Titration Curve

of amino acids

Titration curves

- It is a curve that monitors the pH of a solution as amounts of alkali or acid is added.

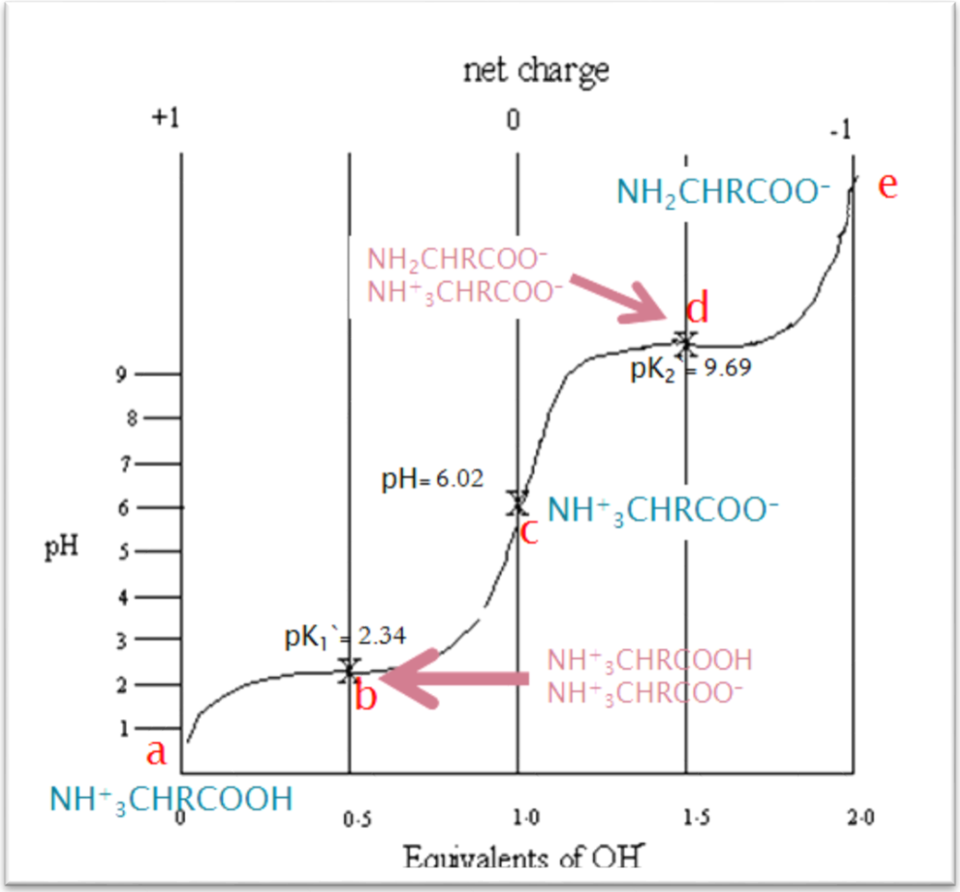
- Amino acids are *simple* weak polyprotic acids.
  - Neutral amino acids (as alanine, glycine) are treated as **diprotic acids**.
  - Acidic amino acids (as aspartic or glutamic acid) and Basic amino acids (as lysine histidine, glutamine, and aspargine) are treated as **triprotic acids**.

Titration curve of Neutral a.a

This curve has two flat zones, at point b and d; meaning it has 2 ionized groups.

- **At point a:**
  - Before titration
  - $NH^+{}_3CHRCOOH$
  - The net charge = +1

