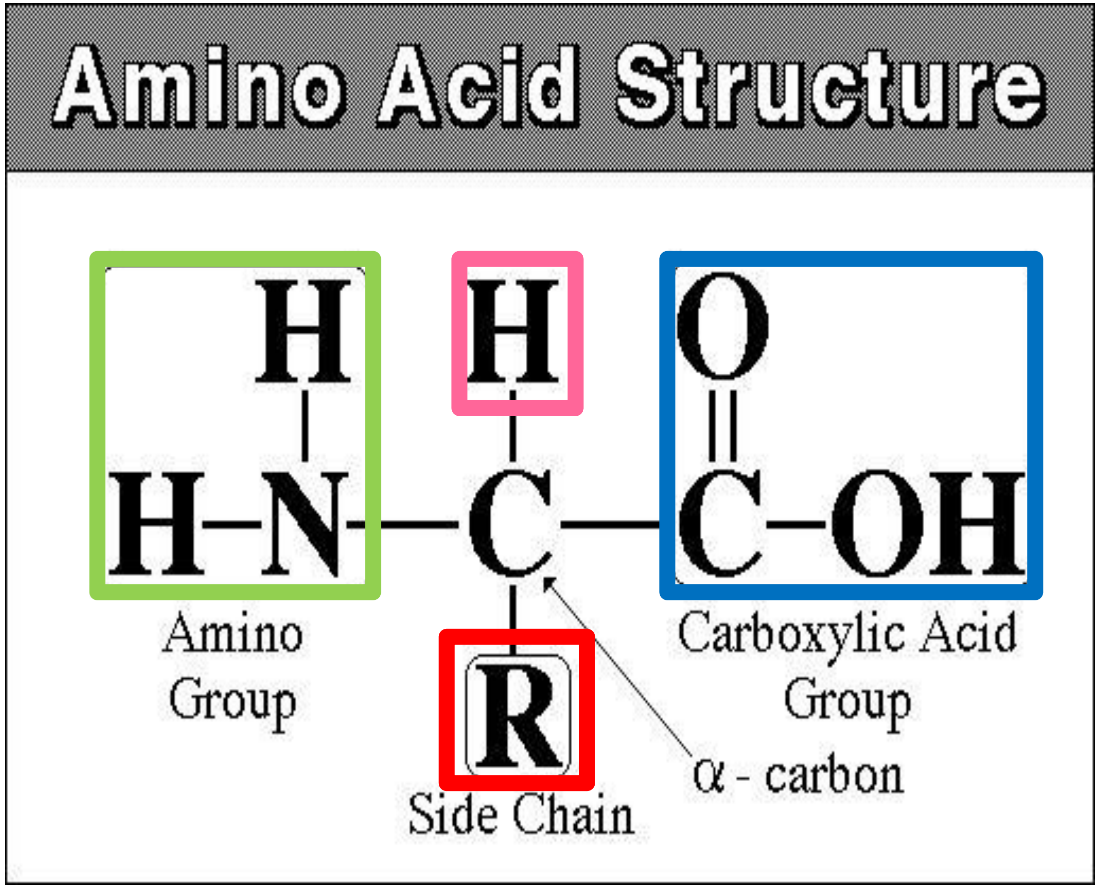
Titration Curves :

- 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.



Amino acid general formula:

- **Amino acids consist of:**
  - A basic amino group ($—NH_2$)
  - An acidic carboxyl group ($—COOH$)
  - A hydrogen atom ($—H$)
  - A distinctive side chain ($—R$).



Amphoteric nature of amino acid:

- When an amino acid is dissolved in water it exists predominantly in the **zwitterion form.**

- Amino acid is an **amphoteric** compound➔ It act as either an acid or a base:

  - **Upon titration with acid** ➔ it acts as a BASE (accept a proton).
  - **Upon titration with base** ➔ it acts as an ACID (donate a proton)

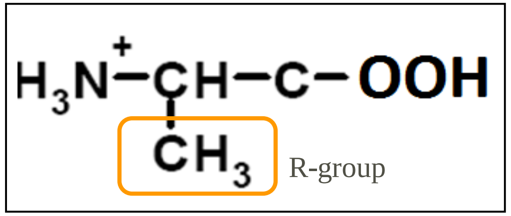
Amino acid as weak acids:

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

(1) Alanine:

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

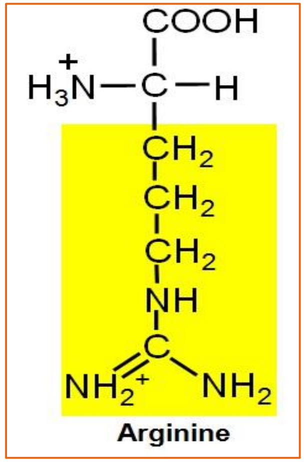
-The COOH will dissociate first then NH_3^+ dissociate later . (Because pKa1<pKa2)



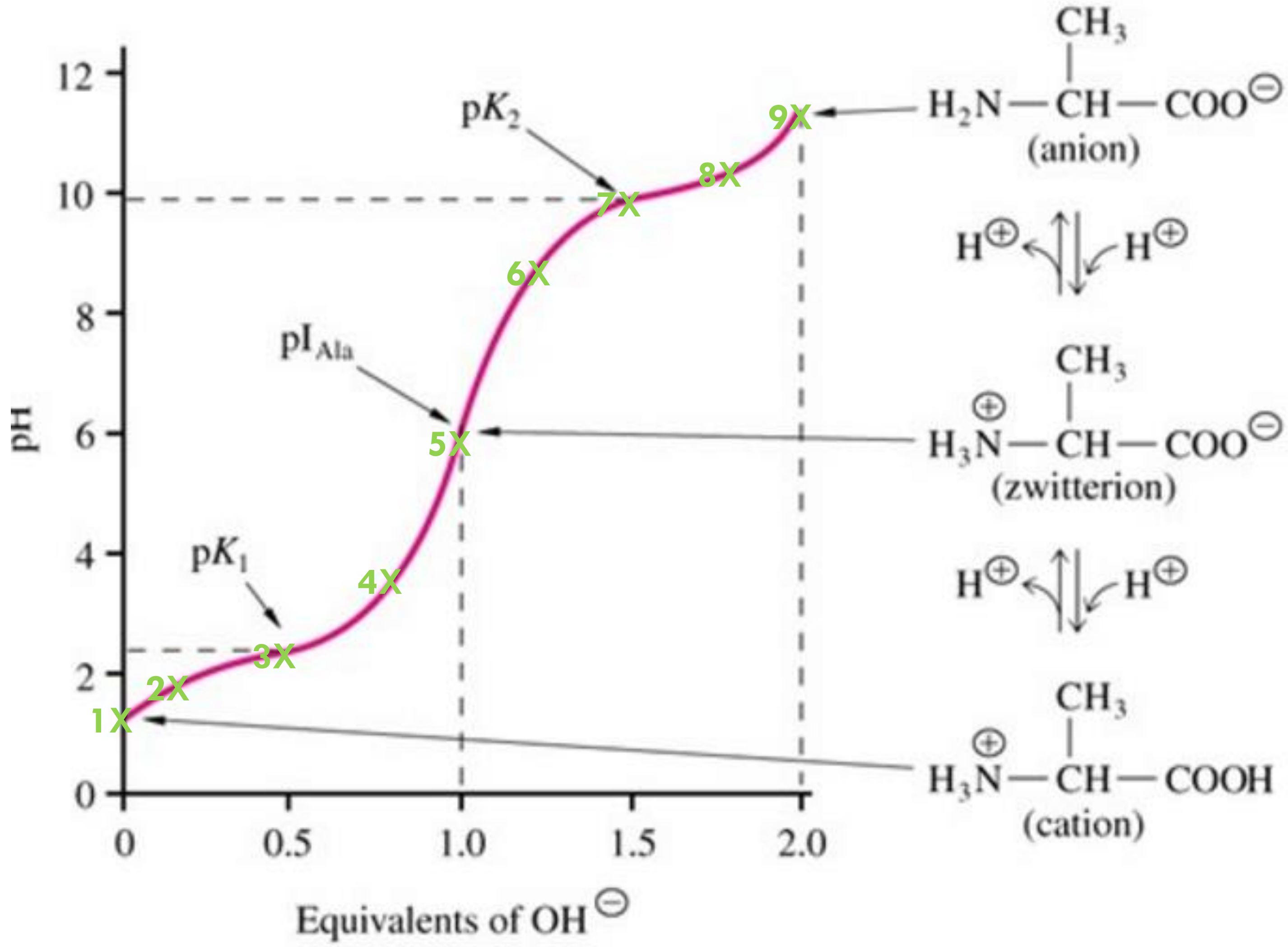
Full protonated alanine

(2) Arginine:

-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



$[A^{2-}]$

pK_1 carboxylic acid = 2.34

pK_2 amino group = 9.69

$pI = (pK_1 + pK_2)/2$

$[HA^-]$

$[H_2A]$

Titration curve of alanine [diprotic]:

[1] In starting point:

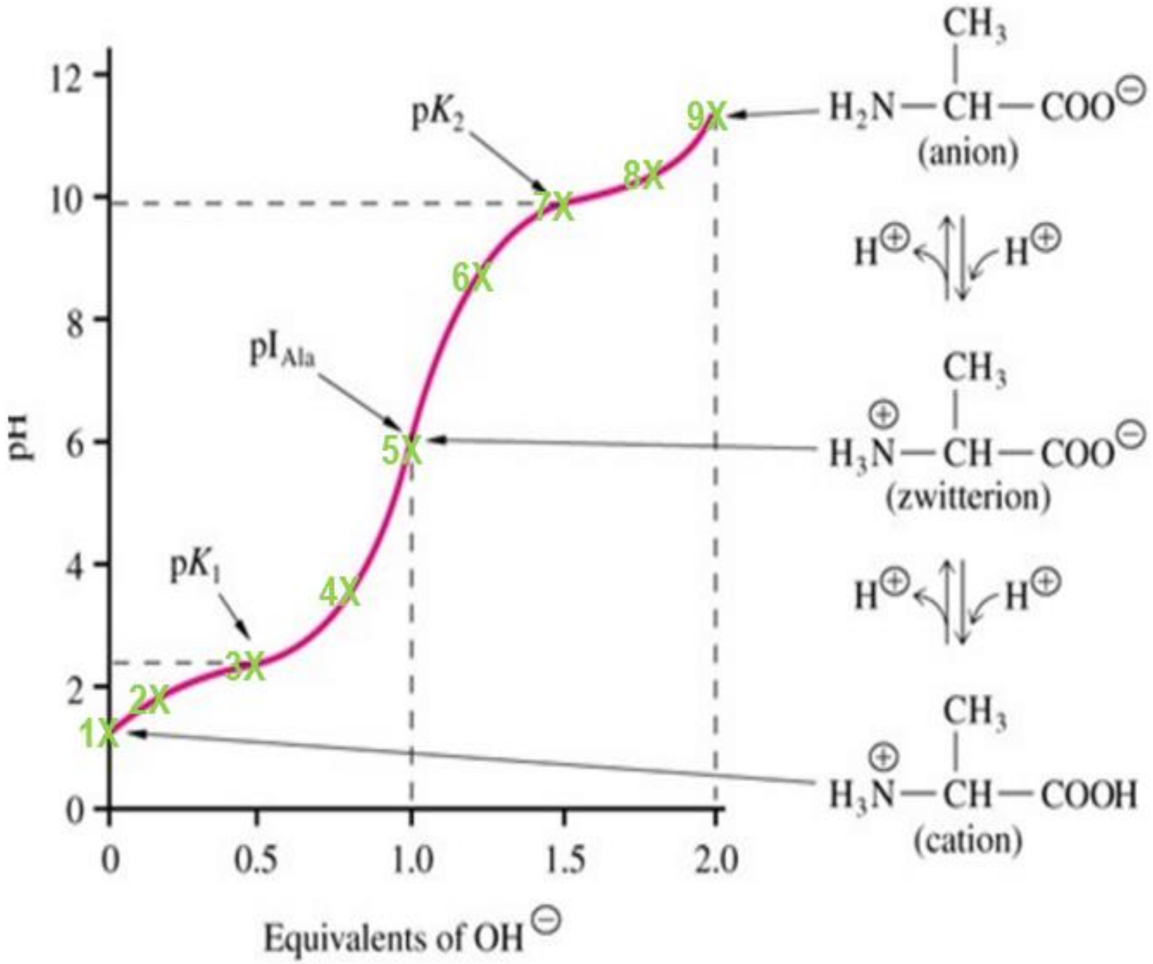
- Alanine is full protonated.
- [NH_3^+-CH-CH_3-COOH].

