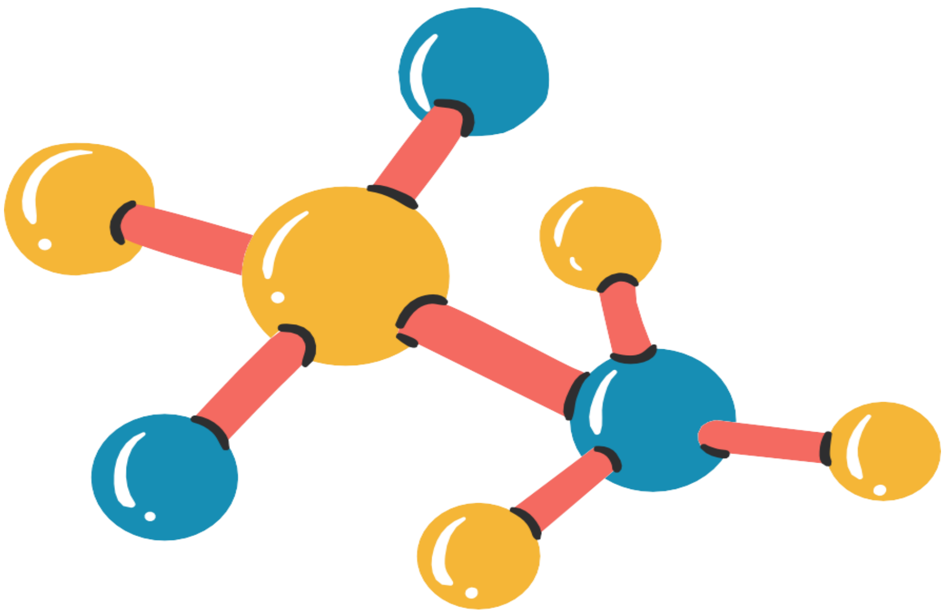
Sheet no.14

Biochemistry



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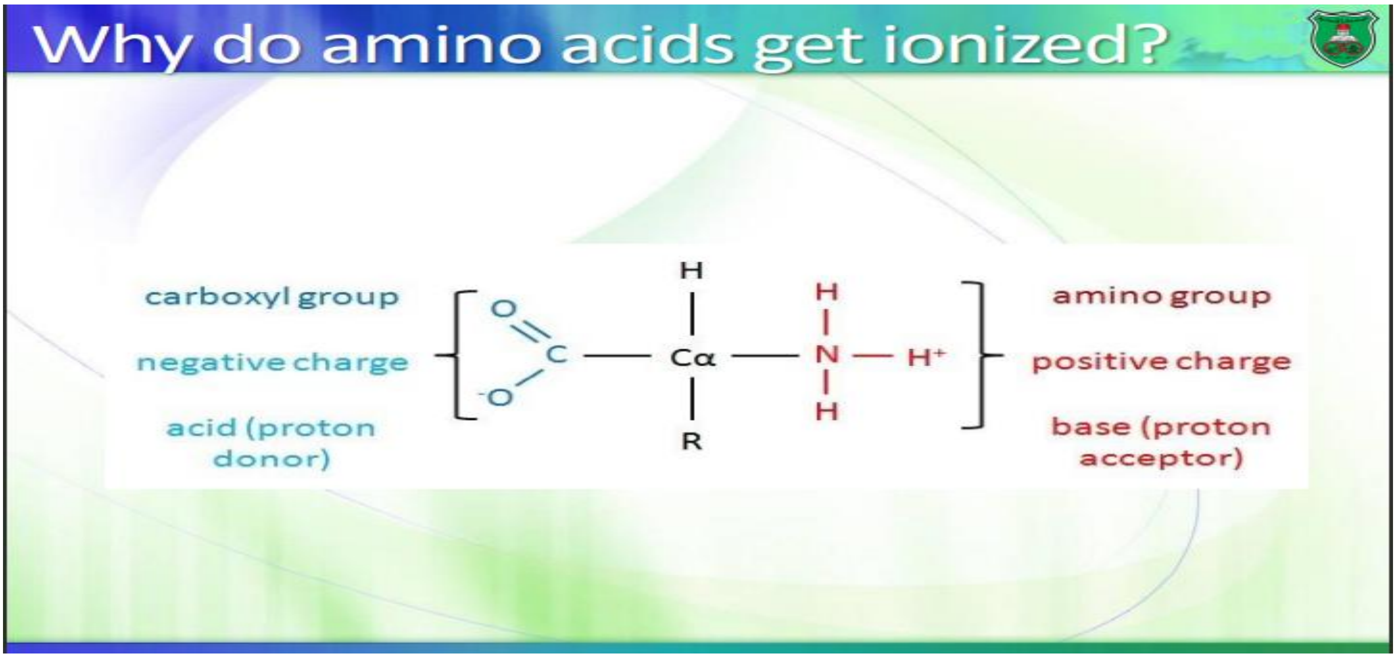
Amino Acids

We will try to link amino acids with acids and bases, as they have an acidic (COO^-) and basic ($NH3^+$) groups.



Under physiological conditions, carboxyl group is going to loss proton (proton donor), it becomes negatively charged . And the amino group is going to accept a proton and it becomes positively charged→this is as free amino acid .

But if the amino acid is among the protein, they make bonds with other amino acids, so the acidity of the amino group and the basicity of the carboxyl group will not appear, the acidic and basic properties of the R group will appear in the protein.

