Titration curve of amino acids

BCH 312 [Practical]

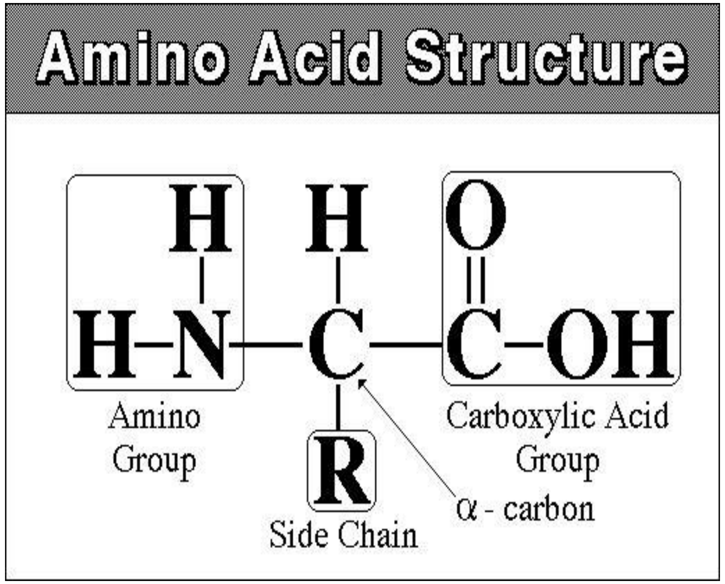
Titration curve

- 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 ($-\mathbf{NH_2}$)
- An acidic carboxyl group ($-\mathbf{COOH}$)
- A hydrogen atom ($-\mathbf{H}$)
- A distinctive side chain ($-\mathbf{R}$).



Titration of amino acid:

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

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

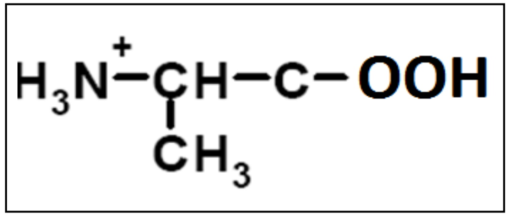
  - **Upon titration with acid** ➔ it acts as a BASE (accept a proton).
  - **Upon titration with base** ➔ it acts as an ACID (donate a proton)

- Amino 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]

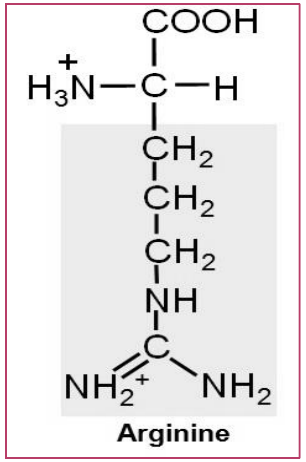
-The COOH will dissociate first then NH_3^+ dissociate later . (Because pKa1<pKa2)