[2] COOH will dissociate first:

- [NH_3^+-CH-CH_3-COOH] > [NH3+-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



Titration curve of alanine or glycine [diprotic]:

[4] In this point:

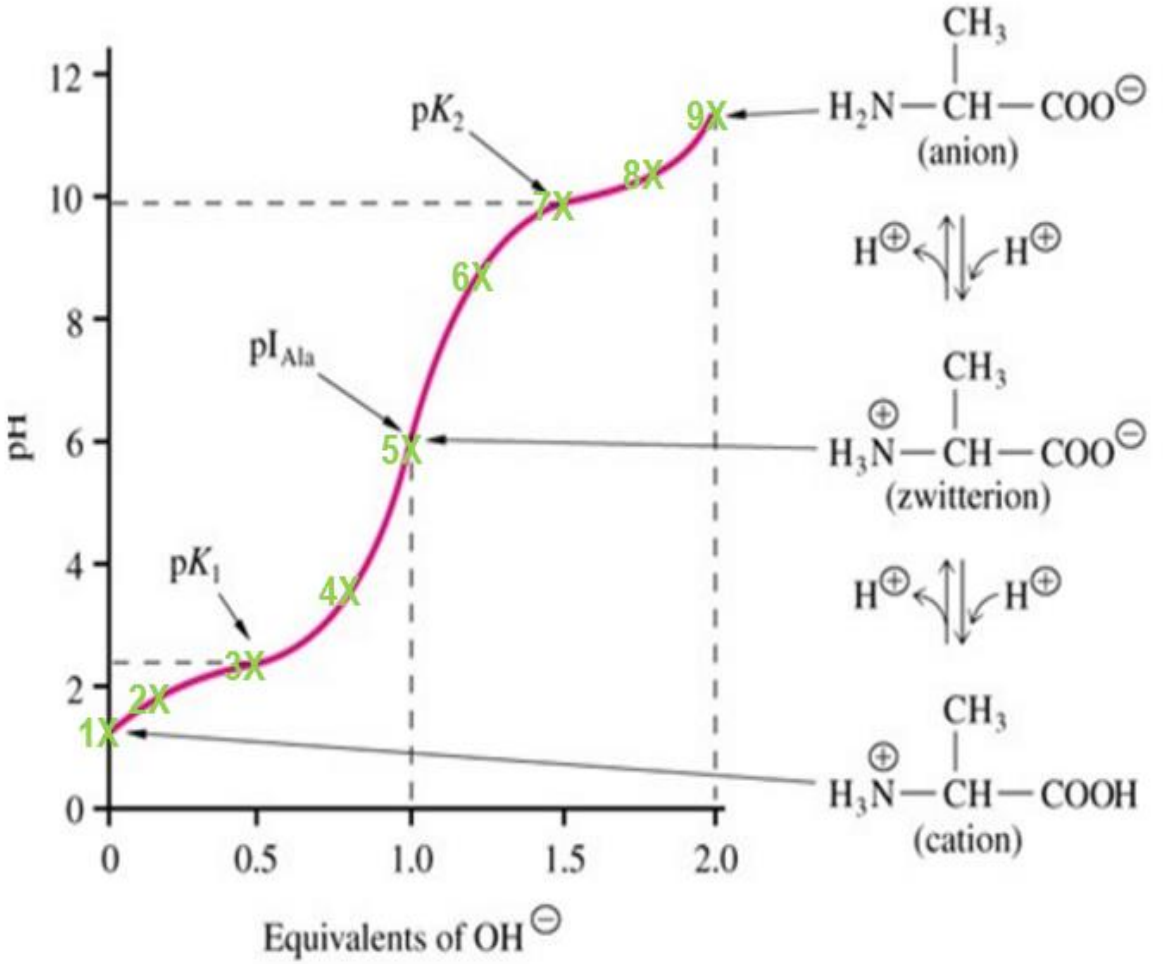
- $[NH_3^+\text{-CH-CH}_3\text{-COOH}] < [NH_3^+\text{-CH-CH}_3\text{-COO}^-]$.
- $pH > pKa_1$.