**Free amino acid :

Carboxyl group → negative charge

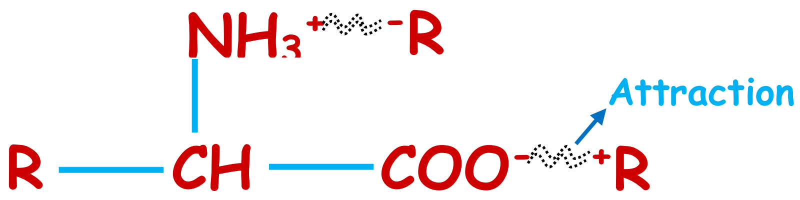
Amino group → positive charge

R group :
- Acid → negative charge
- Base → positive charge

(Except Histidine)

Histidine → It varies between the two cases depending on the surrounding conditions, But ,in general , it is considered a base (which accepts the proton)

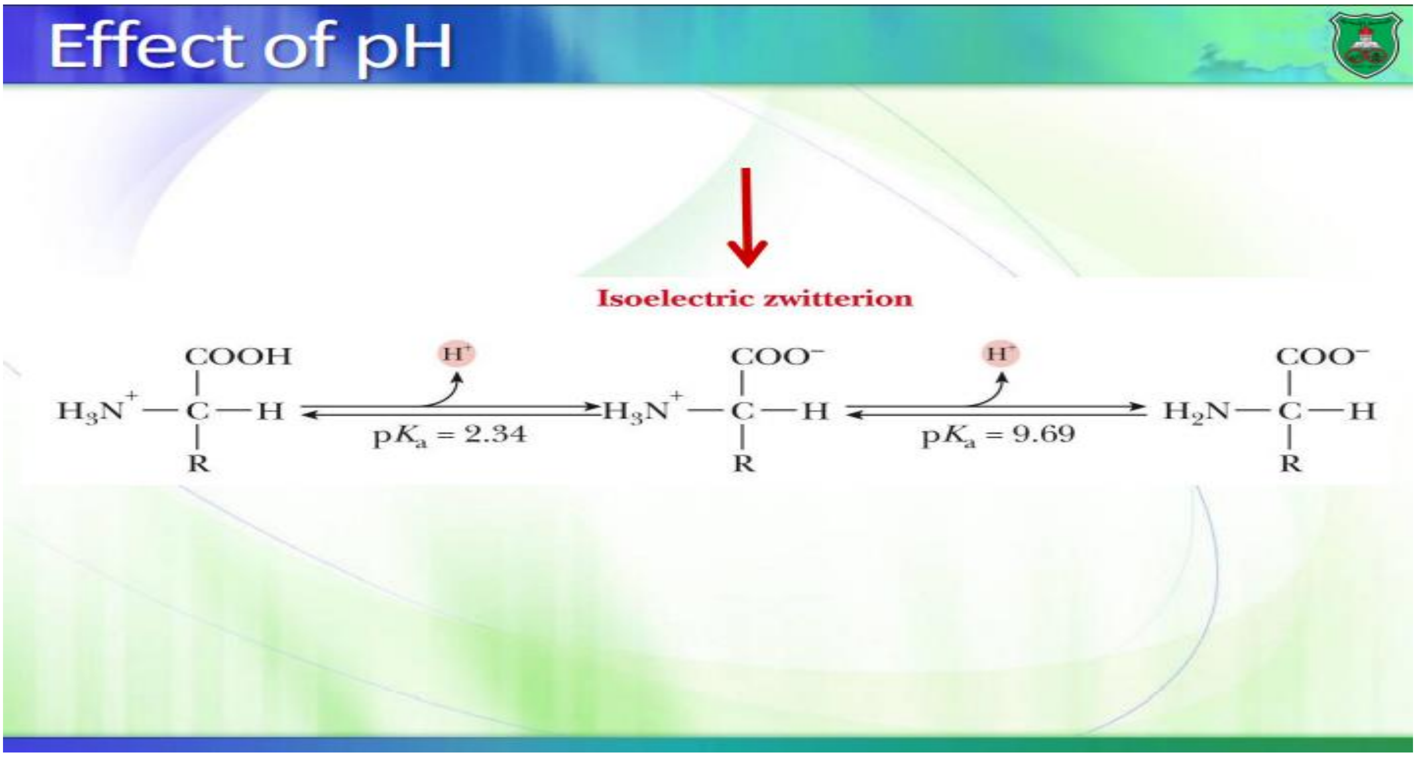
The net amino acid charge = 0, but there are charges.



If we approach the carboxyl side (negatively charged) with a molecule that has a positive charge, attraction will occur and the same is true for the amino group.

