

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 asparagine) are treated as **triprotic acids**.

Titration curve of Neutral a.a

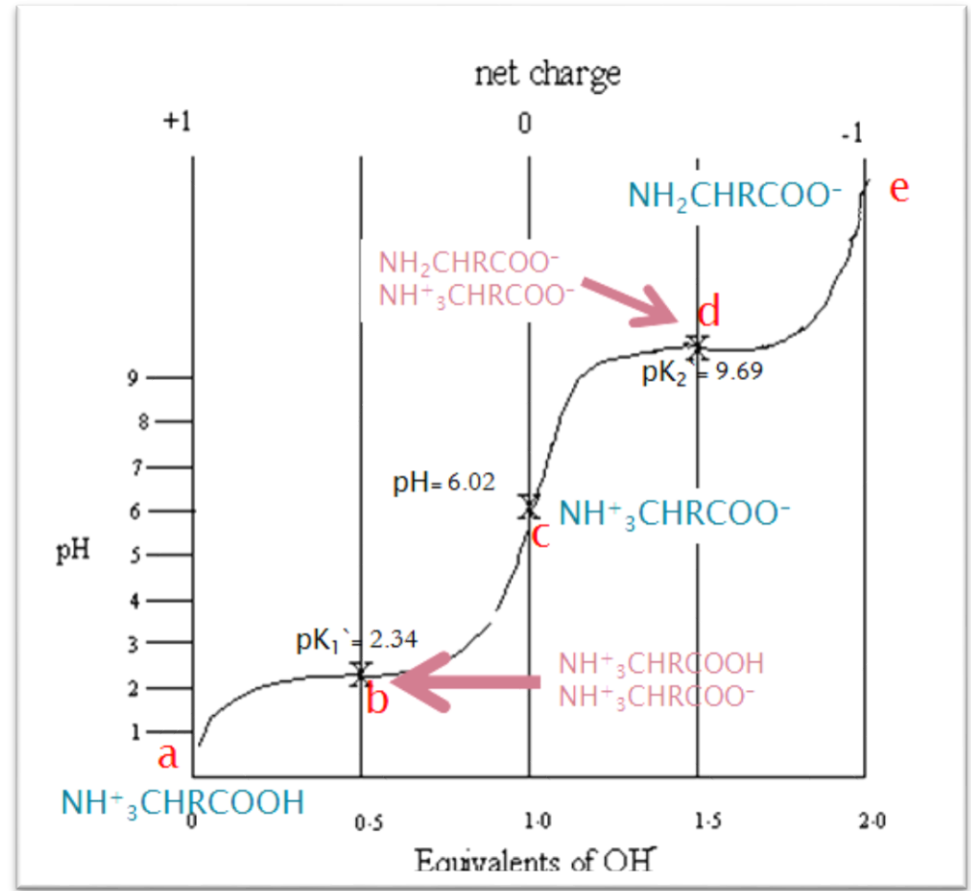
This curve has two flat zones, at point b and d; meaning it has 2 ionized groups.

▶ At point a:

- Before titration
- $\text{NH}_3^+\text{CHRCOOH}$
- The net charge = +1

▶ At point b:

- $\text{pK}_1 = \text{pH}$
- Here it has buffering capacity
- $\text{NH}_3^+\text{CHRCOOH} = \text{NH}_3^+\text{CHRCOO}^-$
- The net charge = +0.5



