Titration curve of amino acids

BCH 312 [PRACTICAL]

- Objective:

-To study titration curves of amino acid,

-Determine the pKa values,

-Determine pI.

-Determine buffering regions

- 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.

- AMINO ACID GENERAL FORMULA:

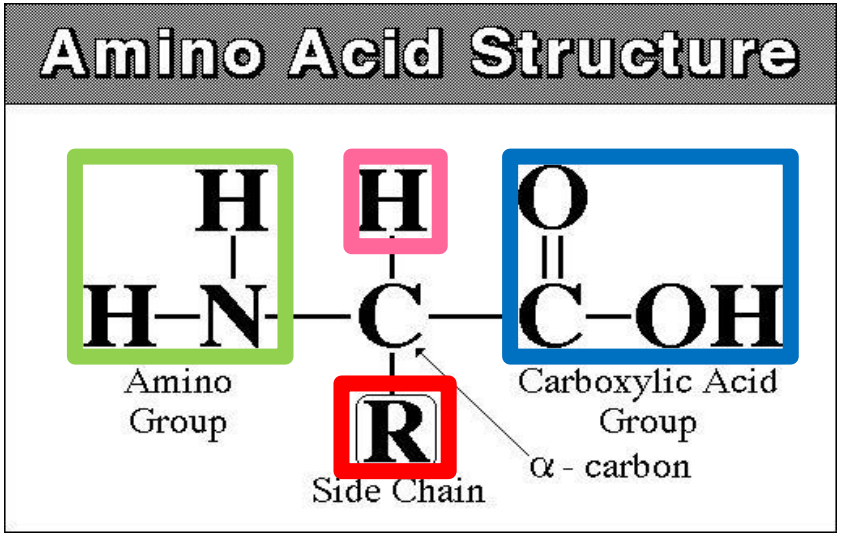
- Amino acids consist of:

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.

-Upon titration with acid, it acts as a base (accept a proton).

-Upon titration with base, it acts as an acid (donate a proton)

-(a compound that can act as either an acid or a base is known as an amphoteric compound).

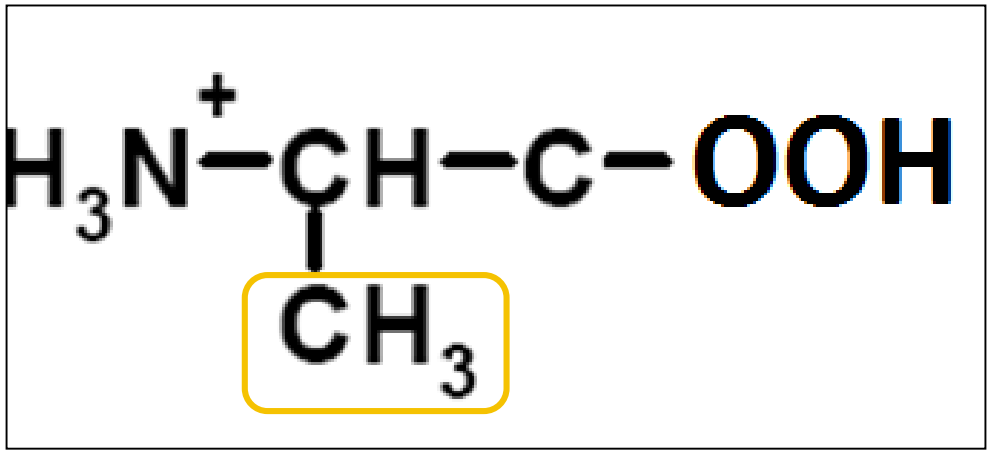
- Amino acids are example of weak acid which contain more than one dissociate group.

- Example :

(1) <u>Alanine</u> contain COOH (PKa1= 2.34)and NH3+ (PKa2= 9.69) groups (it has one PI value=6.010) **[diprotenation]**

The COOH will dissociate first then NH3+ dissociate later .

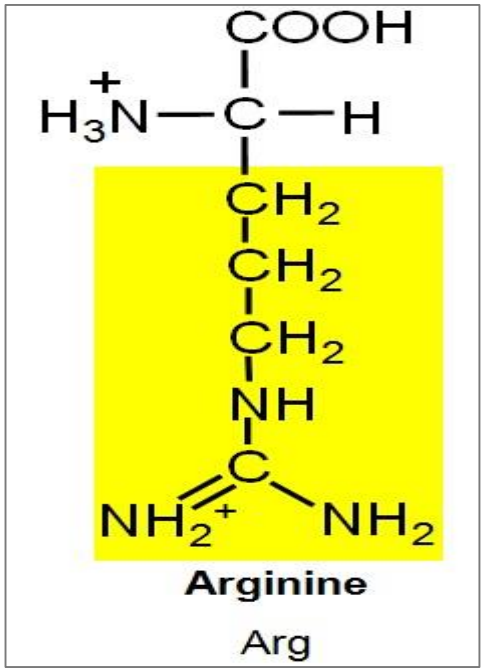
(Because PKa1<PKa2)