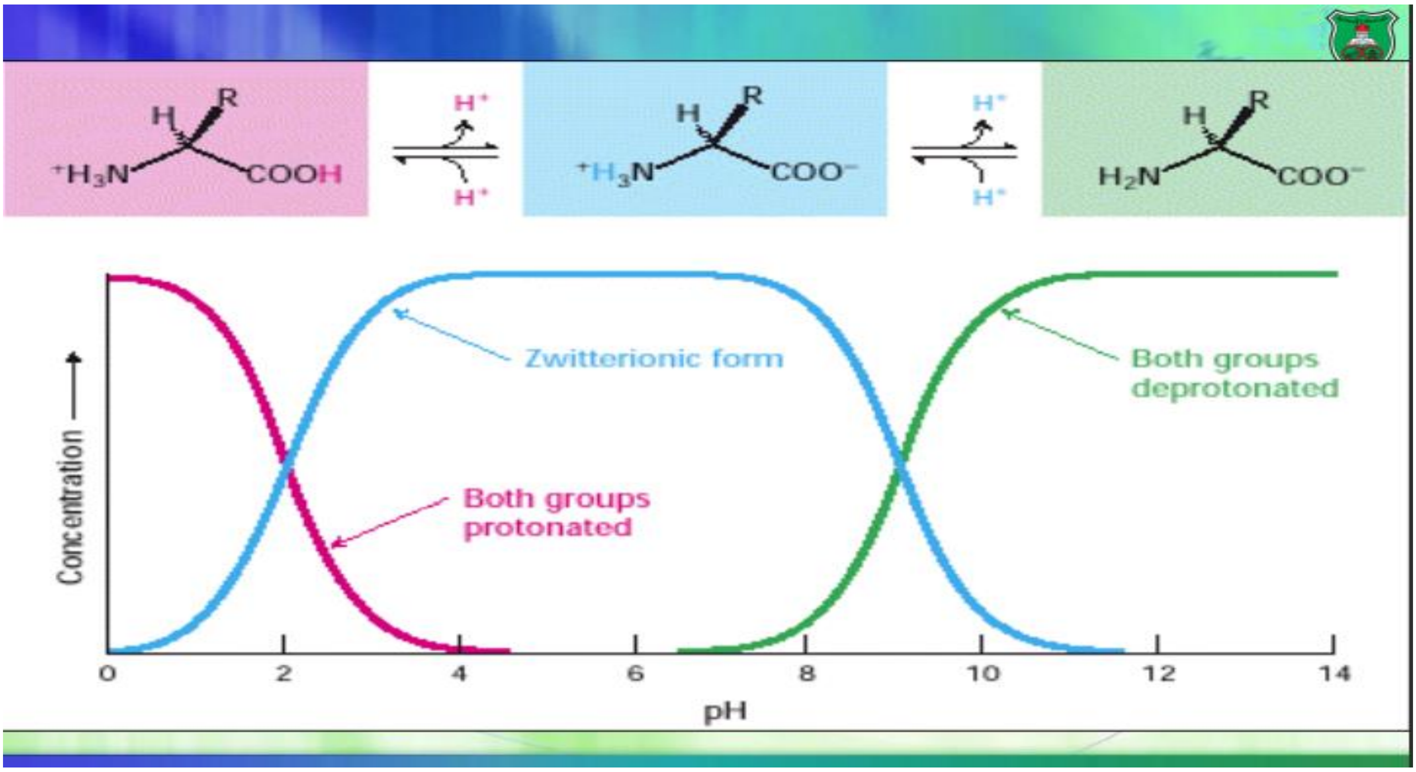
*A molecule that has charges but the total charge is zero is called Zwitterion.

** Non-polar amino acids form a zwitterion simply because the R group does not contain any other groups. As for the polar amino acids (acidic and basic) they have other groups on the R group, so they will have an effect on its formation (when a zwitterion will form, when the total charge (R group + backbone) will be zero). Using titration of different amino acids to know when a zwitterion will form.



-pKa of carboxyl group is very small , that's why under physiological conditions will be ionized.

-pKa of amino group is very high , under physiological conditions accepts proton . That's why is positively charged.



If we follow the path, we start with a high concentration at a very low pH, less than pKa (approx. 2),both groups protonated. This molecule will decrease in concentration with increasing base (or increasing pH)→ Titration .

In the middle, it becomes relatively more basic to form a zwitterion (It will increase until it reaches the max)→all that exists is zwitterion .

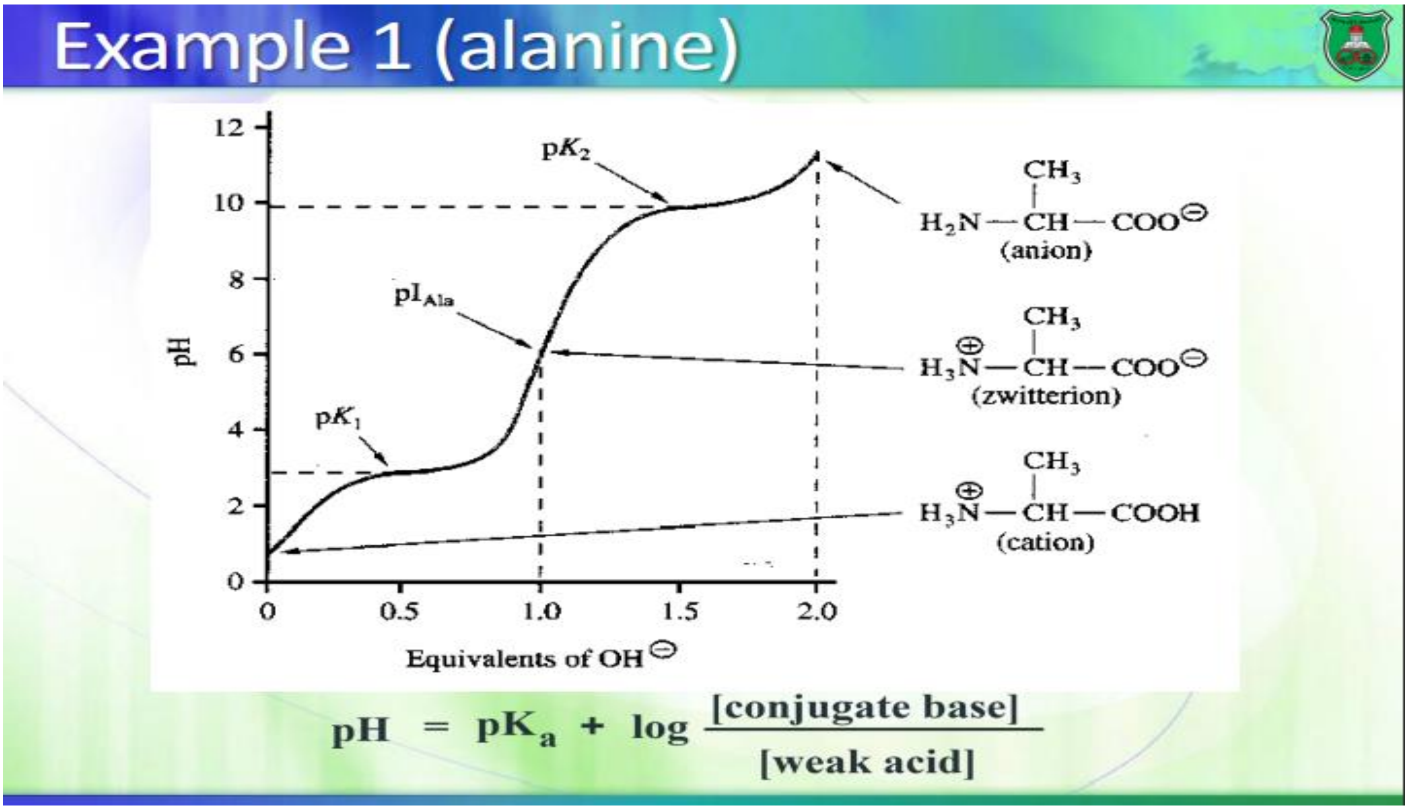
After that , the pH value was increased even more , This molecule will lose its last proton, and the zwitterion concentration will decrease, forming the shape of the molecule in which both of the groups are deprotonated.

