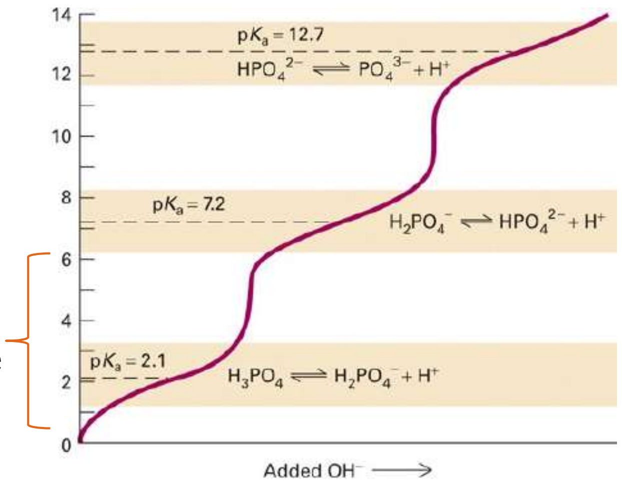
# Titration curve of amino acids

# Titration Curves :

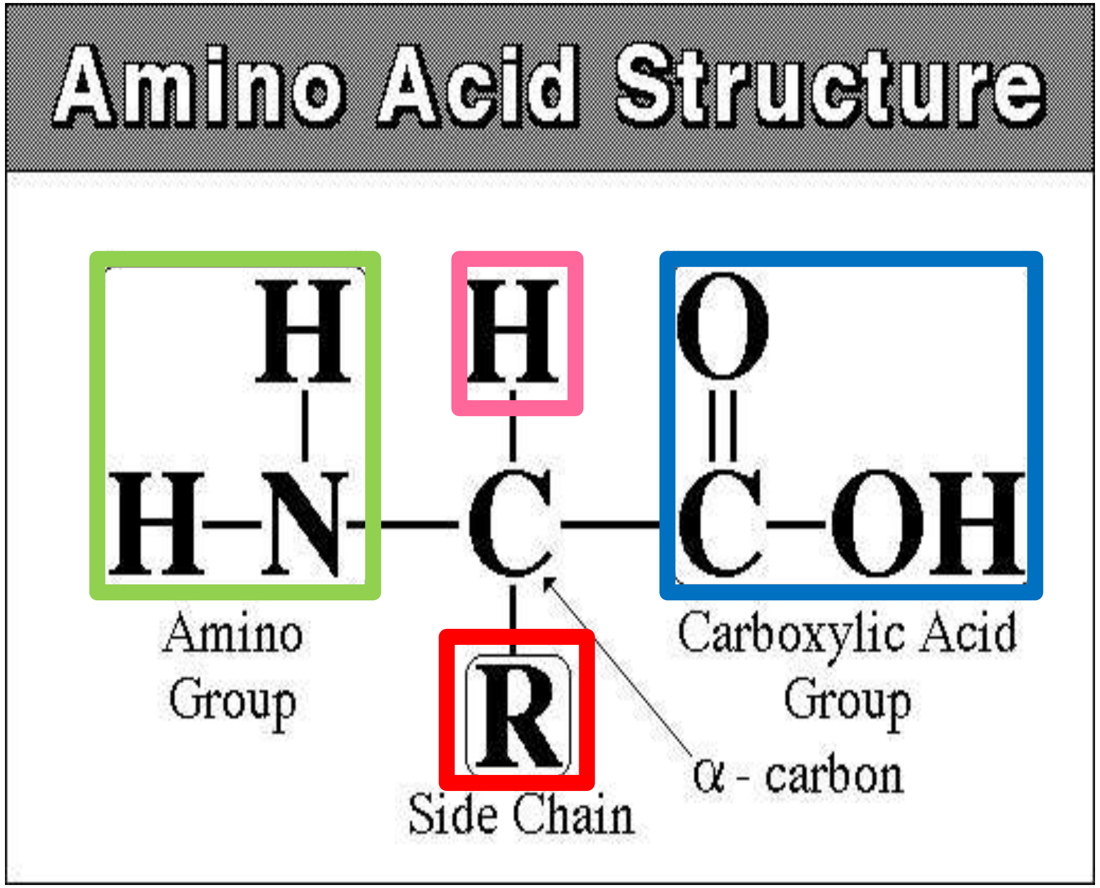
- Titration Curves are produced by monitoring the pH of a given volume of a sample solution after successive **addition of acid or alkali**.
- The curves are usually plots of pH against the volume of titrant added (acid or base).
- Each dissociation group represent **one stage** in the titration curve.