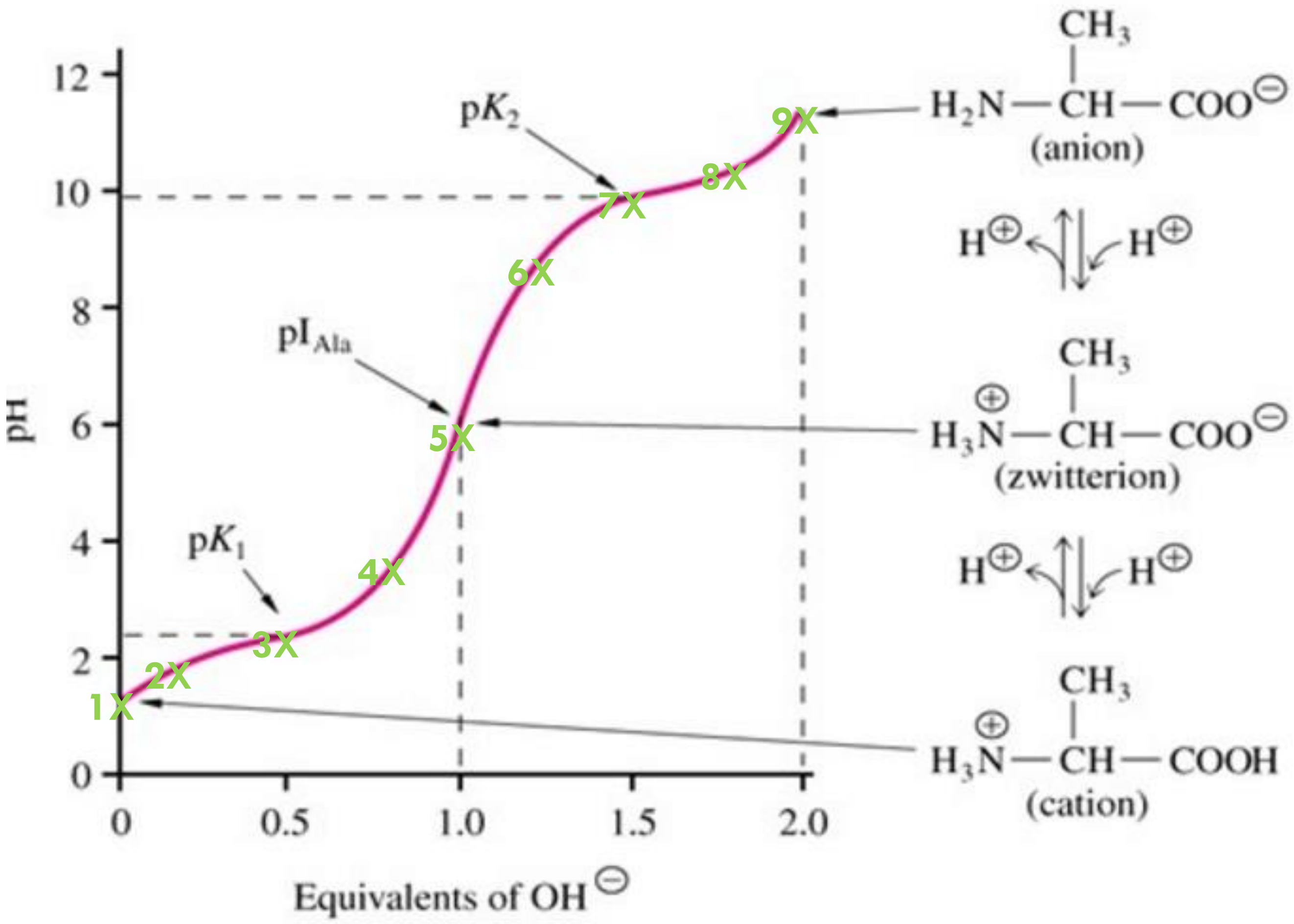
Full protonated alanine

(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



pK_1 carboxylic acid = 2.34

pK_2 amino group = 9.69

$pI = (pK_1 + pK_2)/2$

Titration curve of alanine or glycine [diprotic]:

[1] In starting point:

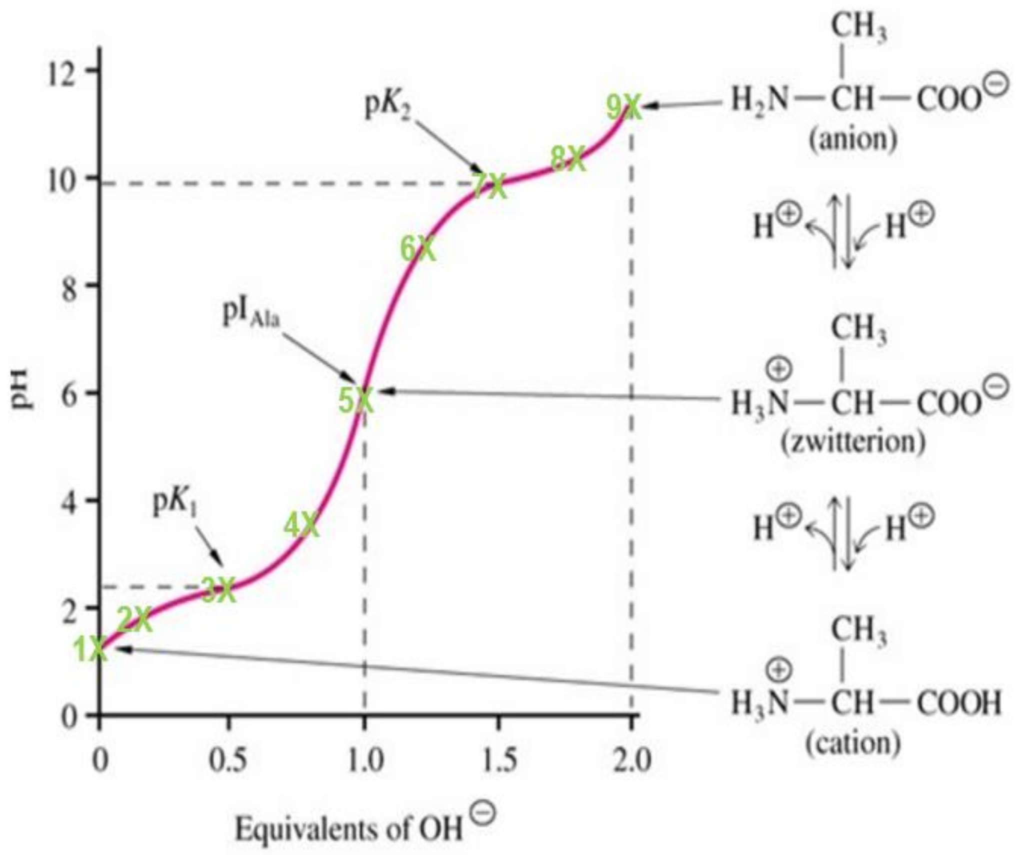
- Alanine is full protonated.
- [NH_3^+-CH-CH_3-COOH].

[2] COOH will <u>dissociate</u> 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^-].
- $pH=pKa_1$



Cont.

[4] In this point:

$[NH_3^+\text{-}CH\text{-}CH_3\text{-}COOH] < [NH_3^+\text{-}CH\text{-}CH_3\text{-}COO^-]$.

$pH > pKa_1$.

[5] Isoelectric point:

The COOH is full dissociate to COO^-.

$[NH_3^+\text{-}CH\text{-}CH_3\text{-}COO^-]$.

Con. of -ve charge = Con. of +ve charge.

The amino acid present as Zwetter ion (neutral form) .

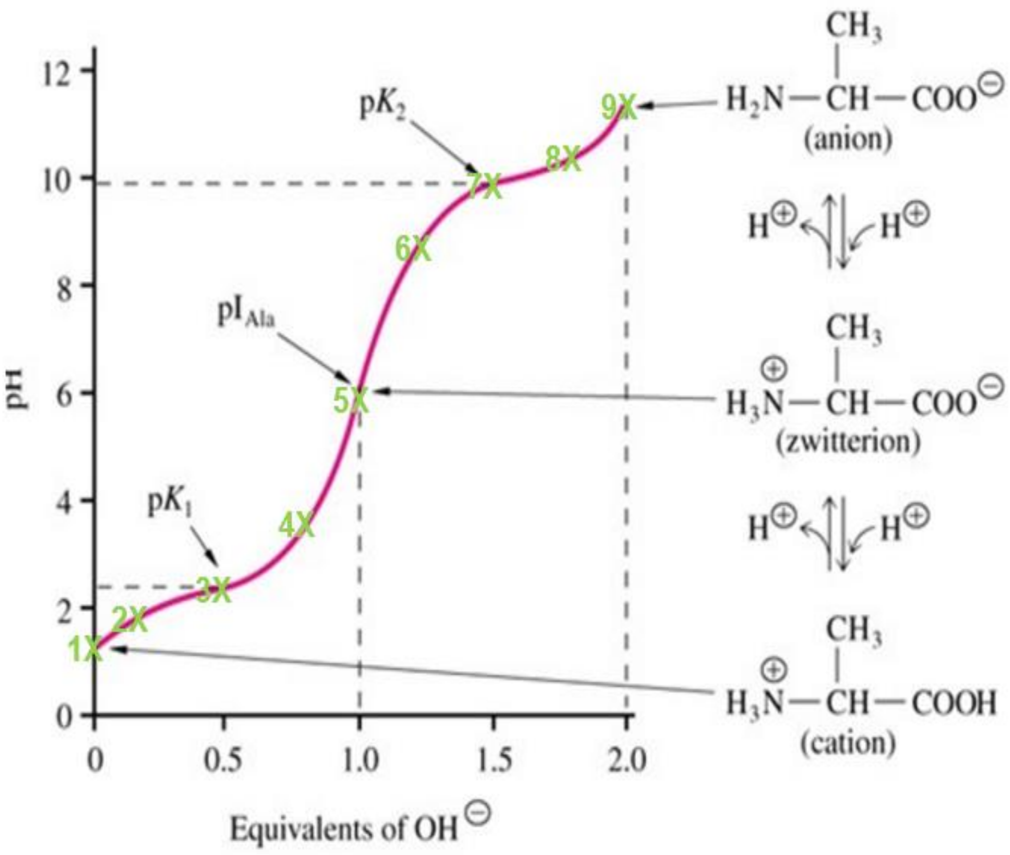
Remember that :PI (isoelectric point) is the pH value at which the net charge of amino acid equal to zero.