**Why do amino acids get ionized?

In the physiological condition, carboxyl group has (-) charge which makes it a proton donor (acid) , where amine group has (+) charge that makes it a proton acceptor (base).

We can explain it by Henderson equation , COOH has a low pKa (by default we consider it = 2) so it has that ability to give proton , NH_2 has a high pKa (consider it = 9) ,so the affinity to accept proton is high .

Note : previously ,we discuss the groups in the backbone , the other groups in the side chain have totally different happy story.



Alanine

We will discuss the titration of the amino acid in chemical vitro,not under physiological environment.

Some basic, since carboxyl group in the backbone is very reactive because it is free of any other group, it will firstly react (give a proton).

First step

Here Ala is completely protonated , cause of the low pH

(Under 2 which is the pKa of carboxyl group that results in COOH won't give a proton)

→ that means the whole molecule is charged by +1.

After we increase the pH , COOH starts to give H^+ until we reach the point where the concentration of cation form equals the concentration of Zwitterion form . we call this point pK_1.

Notice that in pK_1, we add 0.5 of the base (OH^-) .

Second step

pl (isoelectric point) where the *Zwitterion form* is completely present in the vitro. Isoelectric means same of the charge in amount (the definition the *Zwitterion form*).

Third step

By more increasing pH above 9 , NH_3^+ begins to loss its H atom . That will form the anion of Ala (the minus comes from COO^-).

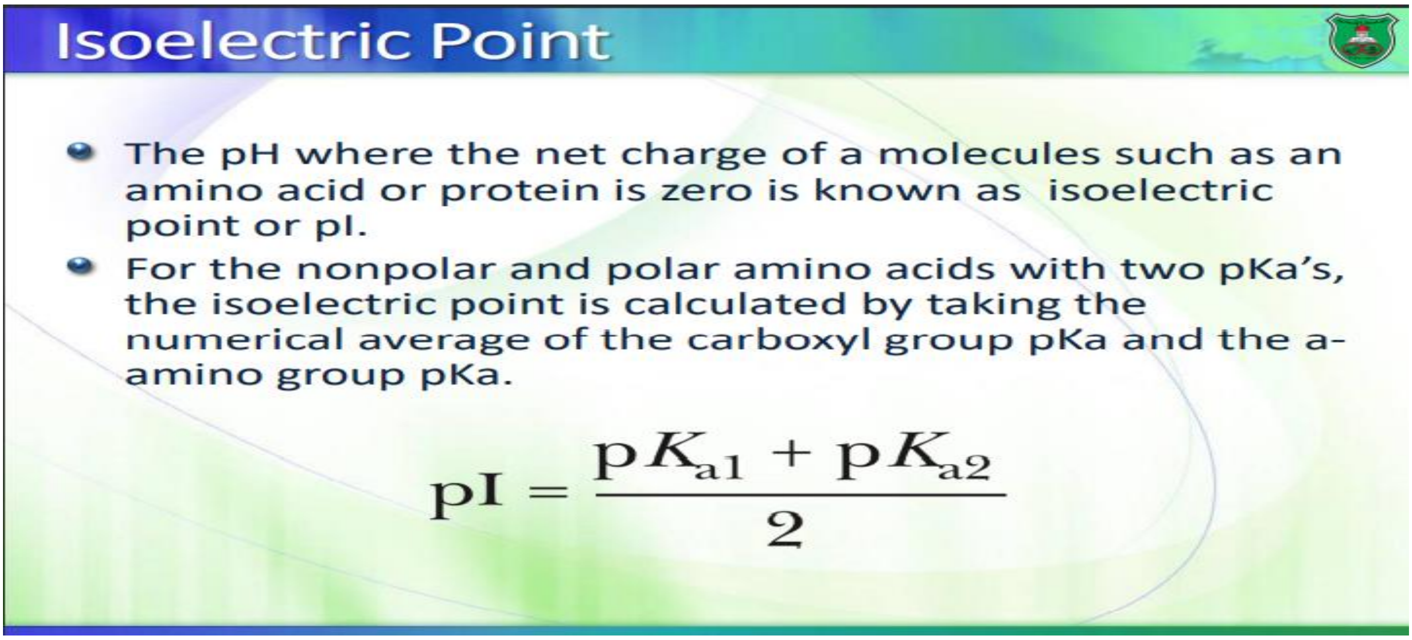
We use 2 mole of OH , so you can deal here as diprotic acid .

