DOPAMINE SCIENTIFIC TEAM

Lecture 12

Amino Acid 2

اللهم إنّي أسألك أن تنصرَ أهل غزة وأن ترزقهم القوة والصبر، وأن تربط على قلوبهم يا رحمٰن ، اللهم أنزل عليهم من رحمتك وداوِ جرحاهُم ، وتقبل موتاهم ، واشفِ مرضاهم ، اللهم إني أسألك أن ترزقهم النصر والثبات والتمكين ، آمين

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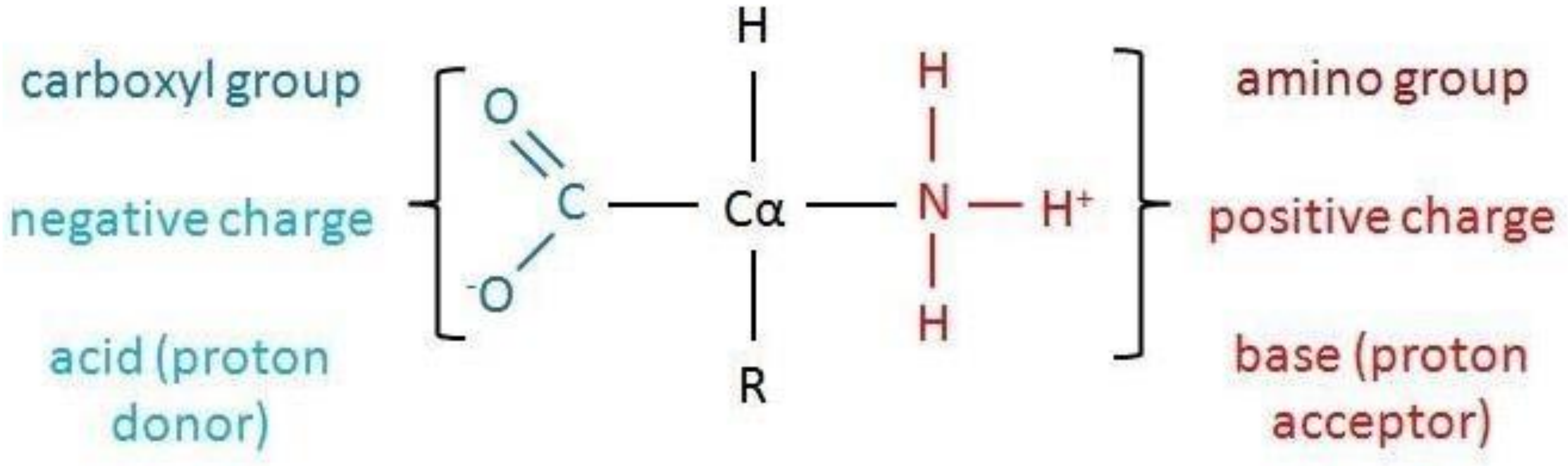
Ionization of amino acids

Why do amino acids get ionized?