**one stage**



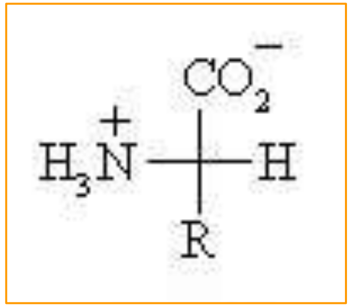
# Amino acid general formula:

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

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

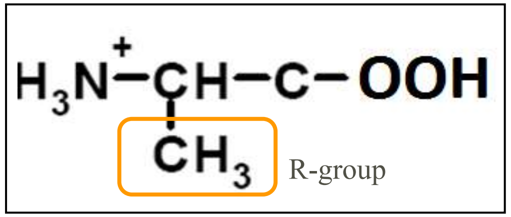
# Titration of amino acid cont':

- 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]

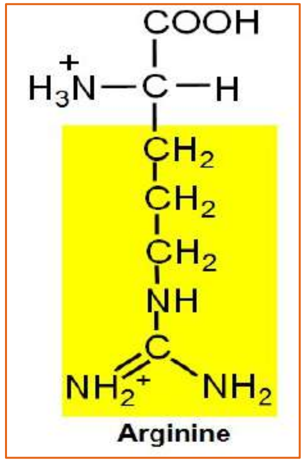
-The COOH will dissociate first then $NH_3^+$ dissociate later . (Because $pKa_1$<$pKa_2$)