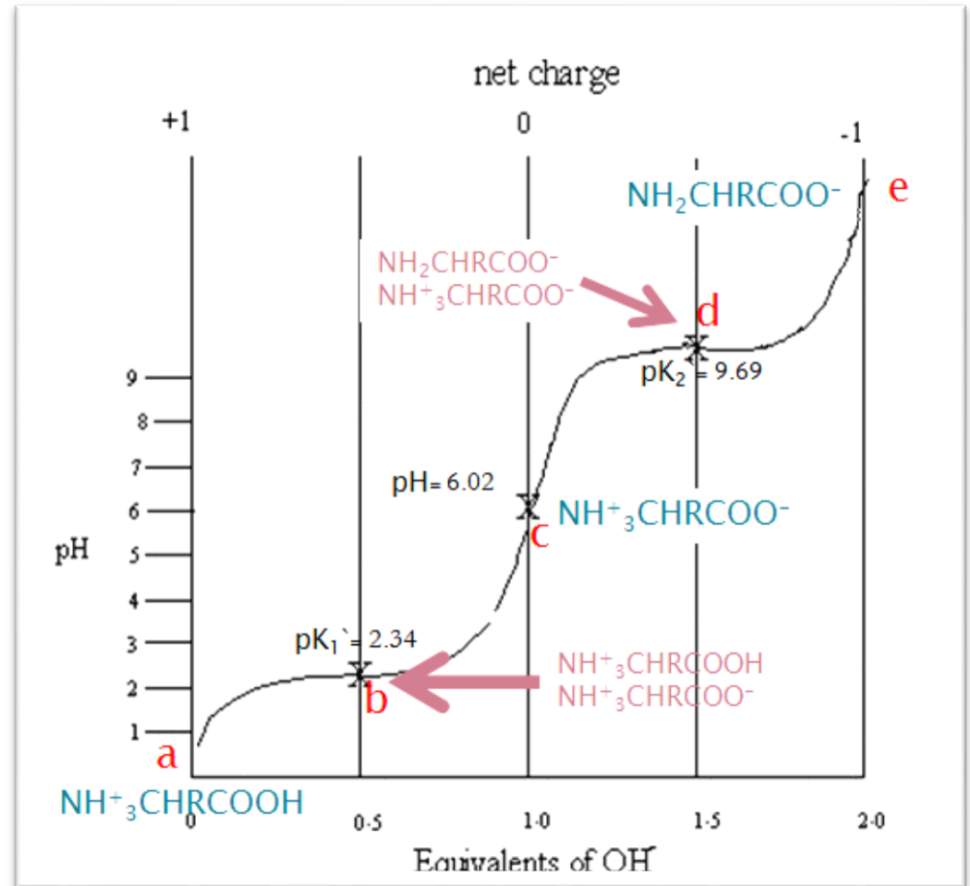
Titration curve of Neutral a.a

▶ At point c:

- Isoelectric point (pI)
- $pI = pH$;
- To calculate pI: $(pK_1 + pK_2) \div 2$
- $NH_3^+CHRCOO^-$ a zwitter ion
- The net charge = 0

▶ At point d:

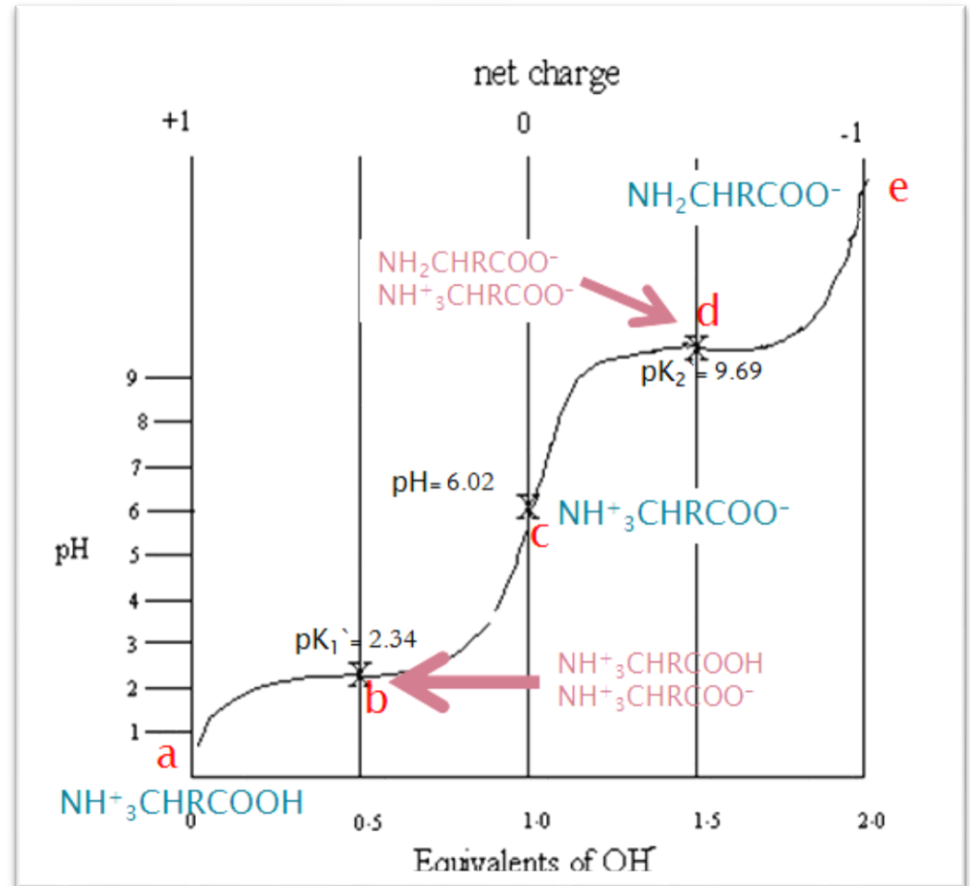
- $pK_2 = pH$
- Here it has buffering capacity
- $NH_3^+CHRCOO^- = NH_2CHRCOO^-$
- The net charge = -0.5



Titration curve of Neutral a.a

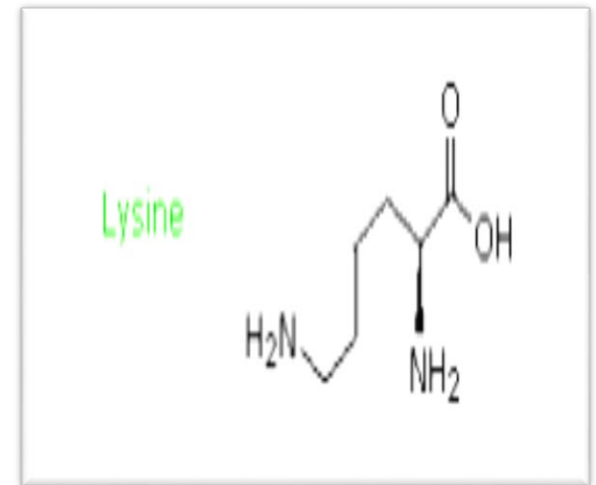
► At point e:

- End of titration
- $\text{NH}_2\text{CHR}\text{COO}^-$
- The net charge = -1

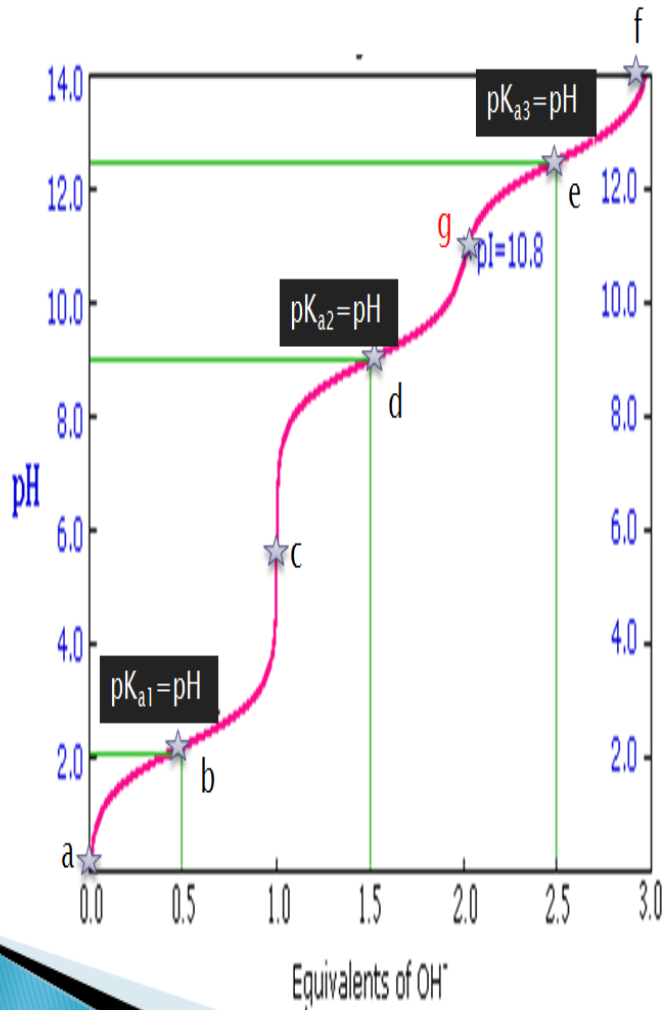


Titration curve of lysine

- ▶ 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 -NH₃⁺ will be titrated next = 8.95
 - 3rd R-NH₃⁺ will be titrated last = 10.53
- ▶ We have three flat zones, i.e. three ionized groups.



Titration curve of lysine



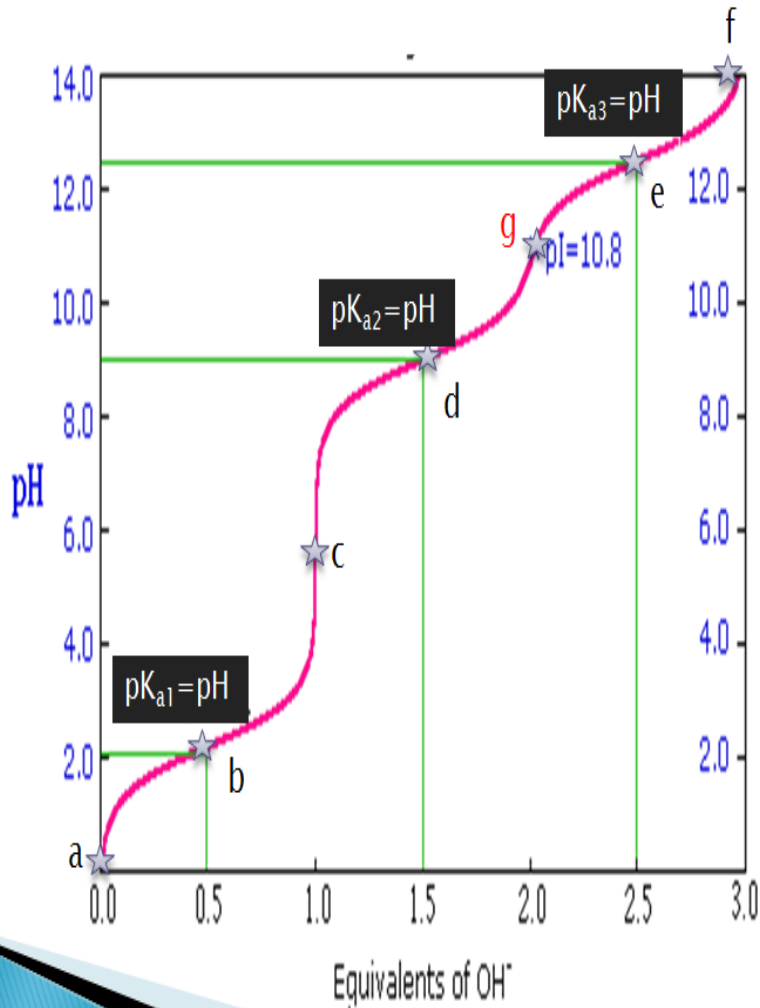
▶ **At point a:**

- Before titration
- $\text{NH}_3^+\text{CH}(\text{CH}_2)_4\text{NH}_3^+\text{COOH}$
- The net charge = +2