[5] Isoelectric point:

- The COOH is full dissociate to COO^-.
- $[NH_3^+\text{-CH-CH}_3\text{-COO}^-]$.
- -ve charge = +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:

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



Titration curve of alanine or glycine [diprotic]:

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

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

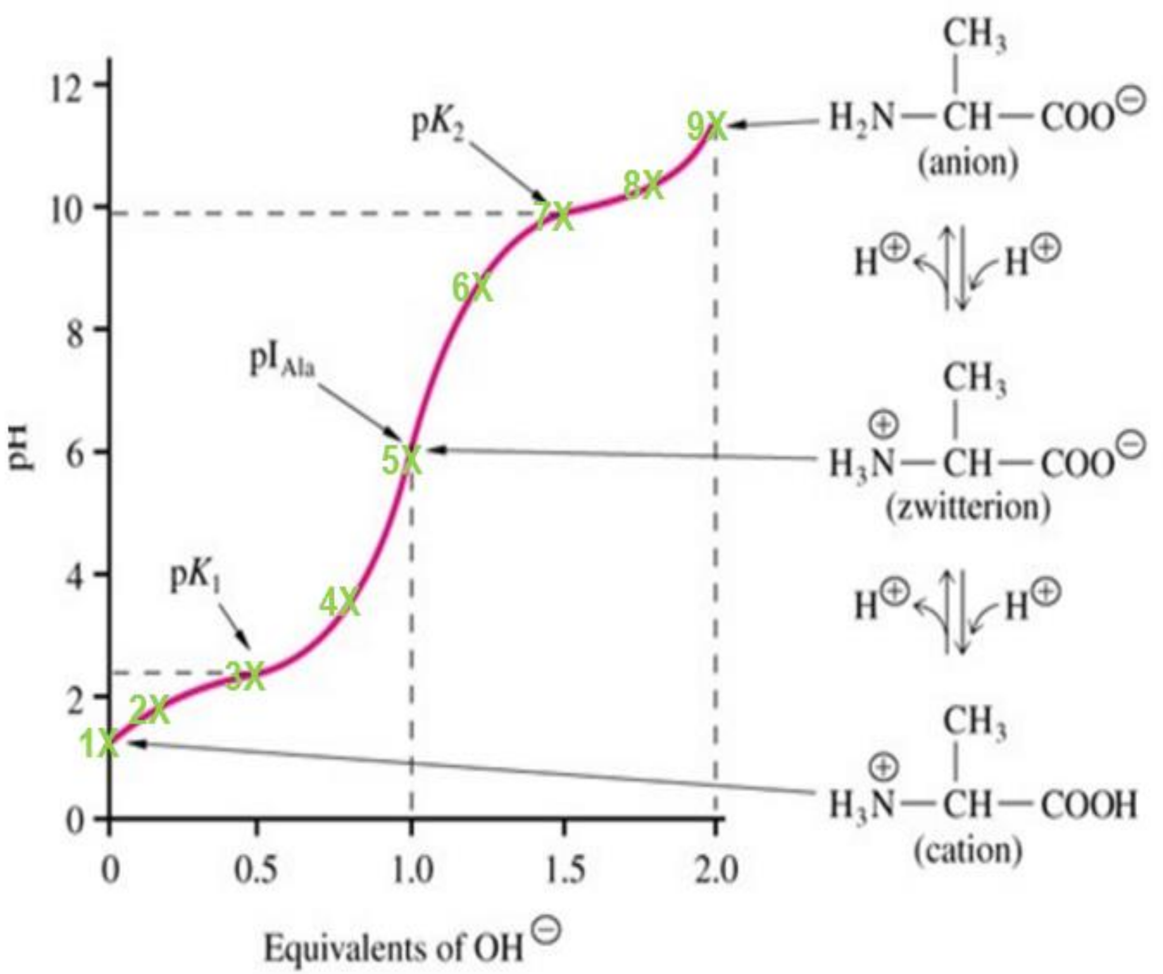
[8] In this point:

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

[9] End point:

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

➔pKb = pKw – pKa2



Calculating the pH at different point of the titration curve :