TO SUM:

1* In strongly acidic pH (at pH zero) alanine is present mainly in the form of positively charged molecule. Its pK (pK_1) = 2.34

2* By adding NaOH, the carboxyl group loses its proton (H^+) and alanine carries both positive and negative charges (zwitter ion).

3*By adding more NaOH, the solution becomes strongly alkaline, and (NH_3^+) group will lose its proton and alanine will become negatively charged. Its pK (pK_2) = 9.69.



pl

The pH where the net charge of a molecules such as an amino acid or protein is zero is known as isoelectric point or pl

can be calculated by the law below:

$$pl = \frac{pK_1 + pK_2}{2}$$

We take the average pf the two steps (before and after forming the *Zwitterion form* . Important to understand).

***To more explain for Ala*:**

For the non-polar and polar amino acids with two pKa's – as alanine , the isoelectric point is calculated by taking the numerical average of the carboxyl group pKa (pk_1)and the an amino group pKa (pk_2)

***Ala* has two pKa because its R group does not participate in giving or accepting H^+, but other amino acids' R groups have their role in titration process .**

Other amino acid- with R group able to be ionized ,

this R group has its own pKa value (range from 2—12) so we have R groups with pKa under 9 and other above → it affects the sequence of giving protons.

It affects the sequence of giving proton:

COO^- → NH_3^+ (side chain) → NH_3^+ (backbone) **OR**

COO^- → NH_3^+ (backbone) → NH_3^+ (side chain)

Ionization of side chains

- Nine of the 20 amino acids have ionizable side chains.
- These amino acids are tyrosine, cysteine, arginine, lysine, histidine, serine, threonine, and aspartic and glutamic acids.
- Each side chain has its own pKa values for ionization of the side chains.

pI of amino acids

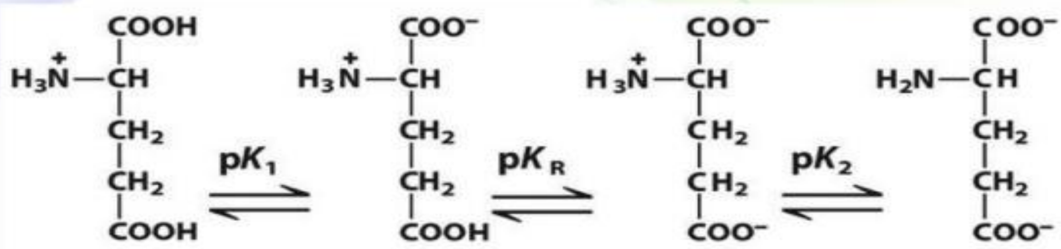
Amino Acid	Side Chain pK_a[3]	pI
Arginine	12.5	10.8
Aspartic Acid	4.0	3.0
Cysteine	8.0	5.0
Glutamic Acid	4.1	3.2
Histidine	6.0	7.5
Lysine	11.0	10

Let's consider pKa of $-NH_2$ = 9 and pKa of $-COOH$ = 2 for all amino acids

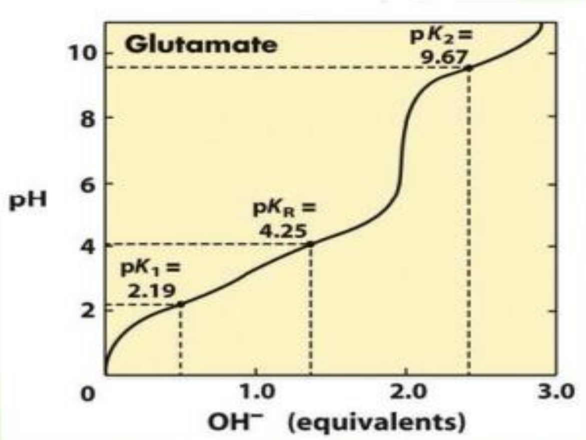
Calculation of pI of amino acids with ionizable R groups

- The isoelectric point for these amino acids is calculated by taking the average of the pKa's of the groups with same charge when ionized
- In this case, the total charge on the groups with like charge must equal one (1) so that it can be balanced by the one (1) opposite charge present on the molecule

Example: Glutamate



- To calculate the isoelectric point of Glu, the pKa's of the two carboxyl groups are averaged.



Glutamate

First step

The R group has a terminal carboxyl group .

At low pH , all of the groups are protonated which makes the total charge = +1 .

In first step of titration, the carboxyl group of the backbone – we agree it's more reactive – will produce H^+ → that results in forming Zwitterion form of Glutamate.

pKa 1 indicates the pKa of the first COOH.