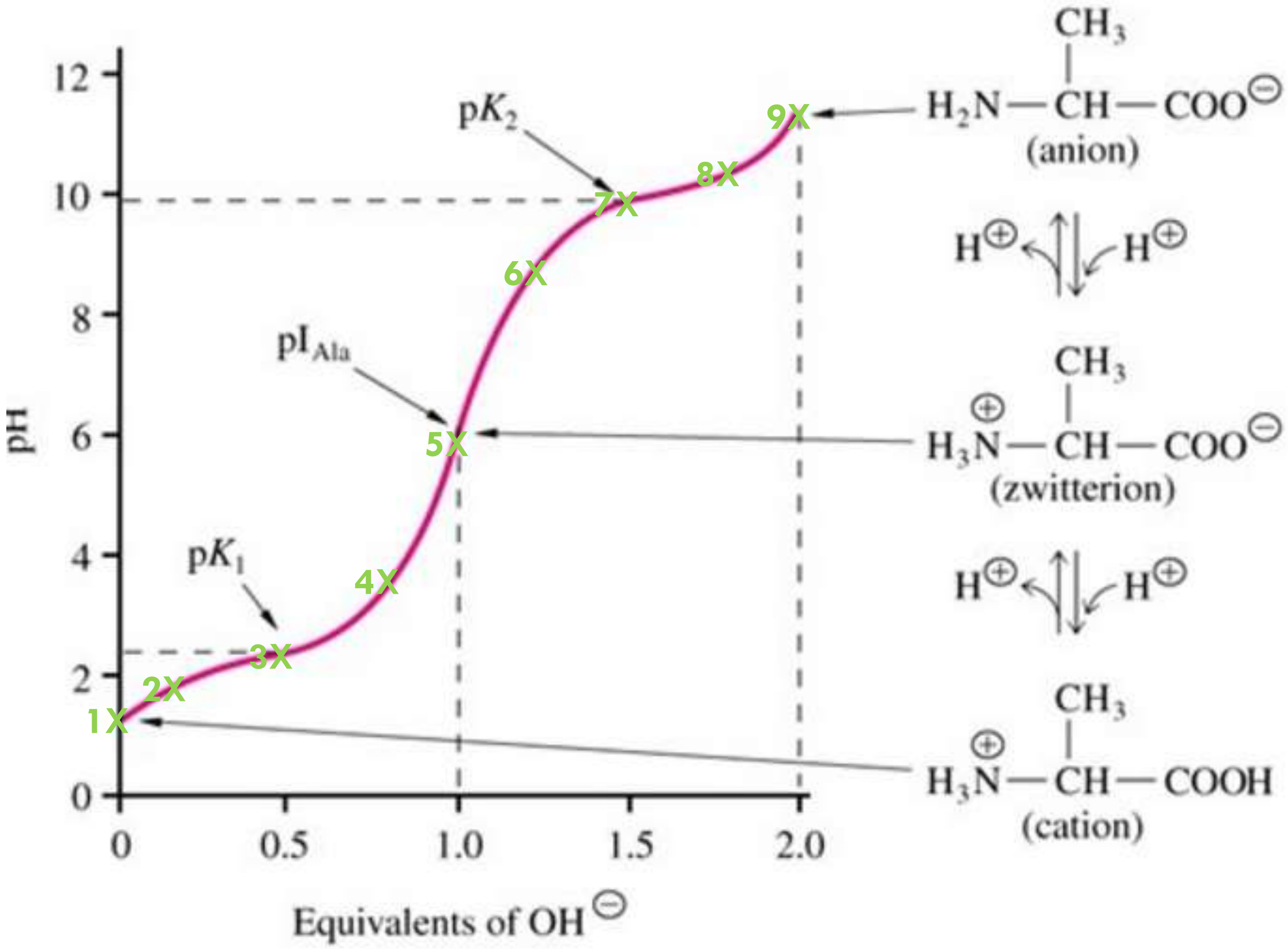

Full protonated alanine

**(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




$pK_1$ carboxylic acid = 2.34

$pK_2$ amino group = 9.69