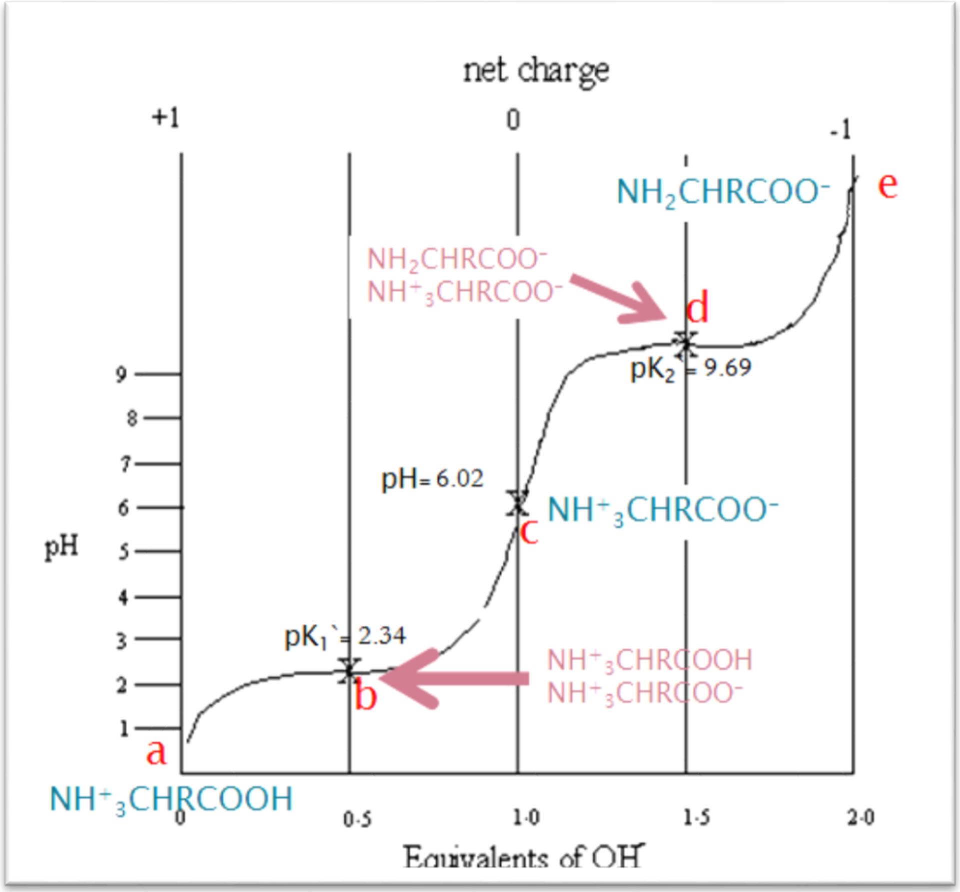
- **At point b:**
  - Pk_1 ` = pH
  - Here it has buffering capacity
  - $NH^+{}_3CHRCOOH = NH^+{}_3CHRCOO^-$
  - The net charge = +0.5



Titration curve of **Neutral** a.a

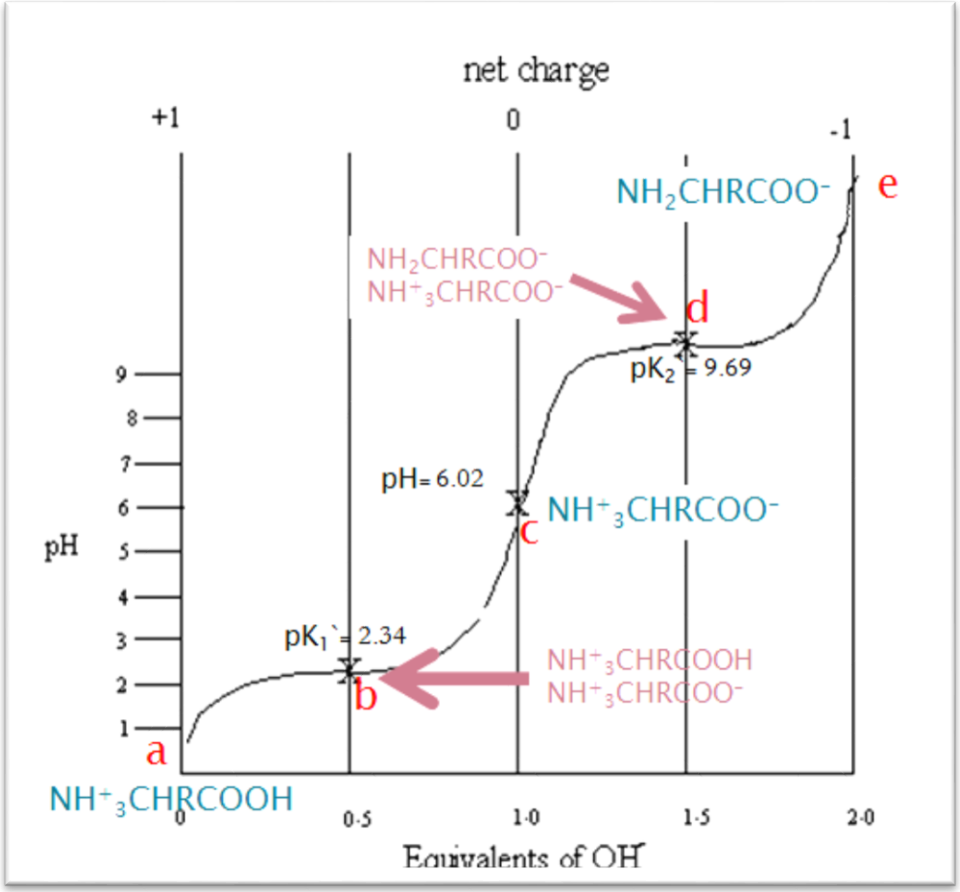
- **At point c:**
    - Isoelectric point (pI)
    - pI = pH;
    - To calculate pI: $(pK_1\text{`}+pK_2\text{`}) \backslash 2$
    - $NH^+{}_3CHRCOO^-$ a zwitter ion
    - The net charge = 0