▶ **At point b:**

- $\text{pK}_{a1} = \text{pH}$
- Here it has buffering capacity
- $\text{NH}_3^+\text{CH}(\text{CH}_2)_4\text{NH}_3^+\text{COOH} \rightleftharpoons \text{NH}_3^+\text{CH}(\text{CH}_2)_4\text{NH}_3^+\text{COO}^-$
- The net charge = +2 | +1 → +1.5

Titration curve of lysine



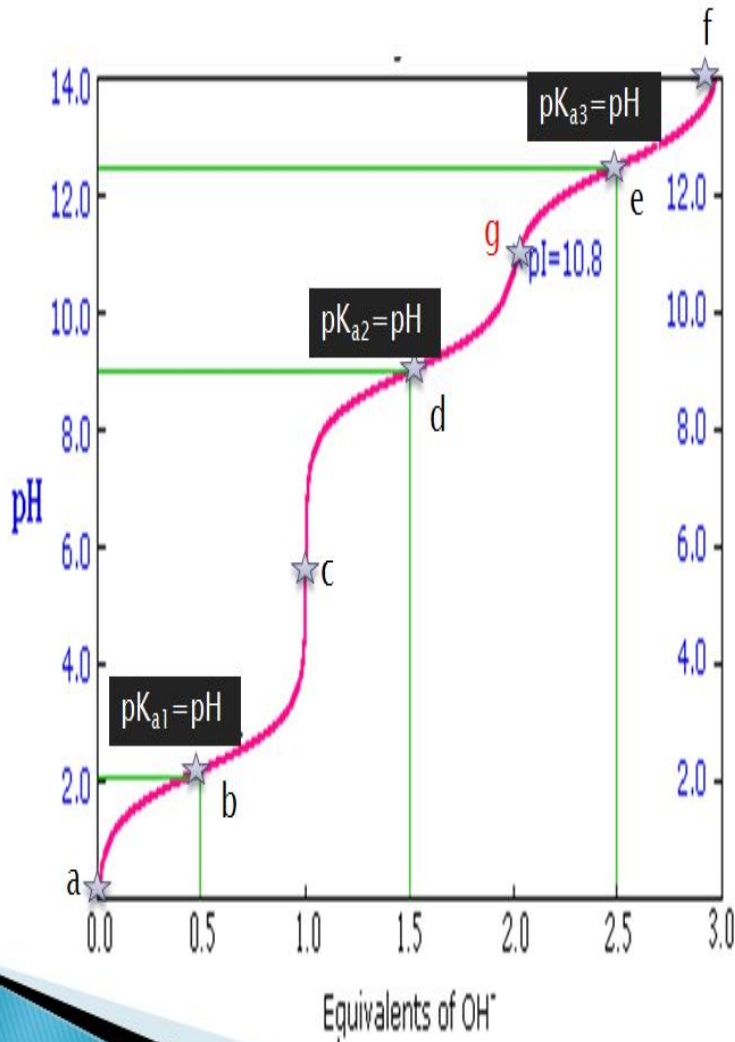
▶ At point c:

- $\text{NH}_3^+\text{CH}(\text{CH}_2)_4\text{NH}_3^+\text{COO}^-$
- All the α -COOH has been titrated.
- The net charge = +1

▶ At point d:

- $\text{pK}_{a2} = \text{pH}$
- Here it has buffering capacity
- $\text{NH}_3^+\text{CH}(\text{CH}_2)_4\text{NH}_3^+\text{COO}^- = \text{NH}_2\text{CH}(\text{CH}_2)_4\text{NH}_3^+\text{COO}^-$
- The net charge = +1 | 0 → +0.5