$pI = (pK_1 + pK_2)/2$

Note that before 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:

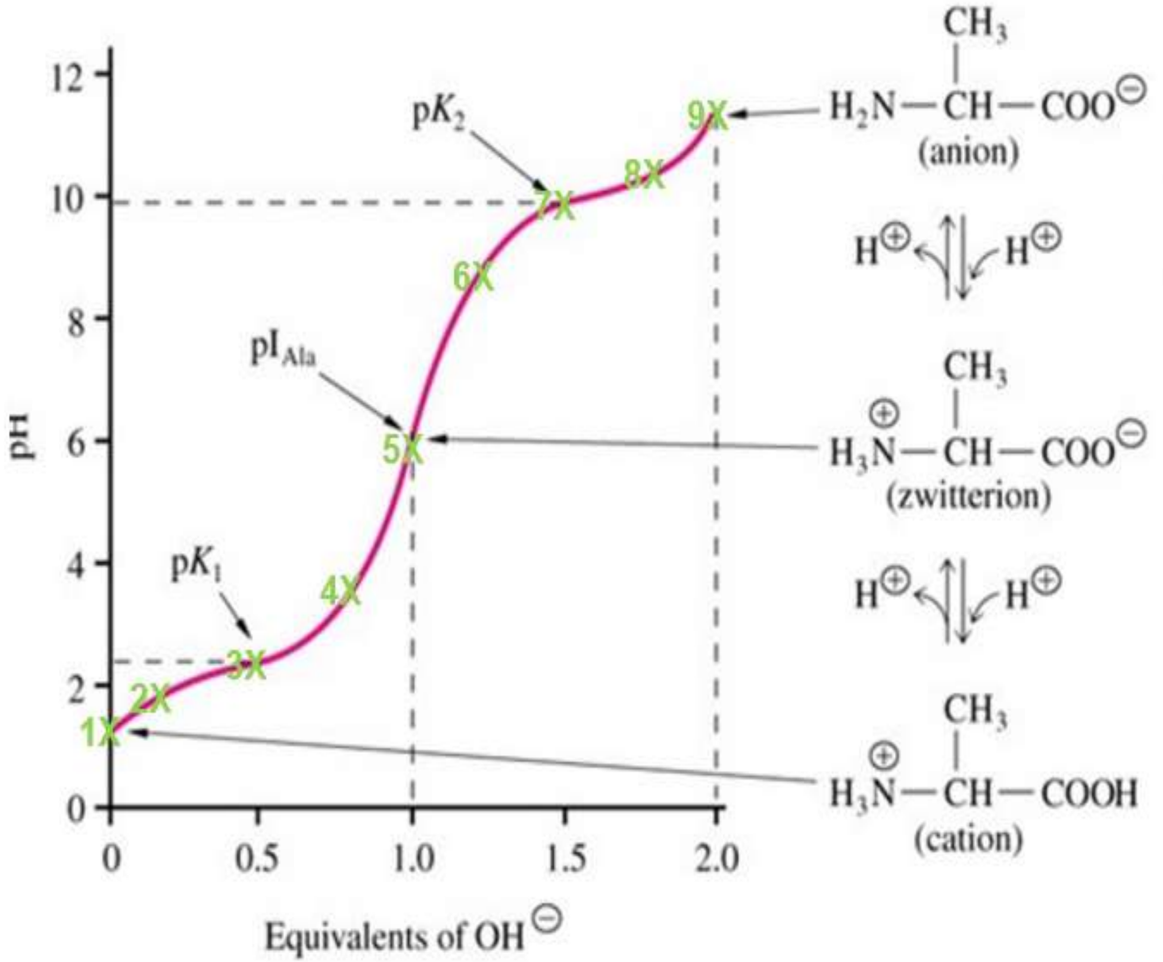
- Alanine is full protonated.
- [$NH_3^+$-CH-$CH_3$-COOH].