Second step

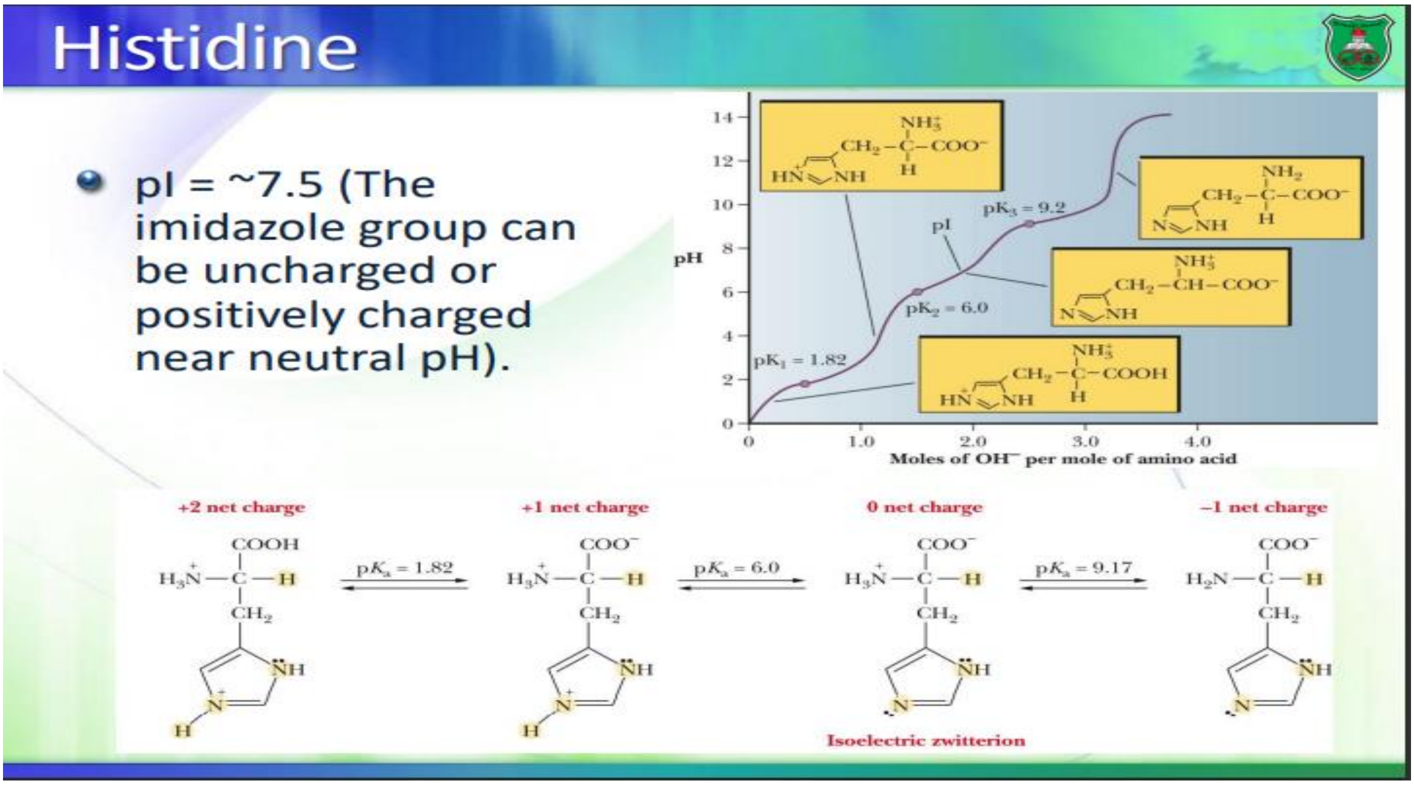
More increasing in pH will activate the second carboxyl group in the side chain to release a proton→ the charge will reduce by one.

Third step

The high increasing in pH makes the amino group reactive to produce H^+→ the total charge would be -2 .

The Zwitterion form of Glutamate formed during the first step by one releasing from COOH group , so if we want to calculate the pl, we will take the pKa 1 and pKa R from the picture , means the pKa before and after forming Zwitterion form.

$$pl = \frac{2.19 + 4.25}{2} = 3.22, as\ in\ the\ table$$



Histidine

From the table of pKa of the R group , the pKa of it is under 9 so it will react before the amino group in the backbone (it doesn't matter to know the sequence of the deprotonation of the molecule, but just in case)

First step

At low pH , the whole molecule is charged by +2 so we need two steps to reach Zwitterion form .By increasing the pH→the COOH in the backbone get deprotonation and the charge will decrease by one→become +1.

Second step

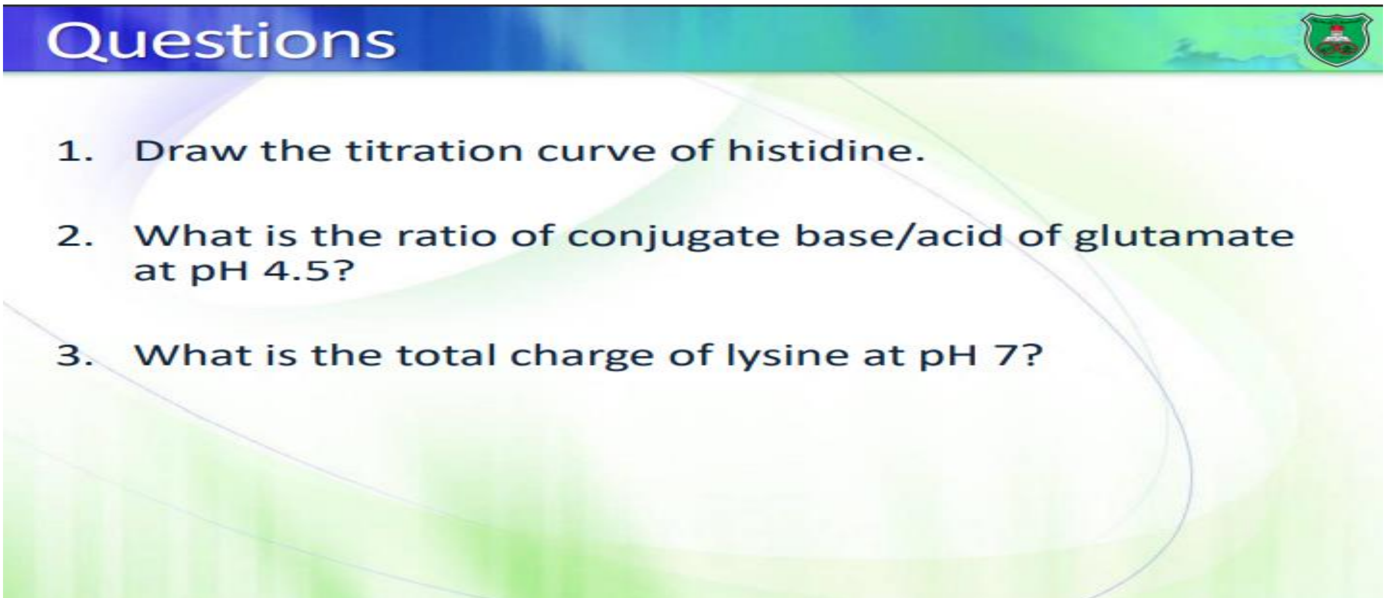
The N atom in the side chain will loss it's H atom by increasing the pH. In this step we get Zwitterion form of Histidine.