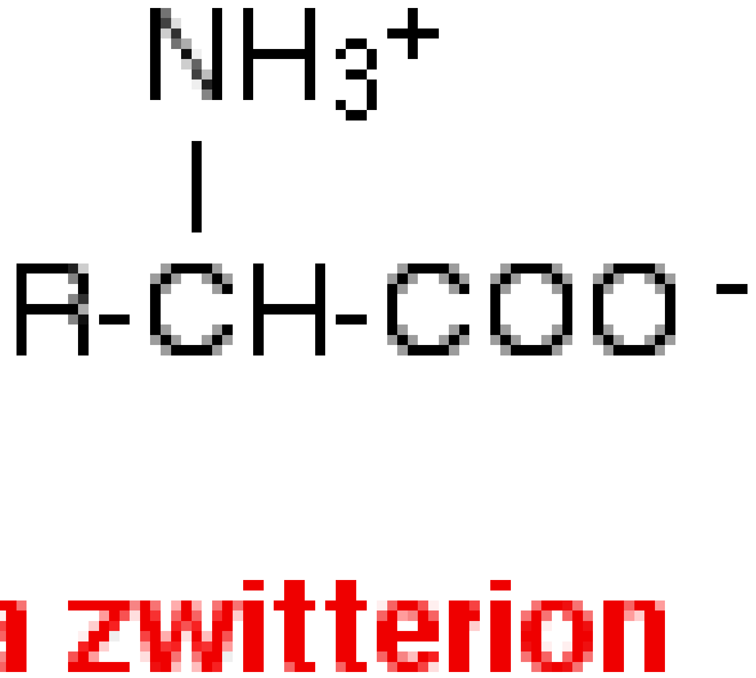


-Positive and negative charge cancel each other

Zwitterion and isoelectric point

- At physiological pH, amino acids (without ionizable groups) are electrically neutral.
- Zwitterion: a molecule with two opposite charges and a net charge of zero.

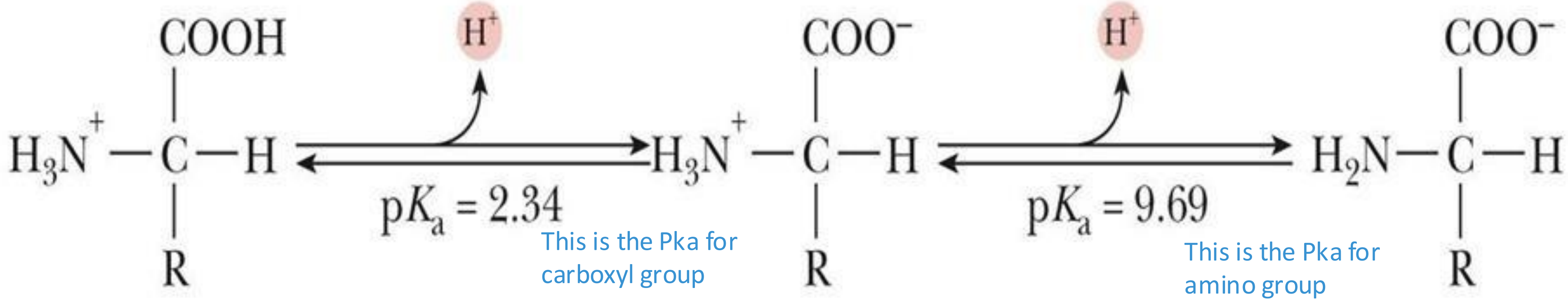
Amino acids generally in their physiological PH are zwitterion/zwitterionic form