- **At point d:**
    - $Pk_2\text{`} = pH$
    - Here it has buffering capacity
    - $NH^+{}_3CHRCOO^- = NH_2CHRCOO^-$
    - The net charge = −0.5



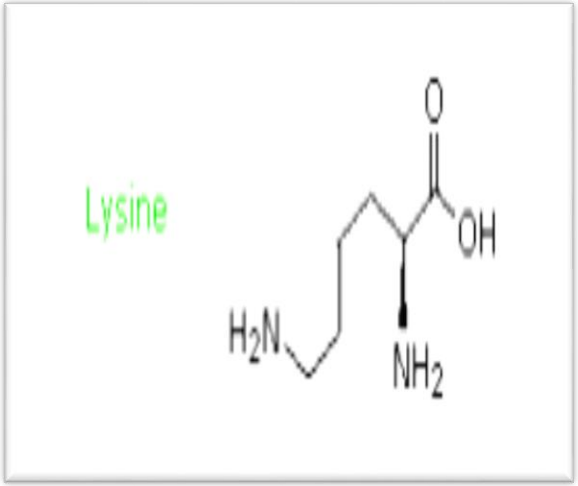
Titration curve of Neutral a.a

- **At point e:**
  - End of titration
  - $NH_2CHRCOO^-$
  - The net charge = −1



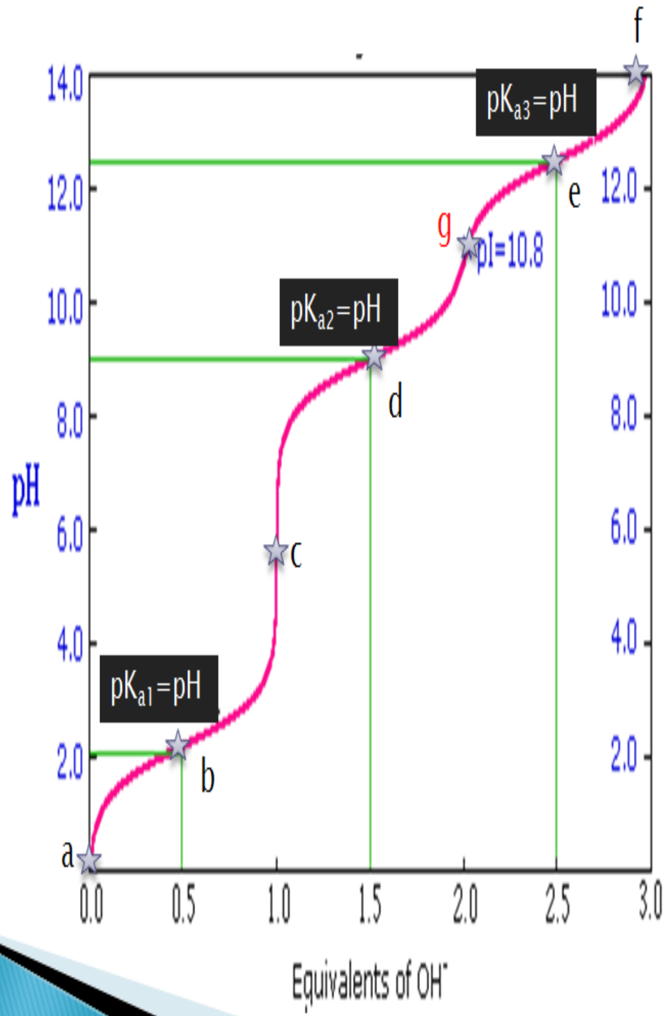
Titration curve of lysine

- Lysine is a basic amino acid with an extra amino group in its side chain.