$pI = (pKa_1 + pKa_2) / 2 = (2.32+9.96)/2= 6.01$

[6] The NH_3^+ start dissociate:

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

$pH < pKa_2$.



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[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:

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

$pH > pKa_2$

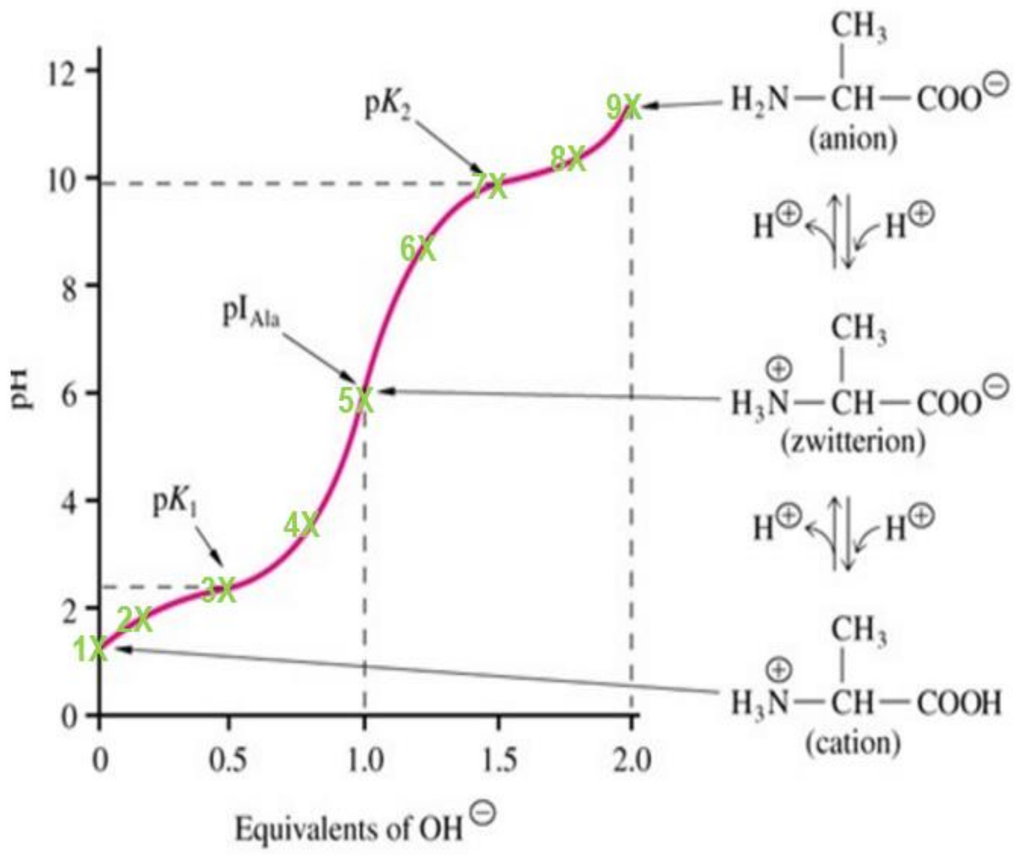
[9] End point:

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:

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

$$pH = pKw - pOH$$

$$pKb = pKw - pKa2$$

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.

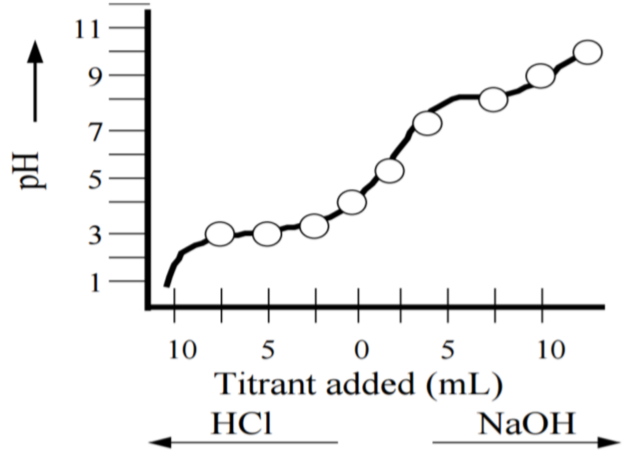
Measured pH value	Amount of 0.1M NaOH added [ml]

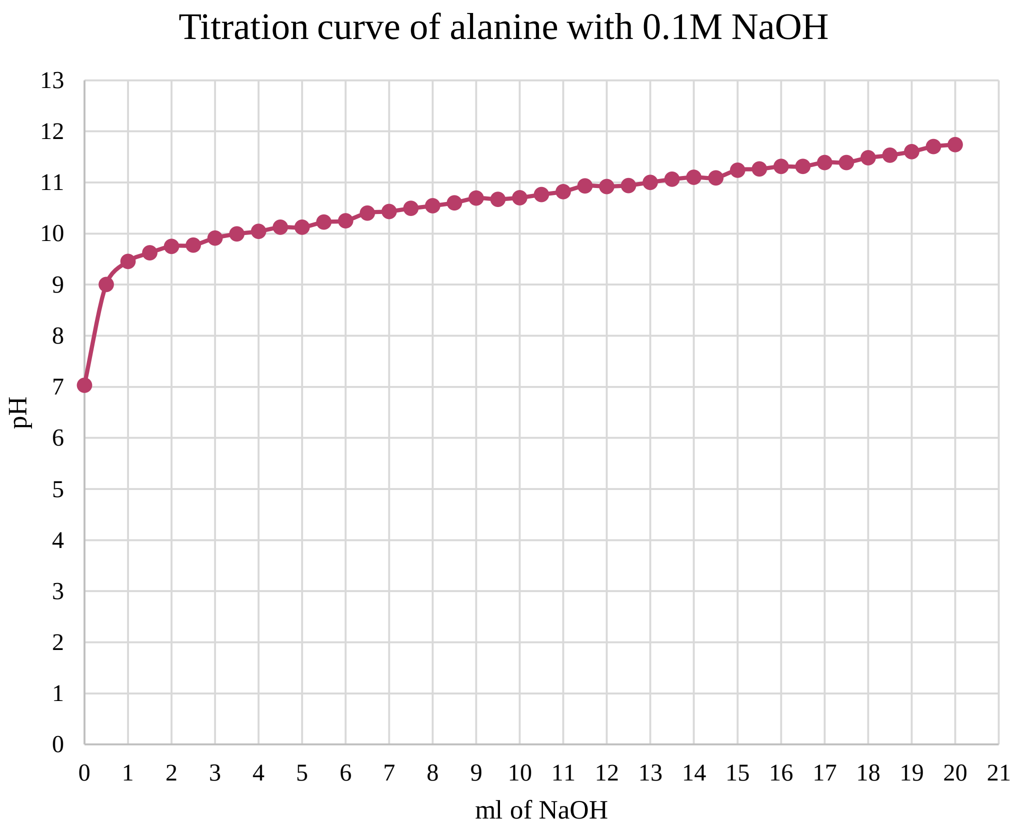
a) Take another 10 ml of a 0.1M alanine solution, titrate it with 0.1 M HCL adding the acid drop wise mixing, and recording the pH after each 0.5 ml HCL added until you reach a pH=2.17.