Titration curve of lysine



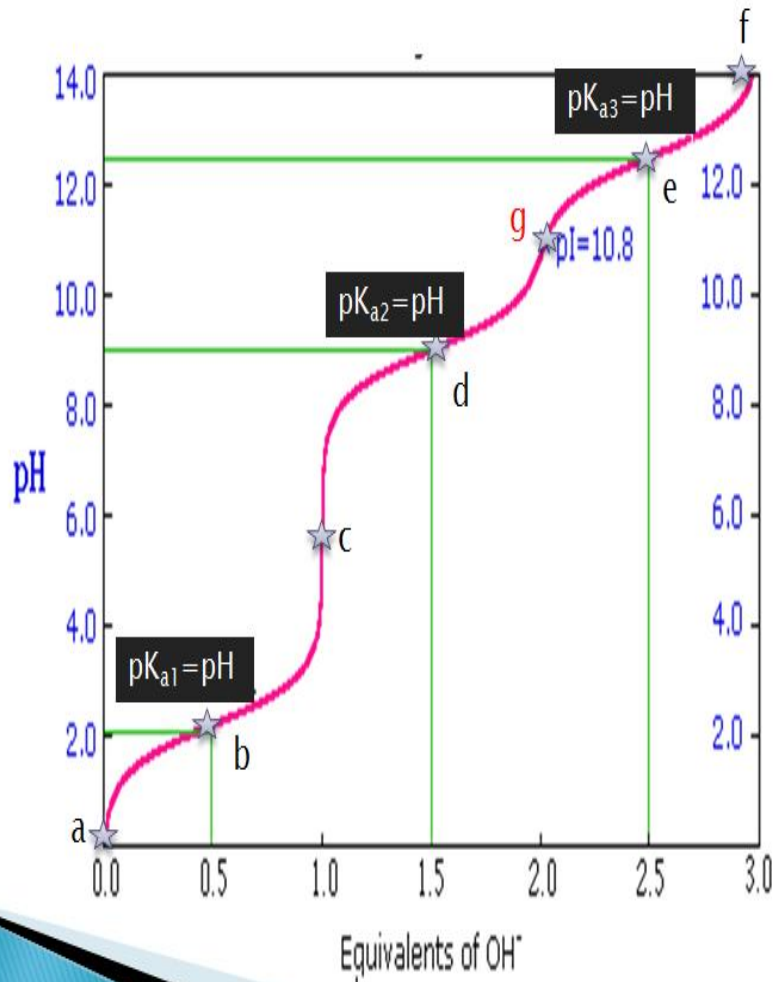
▶ **At point g:**

- It is the pI point
- $\text{NH}_2\text{CH}(\text{CH}_2)_4\text{NH}_3^+\text{COO}^-$
- The net charge = 0
- $\text{Ip} = \text{pH}$, $\text{pI} = (\text{pKa}_2 + \text{pKa}_3) / 2$

▶ **At point e:**

- $\text{NH}_2(\text{CH}_2)_4\text{NH}_3^+\text{COO}^- \rightleftharpoons \text{NH}_2(\text{CH}_2)_4\text{NH}_2\text{COO}^-$
- The net charge = 0 | -1 → -0.5
- $\text{PK}_{a3} = \text{pH}$
- Here it has buffering capacity