- pKa:
    - 1st α–COOH will be titrated first = 2.18
    - 2nd α –NH_3^+ will be titrated next = 8.95
    - 3rd R–NH_3^+ will be titrated last = 10.53
- We have three flat zones, i.e. three ionized groups.

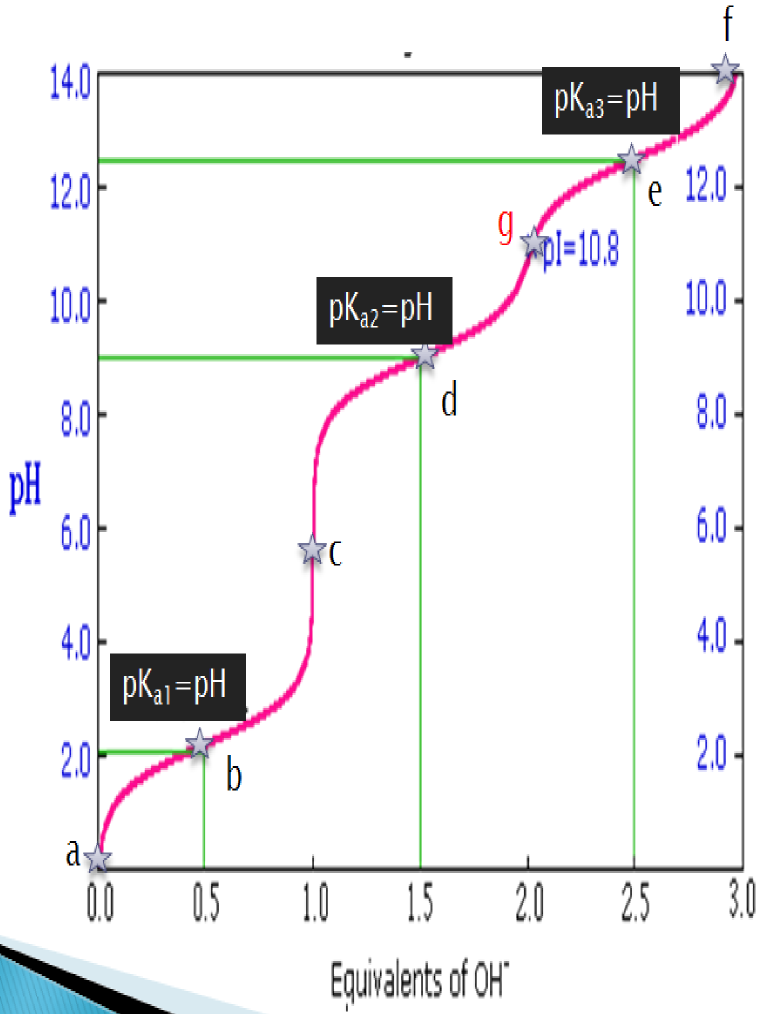
Titration curve of lysine



- **At point a:**
  - Before titration
  - $NH^+_3CH(CH_2)_4NH^+_3COOH$
  - The net charge = +2

- **At point b:**
  - $Pk_{a1} = pH$
  - Here it has buffering capacity
  - $NH^+_3CH(CH_2)_4NH^+_3COOH = NH^+_3CH(CH_2)_4NH^+_3COO^-$
  - The net charge = +2 | +1 ➔ +1.5