Effect of pH

Isoelectric zwitterion

doctor supposed 2.34 =2 , 9.69=9

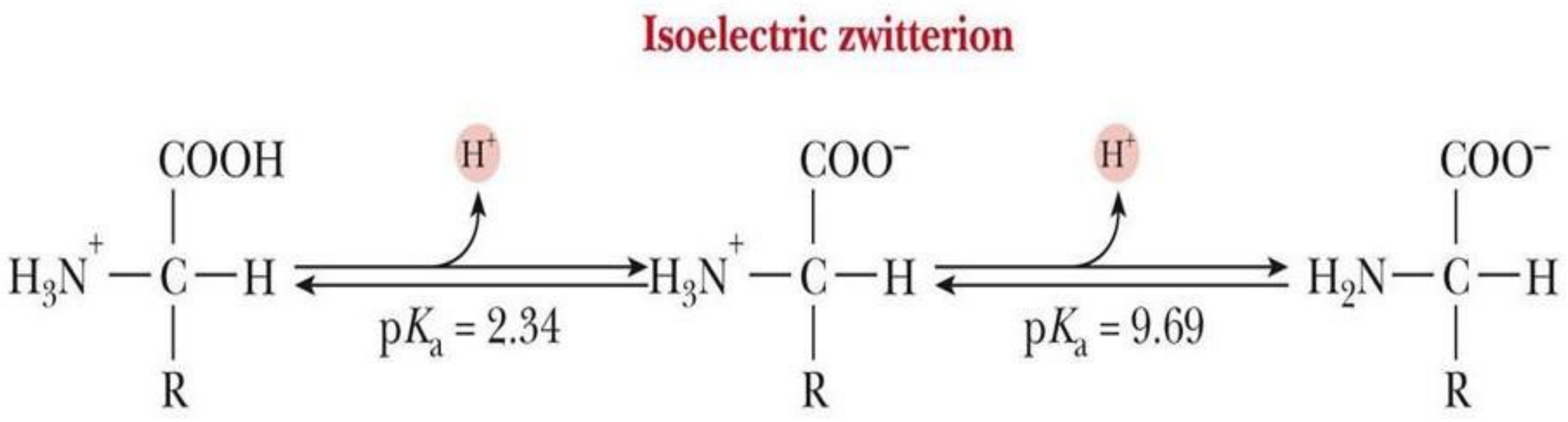


-When PH is very low (Lower than 2 this number indicates to the pka of carboxyl group not the pka of amino acid), **carboxyl group** and **amino group** will be protonated so carboxyl group will be neutral and amino group will have positive charge , why?
PH low that means we have high concentration of protons for both (carboxyl group and amino group)

-When PH =2, 50% of **carboxyl group** is protonated and 50% unprotonated and **amino group** 100% in protonated form

-When PH is higher than 2 and less than 9, the **carboxyl group** will start losing it's proton so it's going to be unprotonated (negative charge) for that the amino acid will be in it's zwitterioic form because the total net charge is zero now, and **amino group** mainly will be protonated (positive charge)