Third step

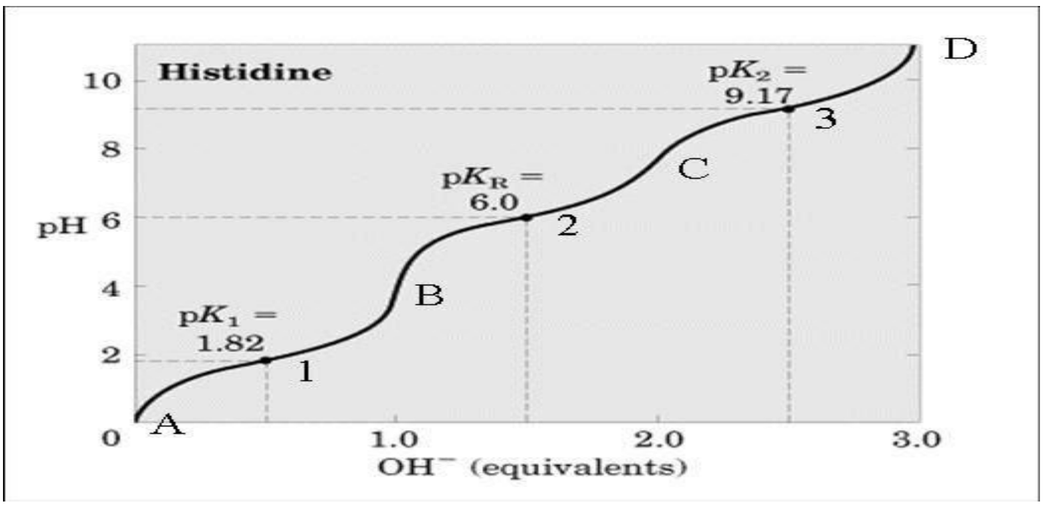
NH_3^+ get deprotonation as the last step in the high pH level , the compound will be charged by -1 . As we see the sequence in the picture .

To calculate the pI , we take the steps before and after the Zwitterion forming . Here the second and the third steps are the involved ones , so we take the pKa values from the curves.