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

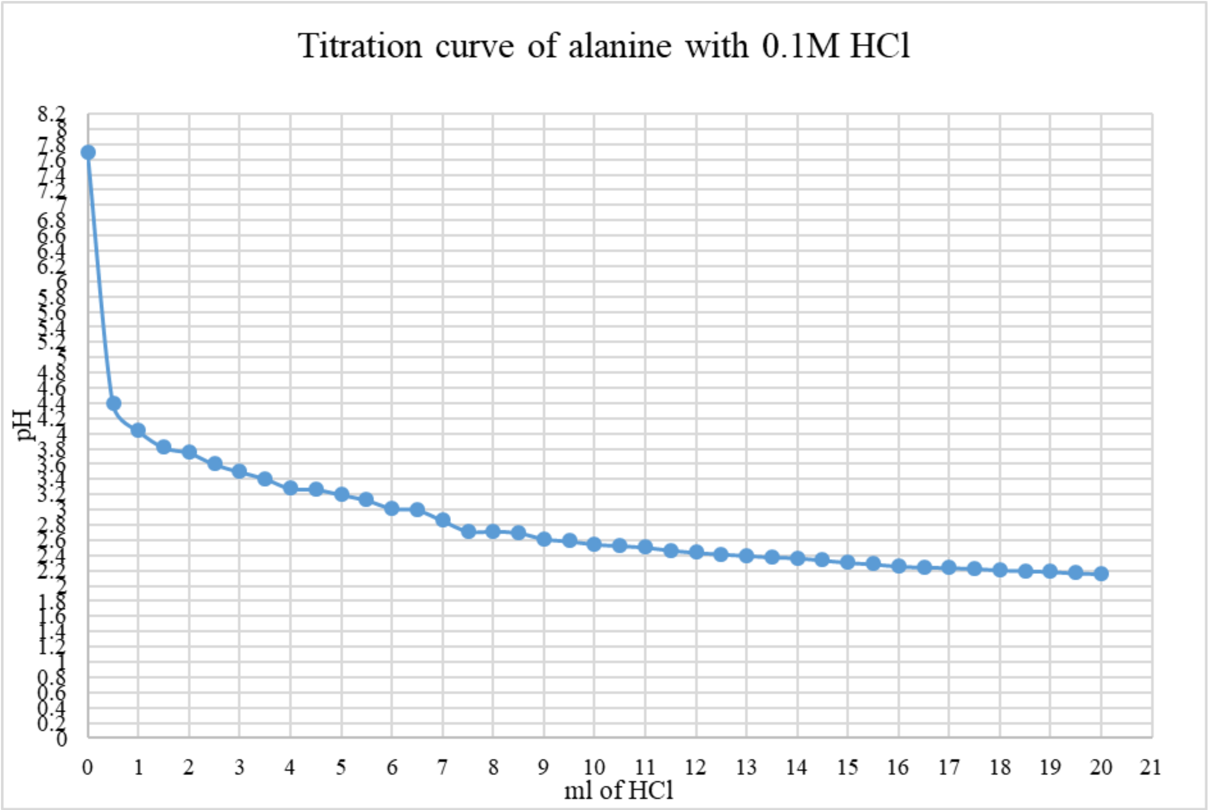
Results

- 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.
- Determine the PI value from your result
- Compare alanine pka and pI values with those obtained from Curve.
- Compare your calculated pH values with those obtained from Curve.
- Determine the buffering points.