Effect of pH

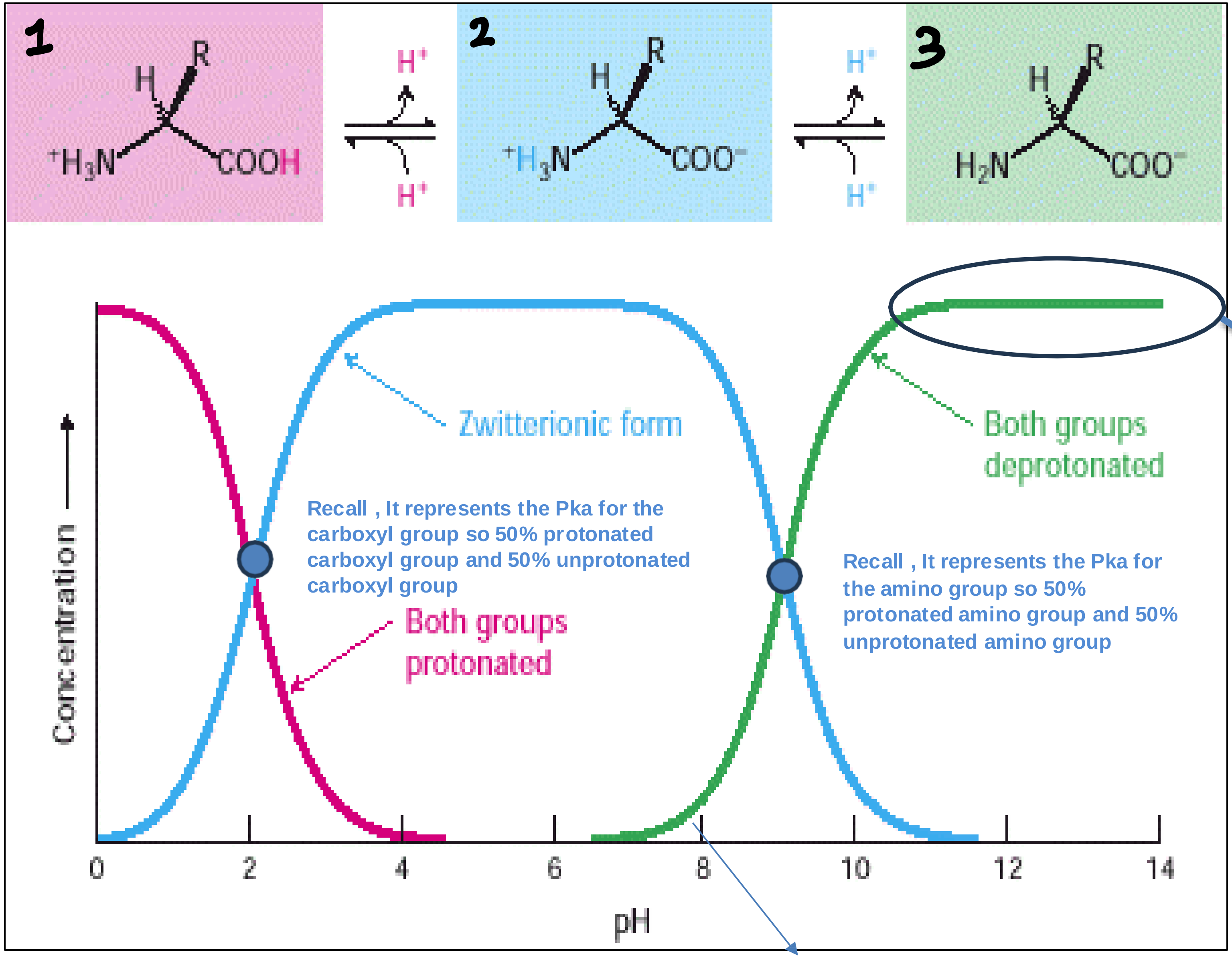


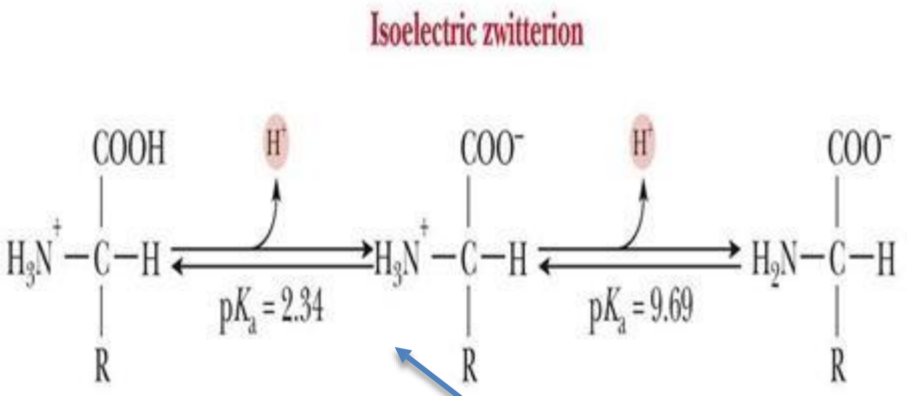
-When PH =9, 50% of **amino group** is protonated/acidic form and 50% unprotonated/ conjugated base form and **carboxyl group** 100% unprotonated

-When PH is higher than 9, **amino group** will start losing it's proton so it will be unprotonated and **carboxyl group** (negative charge) , the total charge now is -1

Amino acid can function as buffers while they have pka and can donate or loss proton , but the question what is the buffering capacity / buffering range for them?
First one. (when Pka=2) the range is 1-3, the midpoint is 2 Second one. (Pka=9) the range is 8-10, the midpoint is 9 , they have two buffering capacity