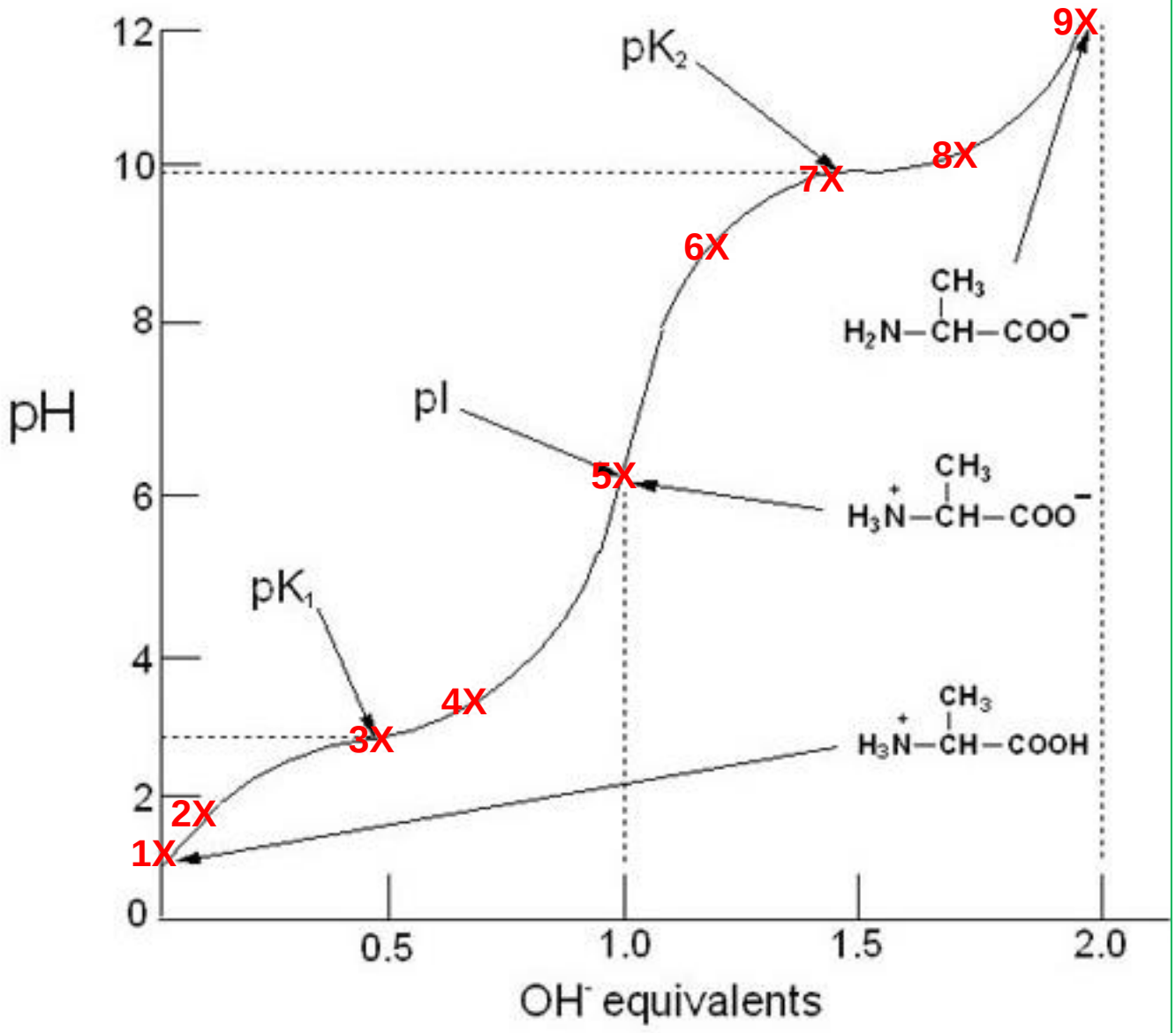
Full protonated alanine

R-group = methyl-group

2) Arginine contain COOH (PKa1= 2.34) , NH3 (PKa2= 9.69) groups and basic group(pKa3=12.5) (It has one pI value=11) **[Triprotenation]**



Alanine Titration Curve



- Titration curve of alanine (or glycine) [diprotenation]:

[1] alanine in starting point is full protonation [NH3+-CH-CH3-COOH].

[2] COOH will dissociate first ,

[NH3+-CH-CH3-COOH] > [NH3+-CH-CH3-COO-]

PH<PKa1.

[3] [NH3+-CH-CH3-COOH]=[NH3+-CH-CH3-COO-] , PH=PKa1,

We already define Pka in the last experiment , in this point the component of alanine act as buffer.

[4] [NH3+-CH-CH3-COOH]<[NH3+-CH-CH3-COO-] , PH > PKa1

[5] The COOH full dissociate to COO- , [NH3+-CH-CH3-COO-] .

At this point the conc. Of negative charge = conc. Of positive charge .the amino acid present as Zwetter ion (neutral form) .

PI (isoelectric point) : PH value at which the net charge of amino acid equal to zero.

PI = (PKa1 + PKa2) /2 = (2.32+9.96)/2= 6.01

[6] The NH3+ start dissociate ,

[NH3+-CH-CH3-COO-] >[NH2-CH-CH3-COO-]

PH <PKa2.

[7] [NH3+-CH-CH3-COO-] = [NH2-CH-CH3-COO-] . PH=PKa2 , the component of alanine act as buffer.

[8] [NH3+-CH-CH3-COO-] < [NH2-CH-CH3-COO-] , PH >PKa2

[9] The NH3 group will dissociate and at the same time the alanine full dissociate in end point , [NH2-CH-CH3-COO]

POH= (Pkb+P[A-])/2

PKb = PKw – PKa2

- Note in calculation method:

The PH calculated by different way :

[1] at starting point ⟶ PH= (Pka+P[HA])/2

[2] At any point within the curve (befor or in or after middle titration)

PH=Pka+log([A-]/[HA])

[3] At end point ⟶ POH=(PKb+P[A-])/2

PH=PKw – POH

Results:

[1] record the titration table and Plot a Curve of pH versus ml of OH- added.

[2]Calculate the pH of the alanine solution after the addition of 0 ml, 5ml, of 0.2M NaOH. And calculate PH after addition of 0.5 ml , 2 ml of HCL

[3] determine the pKa of ionizable groups of amino acids

[4]Compare your calculated pH values with those obtained from Curve.

[5] determine the PI value from your result .