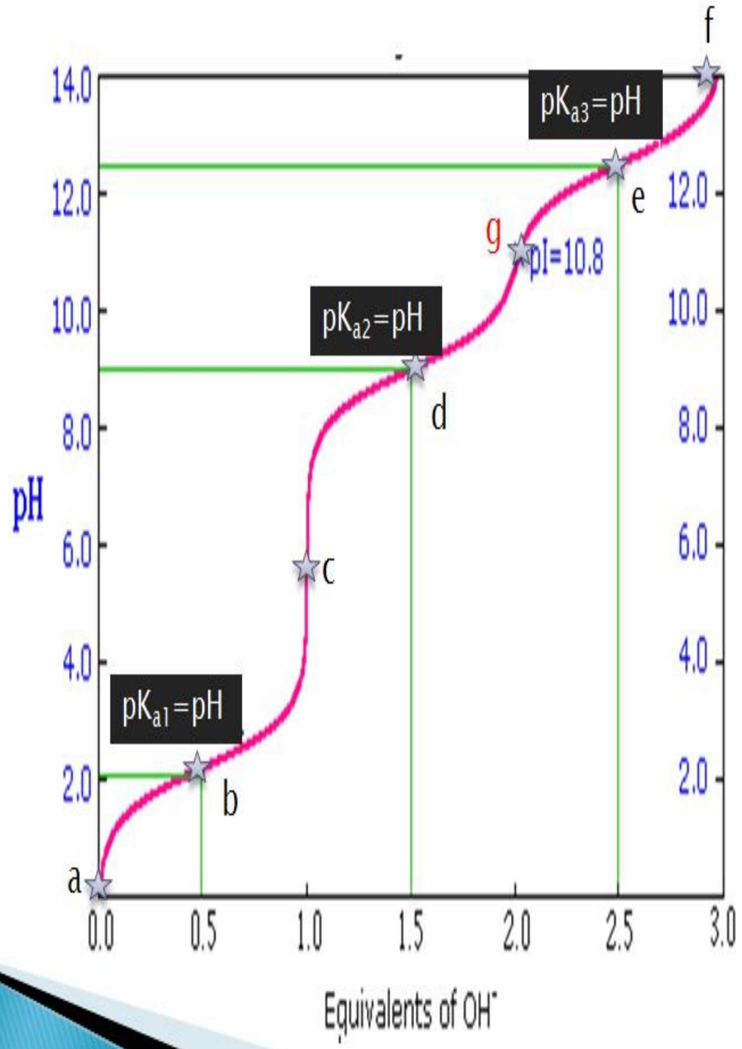
Titration curve of lysine



- **At point c:**
  - $NH^+_3CH(CH_2)_4NH^+_3COO^-$
  - All the α–COOH has been titrated.
  - The net charge = +1

- **At point d:**
  - Pk_{a2} = pH
  - Here it has buffering capacity
  - $NH^+_3CH(CH_2)_4NH^+_3COO^-$ = $NH_2CH(CH_2)_4NH^+_3COO^-$
  - The net charge = +1 | 0 ➔ +0.5