Outside the range, amino acid can't act as buffer





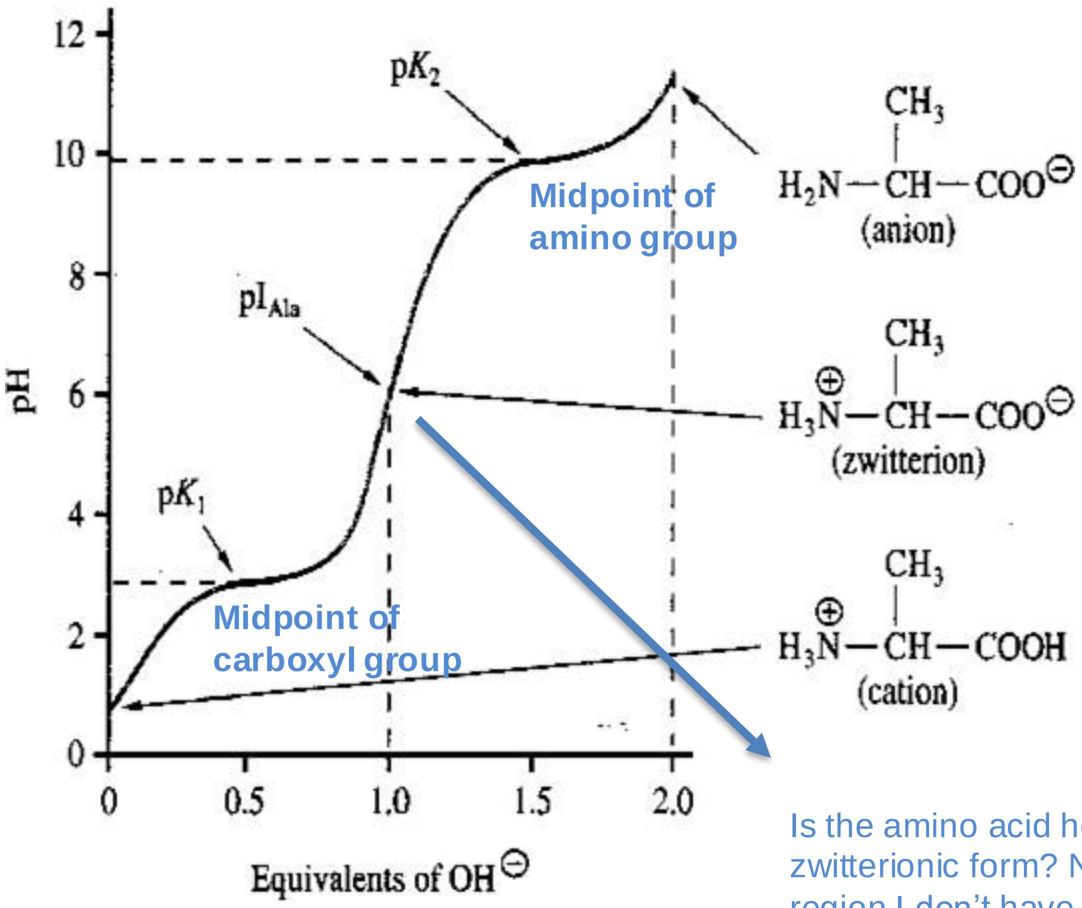
eventually when PH be very high , both groups will bo 100% unprotonated form

As we increase the PH more than 2 , this form **(2)** will increase because the reaction goes to the right and **(1)** will decrease

Remember when PH = 2 , 50% will be in form **(1)** and 50% will be in form **(2)** , when PH = 9, 50% will be in form **(2)**and 50% will be in form **(3)**

eventually when pH be at 8 , the amino group will start losing it's proton so it will be unprotonated and it will lose it's charge

Example 1 (alanine)



1.Alanine have two ionized groups (amino group and carboxyl group) , when the equivalent of the strong base is zero and the solution is acidic,100% of *alanine's groups* will be protonated so the carboxyl group (no charge) and amino group (positive charge) so total charge is +1