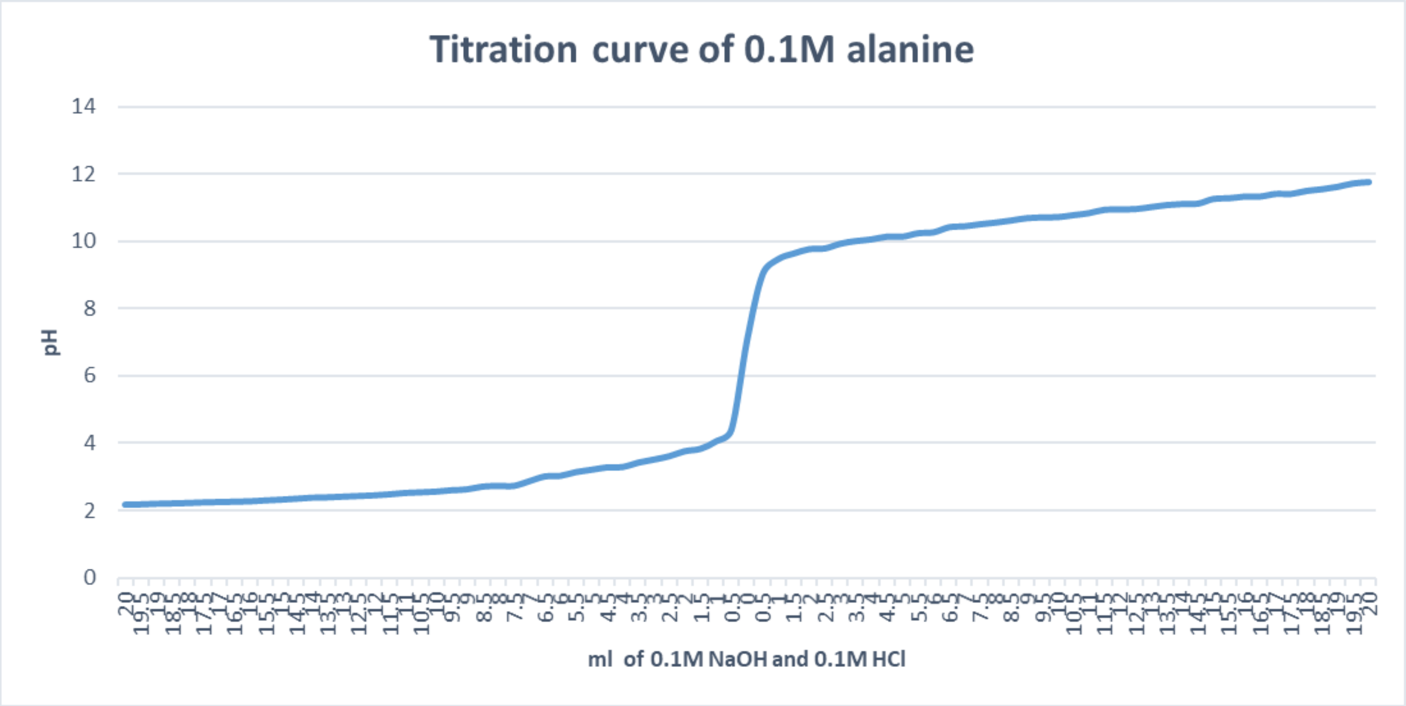




Titration curve of alanine with 0.1M NaOH
pH
ml of NaOH



Titration curve of alanine with 0.1M HCl
pH
ml of HCl


Titration curve of 0.1M alanine
pH
14
12
10
8
6
4
2
0
ml of 0.1M NaOH and 0.1M HCl

- Note: in calculating the pH:
- At any point within the curve pH=pka+log([A-]/[HA])

If a base is added:

The amino acid will be treated as an **acid**

The pKa used is of the **amine group.**

The upper stage

If acid is added:

The amino acid will be treated as a **base**

The pKa used is of the **carboxyl group**

The lower stage