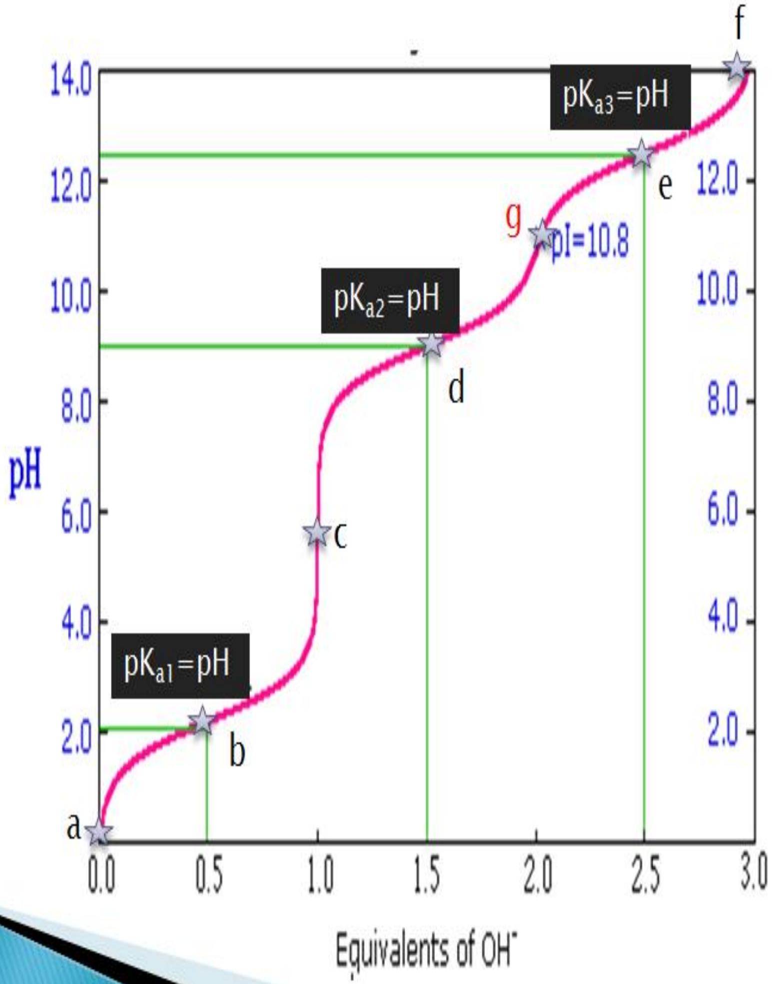
Titration curve of lysine



- **At point g:**
  - It is the pI point
  - $NH_2CH(CH_2)_4NH^+{}_3COO^-$
  - The net charge = 0
  - Ip = pH, pI= $(pKa_2+pKa_3)/2$

- **At point e:**
  - $NH_2(CH_2)_4NH^+{}_3COO^- = NH_2(CH_2)_4NH_2COO^-$
  - The net charge = 0 | -1 ➔ -0.5
  - Pk_{a3} = pH
  - Here it has buffering capacity

Titration curve of lysine

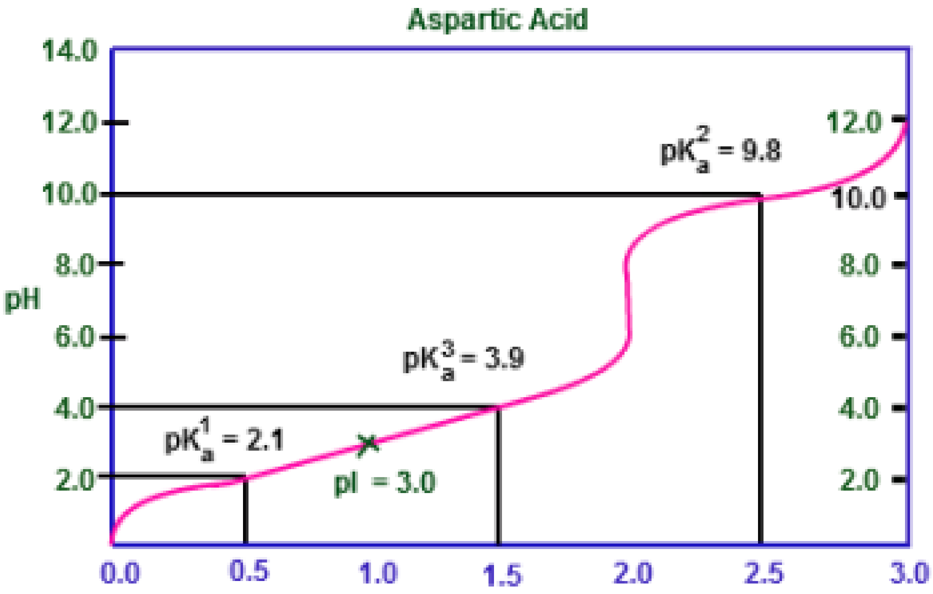


- **At point f:**
  - End of titration
  - $NH_2(CH_2)_4NH_2COO^-$
  - The net charge = –1
  - All has been titrated.

Information obtained from a titration curve

1- The number of ionizable groups in that amino acid, can be detected from the number of titration stages in the curve, (or the number of pK_a's or number of flat zones in the curve).

2- In acidic amino acids: 1st α-COOH will be titrated first , 2nd R-COOH will be titrated next, 3rd α -NH_3^+ will be titrated last.



3- The pK_a values of the amino acid can be obtained from the curve which is equal to the pH value at the mid-point of each stage.

4- The isoelectric point , **pI** for each amino acid can be obtained from the curve by detecting the point where the amino acid is **ALL** in the zwitterion form (net charge = 0.0). The pH at that point is the pI.

- Or it can be obtained mathematically from:

$$pI = \frac{pKa_1 + pKa_2}{2}$$ (in the case of a neutral amino acid)

$$pI = \frac{pKa_1 + pKa_2}{2}$$ (in the case of acidic amino acids)

$$pI = \frac{pKa_2 + pKa_3}{2}$$ (in the case of basic amino acids)

3- Whether the triprotic amino acid is basic or acidic, that can be detected from the pKa_2.