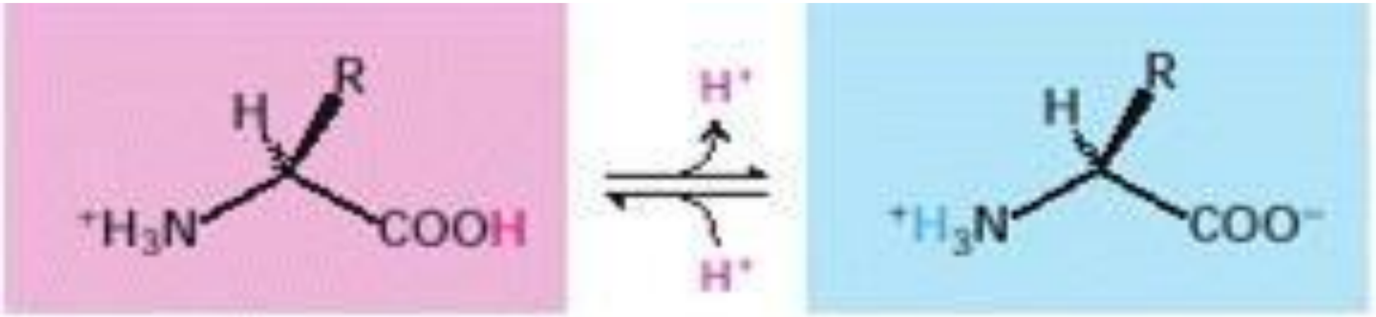
2.As we are adding the strong base ,the solution is becoming more and more alkaline(or we can said less Acidic) and PH is increasing of course so we will reach to the buffering range of Carboxyl group **(1-3)** therefore the amino acid will start buffering/ resistance the change of PH

3. Now we are out of the carboxyl range , note that the change of PH become dramatic because I don't have buffering

Is the amino acid here will be 100% in zwitterionic form? No , Although in this region I don't have buffering , the conjugated base doesn't present 100% because I still have the acidic form ,may the ratio between the conjugated base and acid be 100:1

If you want to know how buffering happens go to the next slide

Someone asked the doctor if we can consider zwitterion as amphoteric (act as acid and base) , doctor mamoon said : "it's possible according to the definition"



If we want to apply it in the Henderson-Hasselbalch Equation

When we add strong base , the protons in the solution will decrease and therefore the reaction will go to the right direction,why? Because the carboxyl group will make releasing and compensation for the protons that we lost so the PH will slowly increase , that's how buffering takes place.

$$pH = pK_a + \log \frac{[\text{conjugate base}]}{[\text{weak acid}]}$$

For carboxyl group

We are looking to the group not the Amino acids

Example 1 (alanine)

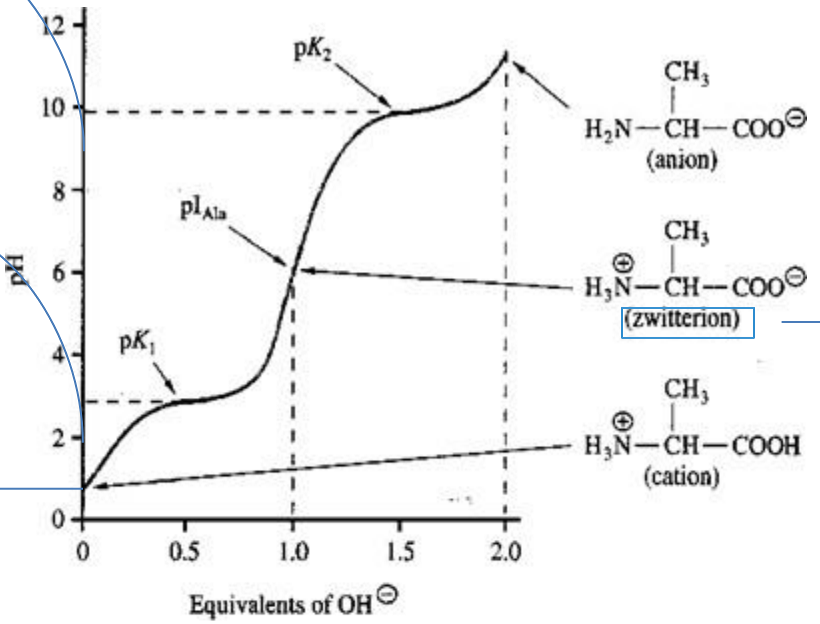
The isoelectric point is the pH when the molecule has charged groups , but the net charge is zero,

pKa? It's the pH when 50% of the group (not the molecule) like amino group or carboxylic group is protonated , and 50% is unprotonated. (50% in the acidic form , and 50% in the conjugate base form).