### [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-$CH_3$-$COO^-$].
- $pH=pKa_1$



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:**

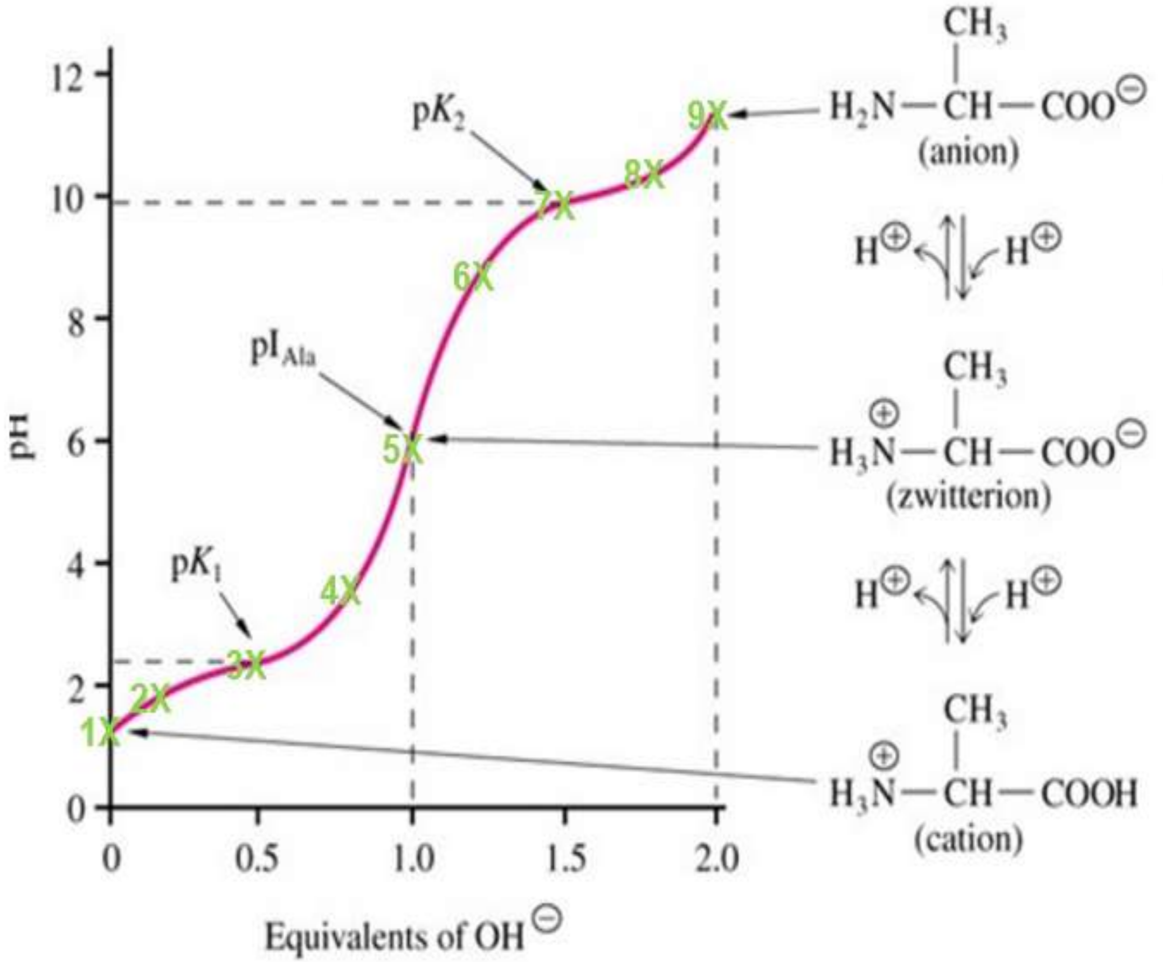
- [$NH_3^+$-CH-$CH_3$-COOH]<[$NH_3^+$-CH-$CH_3$-$COO^-$].
- pH > $pKa_1$.