- If it's value is closer to the value of pKa_1 (that of the α- carboxyl group), then it is an acidic amino acid.
- If it's value is closer to the value of pKa_3 (that of the R-amino group), then it is basic amino acid

5- You can also determine from the curve the pH values at which the amino acid can act as a buffer. (the pH ranges ± 1 from the pH value of each midpoint).

How to Obtain a Titration Curves of Amino Acids?

1- Calculate the no. of moles of a.a.

2- Calculate the first moles of OH^- **(at pK_{a1})**

A= no. of moles of a.a / pK_{a1}

3- Calculate the second moles of OH^- added **(at pK_{a2})**

B= No of moles of a.a + A

4- Calculate the third moles of OH^- added **(at pK_{a3})**

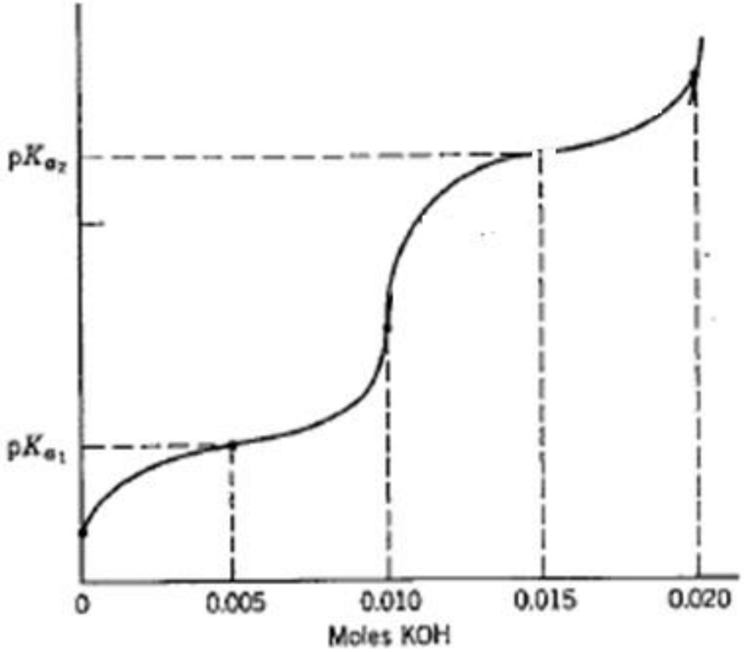
C= No of moles of a.a + B

5- Calculate **no. of moles of OH^- added** **(at pI)**

Neutral and Acidic a.a → (A+B) /2   Basic a.a → (B+C)/2

Example 1

- Sketch the pH curve for the titration of 100 ml of 0.1M glycine with KOH? $pk_{a1} = 1.71$, $pk_{a2} = 9.6$?



No. of moles of a.a $= M \times V$

$= 0.1 \times 0.1$

$= 0.01$ mole

The first moles of OH^- (at pK_{a1}) :

A $= 0.01 / 1.71 = 0.005$ moles

The second moles of OH^- added (at pK_{a2}) :

B $= 0.01 + 0.005 = 0.015$ moles

****PI = $(pk_{a1} + pk_{a2}) / 2 = 5.66$****

No. of **moles of OH^- added (at pI)**= $(0.005+0.015)/2=0.01$ **moles**

Example 2

- Plot the titration curve of aspartic acid it has a volume of 100 ml and 0.1M when titrated with 0.1M KOH? $pk_{a1} = 2.09$, $pk_{a2} = 3.86$, $pk_{a3} = 9.82$?

No. of moles of a.a = M × V
= 0.1 × 0.1 = 0.01 mole

The first moles of OH^- (at pK_{a1}) :

A = 0.01 / 2.09 = 0.0048 moles

The second moles of OH^- added (at pK_{a2}) :

B = 0.01 + 0.0048 = 0.0148 moles

The third moles of OH^- added (at pK_{a3}) :