Titration curve of lysine

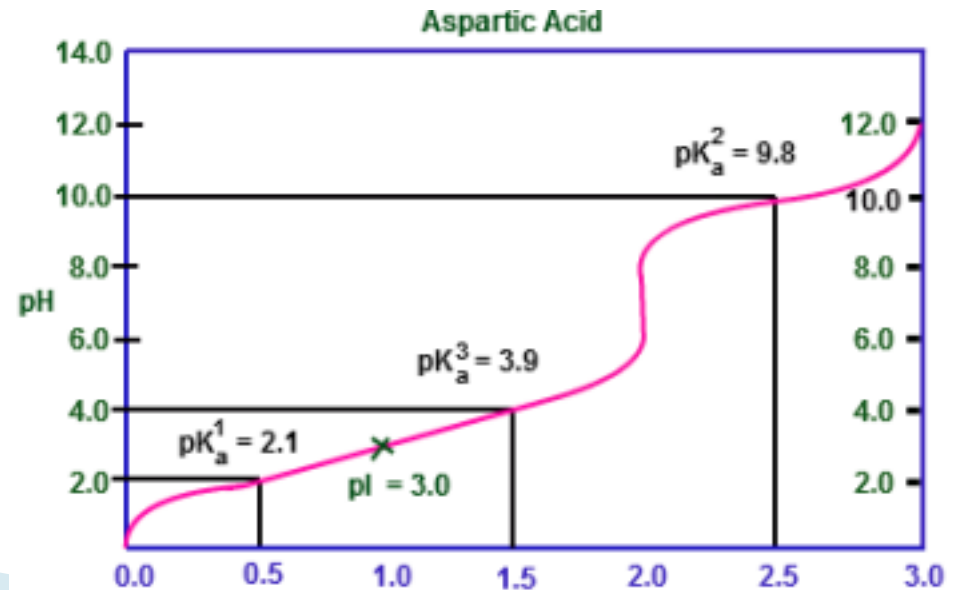


► At point f:

- End of titration
- $\text{NH}_2(\text{CH}_2)_4\text{NH}_2\text{COO}^-$
- 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 -NH₃⁺ 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 , **pl** 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 pl.

➤ Or it can be obtained mathematically from:

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

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

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

3– Whether the triprotic amino acid is basic or acidic, that can be detected from the pK_{a_2} .

- If it's value is closer to the value of pK_{a_1} (that of the α - carboxyl group), then it is an **acidic** amino acid.
- If it's value is closer to the value of pK_{a_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})

$$A = \text{no. of moles of a.a.} / \text{pK}_{a1}$$

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

$$B = \text{No of moles of a.a.} + A$$

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

$$C = \text{No of moles of a.a.} + B$$

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

$$\text{Neutral and Acidic a.a.} \rightarrow (A+B) / 2$$

$$\text{Basic a.a.} \rightarrow (B+C) / 2$$

Example 1

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

$$\begin{aligned}\text{No. of moles of a.a} &= M \times V \\ &= 0.1 \times 0.1 \\ &= 0.01 \text{ mole}\end{aligned}$$

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

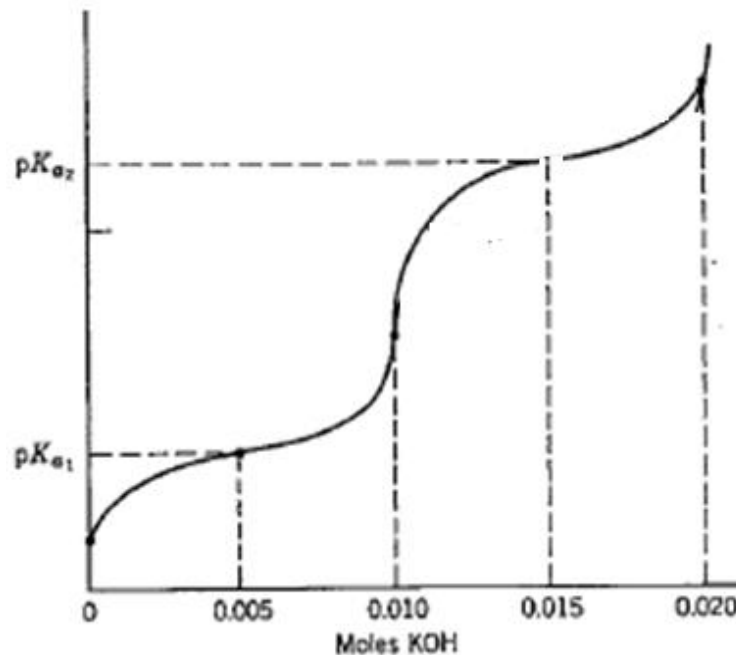
$$A = 0.01 / 1.71 = 0.005 \text{ moles}$$

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

$$B = 0.01 + 0.005 = 0.015 \text{ moles}$$

$$\text{**PI} = (pK_{a1} + pK_{a2}) / 2 = 5.66\text{**}$$

$$\text{No. of moles of } \text{OH}^- \text{ added (at PI)} = (0.005 + 0.015) / 2 = 0.01 \text{ moles}$$