**[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:**

- $[NH_3^+\text{-CH-}CH_3\text{-}COO^-] = [NH_2\text{-CH-}CH_3\text{-}COO^-]$ .
- $pH = pKa_2$.

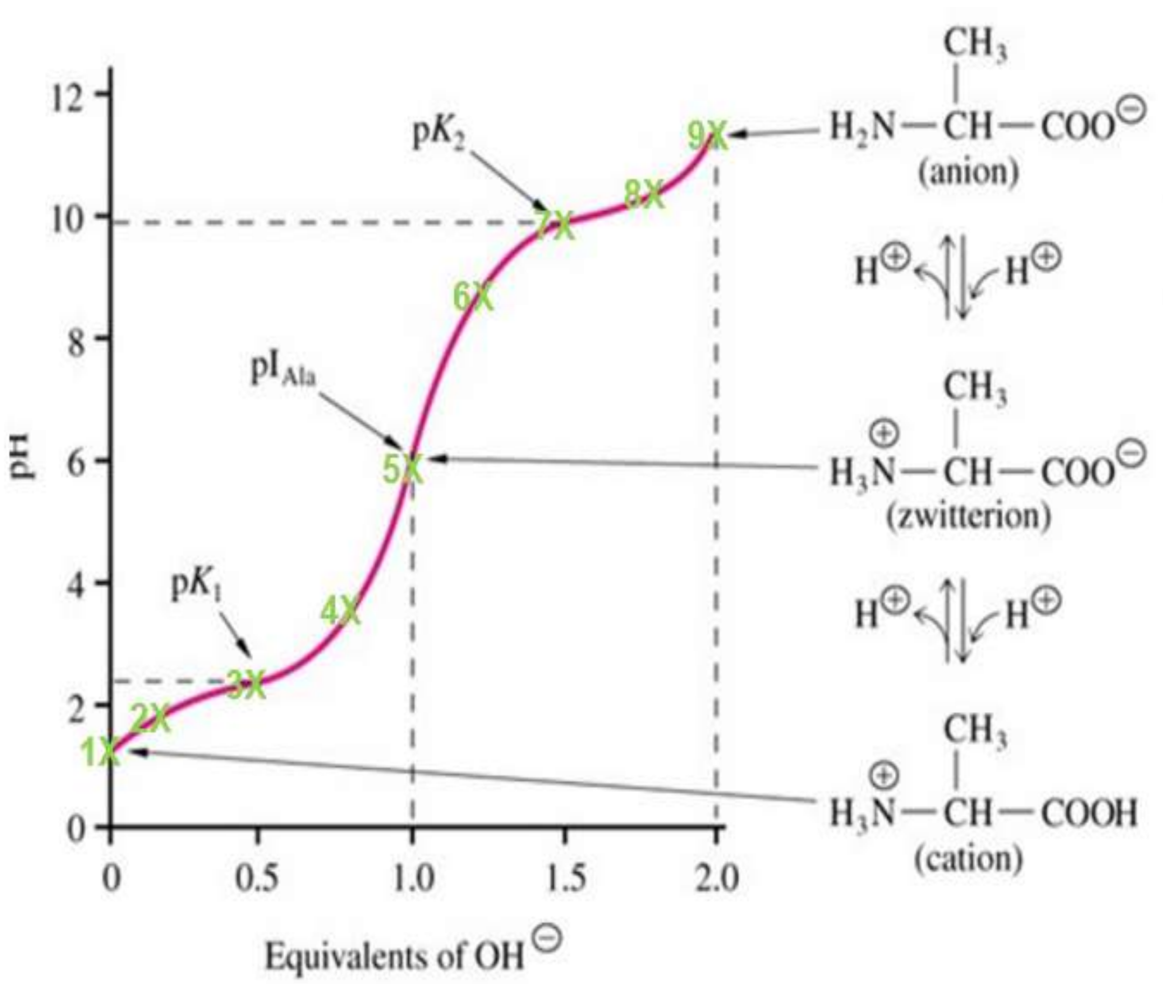
**[8] In this point:**

- $[NH_3^+\text{-CH-}CH_3\text{-}COO^-] < [NH_2\text{-CH-}CH_3\text{-}COO^-]$ .
- $pH > pKa_2$

**[9] End point:**

- The alanine is full dissociated.
- $[NH_2\text{-CH-}CH_3\text{-}COO^-]$
- pOH= (pkb+P[A-])/2

➔pKb = pKw – pKa2



# 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:**

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

$$pH = pKw - pOH$$

$$pKb = pKw - pKa2$$

# Example:

**Remember !!**
Before the titration with acid and base, the amino acid is in its **isoelectric form**
**[$NH_3^+$-CH-$CH_3$-$COO^-$]**

- **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**

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

➔ [**$NH_3^+$**-CH-$CH_3$-$COO^-$] > [**$NH_2$**-CH-$CH_3$-$COO^-$].

HA + NaOH ➔ A + $H_2O$

**Mole of HA ($NH3^+$) [original] – mole of $A^-$ (NaOH) [added]**
**= mole of HA ($NH3^+$) 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<$pKa_2$)

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

➔ [$NH_3^+$-CH-$CH_3$-**COOH**]<[$NH_3^+$-CH-$CH_3$-**$COO^-$**].

$A^-$ + HCl ➔ HA

**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 > $pKa_1$)

# 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.

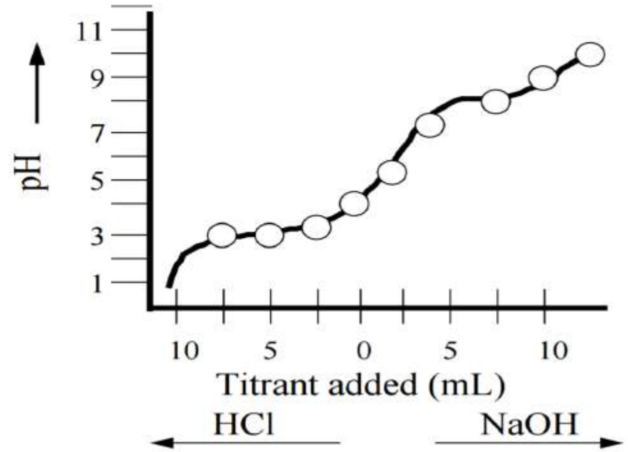
# Method:

- 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



# Results:

How to determine $pKa_1$ , $pKa_2$ and pI from the curve?