$$pI = \frac{6.0 + 9.17}{2} = 7.5 \ \textit{as mentioned in the table}$$



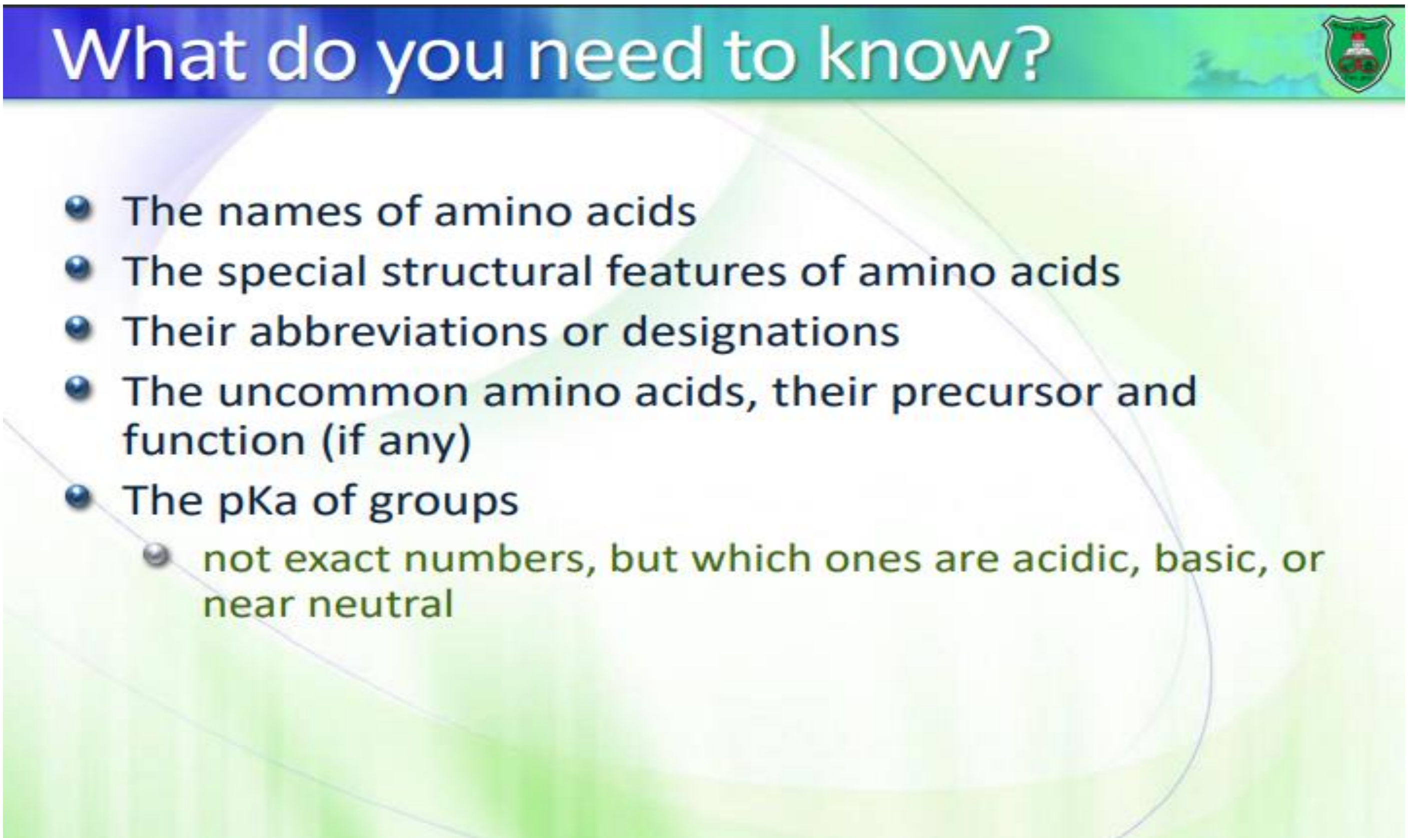
1*



2* $4.5 = 4.25 + log\left(\frac{base}{acid}\right) \rightarrow log\left(\frac{base}{acid}\right) = 0.25$

$$\frac{base}{acid} = 1.778$$

3* +1



Q:

1* The amino acid arginine contains a guanidino R-group and has pKa values of 2.2, 9.0, and 12.5. A sample of arginine is titrated from pH=1.0 to pH=14.0 with NaOH.

At pH=2.2

a) all of the amino acid molecules will be in the fully protonated form.

b) half of the amino acid molecules will be in the fully protonated form.

c) all of the amino acid molecules will be in the zwitterion form.

d) half of the amino acid molecules will be in the zwitterion form.

2* Which property is shared by both arginine and aspartate as each is titrated with NaOH from pH=1.0 to pH=14.0?

a) Both will require the same number of NaOH equivalents to complete the titration.

b) Both will have the same number of equivalence points at the same pH values.

c) Both will have the same net charge at pH=1.0.

d) Both will have the same net charge at pH=14.0.

3*The amino acids have a carboxyl group with a pK around ---- , and an amino group with a pK near ---

a)1.1 and 12.1

b)6.5 and 8.0

c)3.3 and 10.5

d)9.0 and 2.5

e) 2.0 and 9.5

4*The amino acid alanine has two pKa values 2.3 for the COOH group and 9.7 for the NH3+ group .What is the pI for this compound??

a) 6.0 b)1.0 c)12 d)3.5

5*When the amino acid alanine (the R group is: CH_3) is added to a solution with a pH of 7.3 , alanine becomes:

a)a cation b)non-polar c) a zwitterion d) an isotope

6*The isoelectric point of an amino acid is defined as :

a) The pH where the molecule carrier no net electric charge .

b)The pH where the carboxyl group is uncharged .

c)The pH where the amino group is uncharged .

d)The pH of maximum electrolytic mobility

e)$-log_{10}(pK_i + pK_J)$

7*1. Which of the following amino acids has a net charge of 12 at low pH?

a) Aspartic acid b) alanine and glutamic acid

c) arginine and lysine d) leucine

Ans: Arginine and lysine have net charges of 12 at low pH because of their basic side chains.

8*Which has a net charge of -2 at high pH?

a) Aspartic acid and glutamic acid b) alanine

c) arginine and lysine d) leucine

Ans:Aspartic acid and glutamic acid have net charges of -2 at high pH because of their carboxylic acid side chains.

9*The pKa of the side-chain imidazole group of histidine is 6.0. What is the ratio of uncharged to charged side chains at pH 7.0 ?

Ans : The ratio is 10:1 because the pH is one unit higher than the pKa.

10*

I) The amino acid tyrosine contains a phenolic R-group and has pKa values of 2.2, 9.0, and 10.2. A sample of tyrosine is titrated from pH = 1.0 to pH = 14.0 with NaOH. At which pH will all the amino acid molecules be in their fully protonated form?

a) 1.0 b) 2.2 c) 5.6 d) 9.0

II)At which pH will half the amino acid molecules have a +1 charge?

a) 10.2 b) 9.0 c) 2.2 d) 1.0

III)For a solution of tyrosine molecules at pH = 10.2 :

a) all the α-carboxyl groups will be uncharged.

b) all the α-amino groups will be uncharged.

c) all the phenolic R-groups will be uncharged.

d) all the ionizable groups will be uncharged.

Additional information

This property (difference in the charges between amino acids in a given pH) is useful in electrophoresis, a common method for separating molecules in an electric field. This method is extremely useful in determining the important properties of proteins and nucleic acids.

The pH at which a molecule has no net charge is called the isoelectric pH, or isoelectric point (given the symbol pl). At its isoelectric pH, a molecule will not migrate in an electric field. This property can be put to use in separation methods.