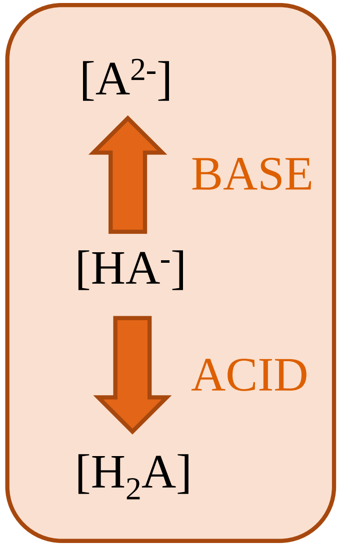
<u>The pH calculated by different way :</u>

[1] at starting point :

$$pH = (pka1 + p[HA])/2$$

[2] At any point within the curve (except start and end points):

$$pH = pka1 + \log([HA^-]/[H_2A]) \quad \text{and} \quad pH = pka2 + \log([A^{2-}]/[HA^-])$$



[3] At end point:

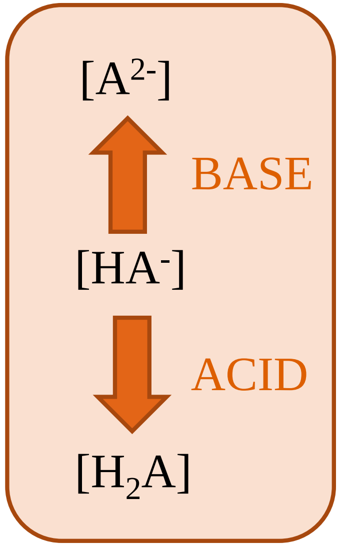
$$pOH = (pKb + p[A-])/2$$

$$pH = pKw - pOH$$

$$pKb = pKw - pKa2$$

Example:

Determine the pH value of 100 ml of alanine (0.2M) , titrated with 0.2M KOH and 0.2M HCl, (pKa1=2.34 and pKa2=9.69), after addition of: (1) 50 ml of HCL. (2) 30 ml of KOH.



[1] pH after addition of 40 ml of HCL?

HCL (acid) ➔ amino acid act as base ➔ COO^- to COOH

pH=pka1+log($[HA^-]/[H_2A]$) ,

HA^-= **Mole of HA [original] – mole of HCl [added] = mole of HA remaining.**
H_2A= **mole of HCL [added]**

-No. of HCL [H_2A] mole = 0.2 X 0.04 L = 0.008 mole
-No. of HA mole originally = 0.2 X 0.1 L =0.02 mole
-No. of HA mole remaining = 0.02 – 0.008 = 0.012 mole

So,
pH=pka1+log([remaining]/[added])
pH = 2.34 + log [0.012]/[0.008]
pH = 2.52

[2] pH after addition of 30 ml of KOH?

NaOH (base) ➔ amino acid act as acid➔ NH_3^+ to NH_2

pH=pka2+log($[A^{2-}]/[HA^-]$)

HA^-= **Mole of HA [original] – mole of KOH [added] = mole of HA remaining.**
A^{2-}= **mole of KOH [added]**

-No. of KOH [A^{2-}] mole = 0.2 X 0.03 L = 0.006 mole
-No. of HA mole originally = 0.2 X 0.1 L =0.02 mole
-No. of HA mole remaining = 0.02 – 0.006 = 0.014 mole

So,
pH=pka2+log([added]/[remaining])
pH = 9.69 + log [0.006] / [0.014]
pH=9.32

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:

a) You are provided with 10 ml of a 0.1M alanine solution, titrate it with 0.1M NaOH adding the base drop wise mixing, and recording the pH after each 0.5 ml NaOH added until you reach a pH=11.

Measured pH value	Amount of 0.1M NaOH added [ml]

b) Take another 10 ml of a 0.1M alanine solution, titrate it with 0.1 M HCL adding the acid drop wise mixing, and recording the pH after each 0.5 ml HCL added until you reach a pH=2.17.

Measured pH value	Amount of 0.1M HCl added [ml]

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, 5ml, of 0.1M NaOH, and calculate pH after addition of 0.5 ml , 2ml of HCl.
- Determine the pKa of ionizable groups of amino acids.
- Compare your calculated pH values with those obtained from Curve.
- Determine the pI value from your result .