C = 0.01 + 0.0148 = 0.0248moles

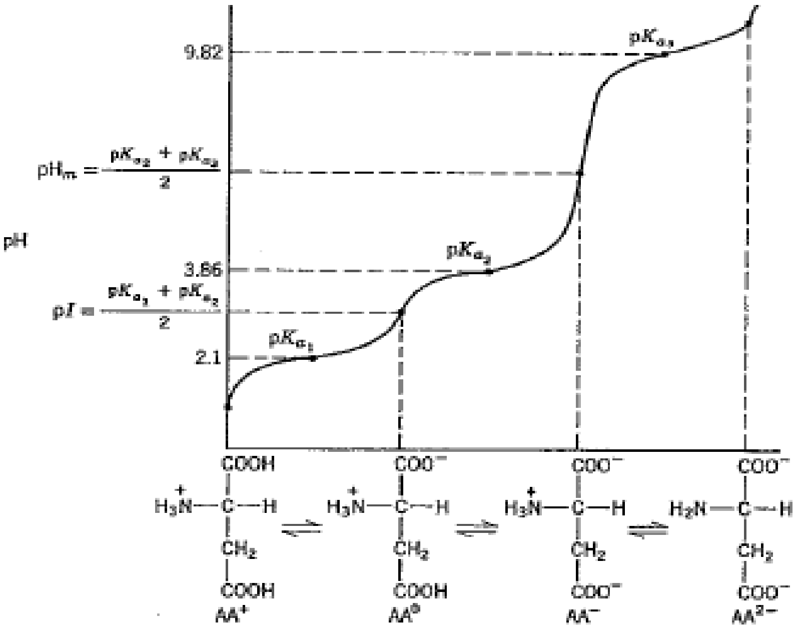


Figure 1-8 Titration curve of aspartic acid. For clarity, the vertical axis is not drawn to scale.

$*PI = (pk_{a1} + pk_{a2}) / 2 = 2.975**$

No. of **moles of OH^- added (at pI)= (0.0048+0.0148)/2=0.0098 moles**

Example 3

- Plot the titration curve of lysine which has a volume of 200 ml and 0.3 M when titrated with 0.1M NaOH? $pk_{a1} = 2.18$, $pk_{a2} = 8.95$, $pk_{a3} = 10.35$?

No. of moles of a.a = M × V

= 0.3 × 0.2= 0.06 mole

The first moles of OH^- (at pK_{a1}) :

A = 0.06 / 2.18 = 0.027 moles

The second moles of OH^- added (at pK_{a2}) :

B = 0.06 + 0.027 = 0.087 moles

The third moles of OH^- added (at pK_{a3}) :

C= 0.06+ 0.087 =0.147 moles

$*PI = (pk_{a2} + pk_{a3}) / 2 = 9.65**$

No. of **moles of OH^- added (at pI)= (0.087+0.147)/2=0.117 moles**

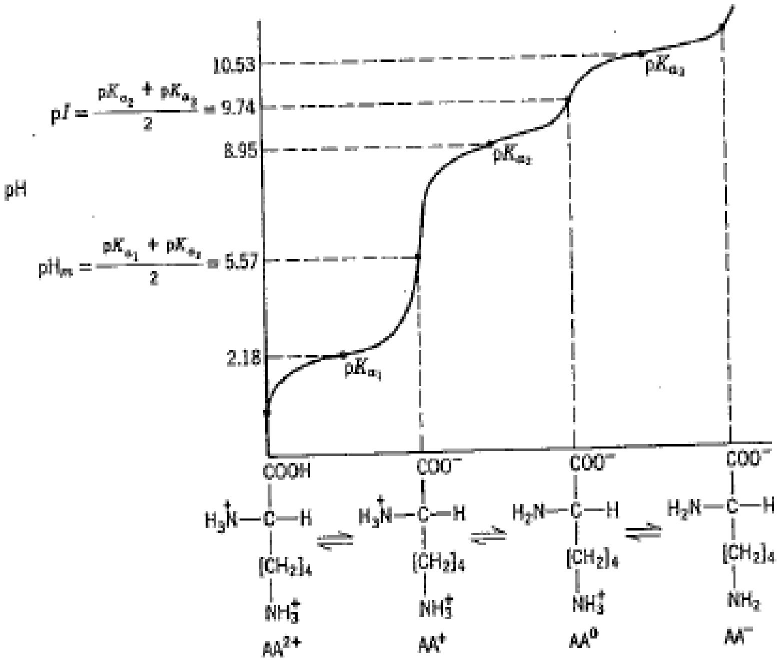


Figure 1-9 Titration curve of lysine. For clarity, the vertical axis is not drawn to scale.