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 = $M \times V$
 $= 0.1 \times 0.1 = 0.01$ 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}):

$C = 0.01 + 0.0148 = 0.0248$ moles

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

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

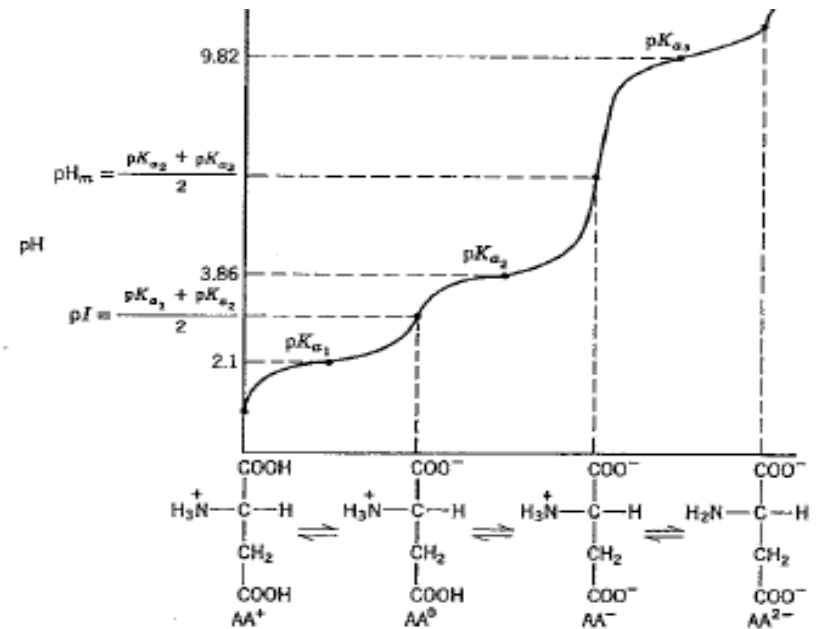


Figure 1-8 Titration curve of aspartic acid. For clarity, the vertical axis is not drawn to scale.

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 = $M \times V$
 $= 0.3 \times 0.2 = 0.06$ 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

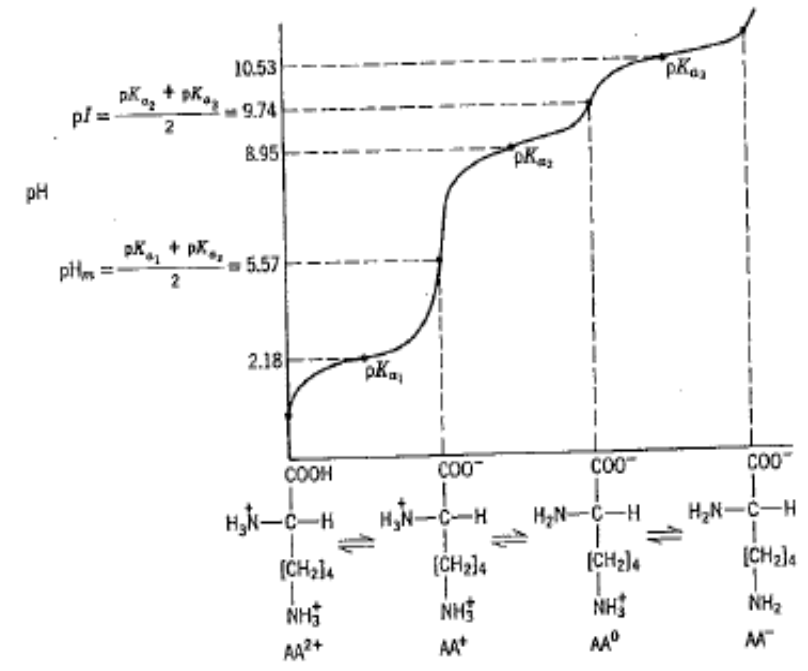


Figure 1-9 Titration curve of lysine. For clarity, the vertical axis is not drawn to scale.