At pH=9, the total carboxylic group is unprotonated , so it has a full -1 charge , and 50% of the amino group is protonated and 50% is unprotonated, so it has +1/2 charge . The net charge is -1/2

At pH=2 , most amino groups are protonated , with +1 charge , while 50% of the carboxylic groups is protonated , and 50% is unprotonated , (the charge of the carboxylic group is -1/2).
The net charge is +1/2 , and it's not the isoelectric point.

At pH=1 , the total charge is +1 , so it's not the isoelectric point.



Here, in the zwitterionic form , most of the amino groups if not all are positively charged, and most of the carboxylic groups if not all are negatively charged, so we have reached the isoelectric point .

At this point , there is no buffering , because we're outside the buffering range , but the amino groups will start loosing their own protons.

The point when the maximal concentration of the amino acid is neutral is about 5.5

$$pH = pK_a + \log \frac{[\text{conjugate base}]}{[\text{weak acid}]}$$

Isoelectric Point

- The isoelectric point or pI is the pH where the net charge of a molecules such as an amino acid or protein is zero.
- For the nonpolar and polar amino acids with two pKa's, the isoelectric point is calculated by taking the average of the pKa's of the carboxyl group and the amino group.

$$pI = \frac{pK_{a1} + pK_{a2}}{2}$$

Ionization of side chains

-7 of the 20 amino acids have ionizable side chains near physiological pH.

-These amino acids are tyrosine, cysteine, arginine, lysine, histidine, and aspartic and glutamic acids.

-Each side chain has its own pKa values for ionization of the side chains.

pI of amino acids

Memorise all

Amino Acid	Side Chain pK_a (approx.	pI
Arginine	12.5	10.8
Aspartic Acid	4.0	3.0
Cysteine	8.0	5.0
Glutamic Acid	4.1 ~4	3.2
Histidine	6.0	7.5
Lysine	11.0	10
Tyrosine	10.0	9.5

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

Example: Glutamate

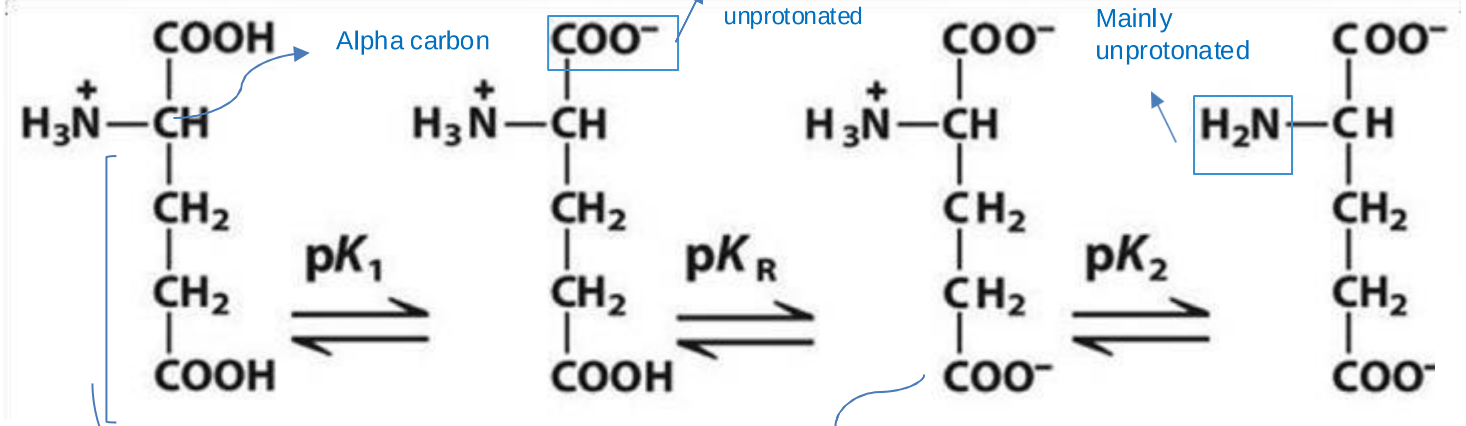
Not zwitterionic

pH=2

50% protonated 50% unprotonated

pH=4

pH=9



R group
Net charge =+1

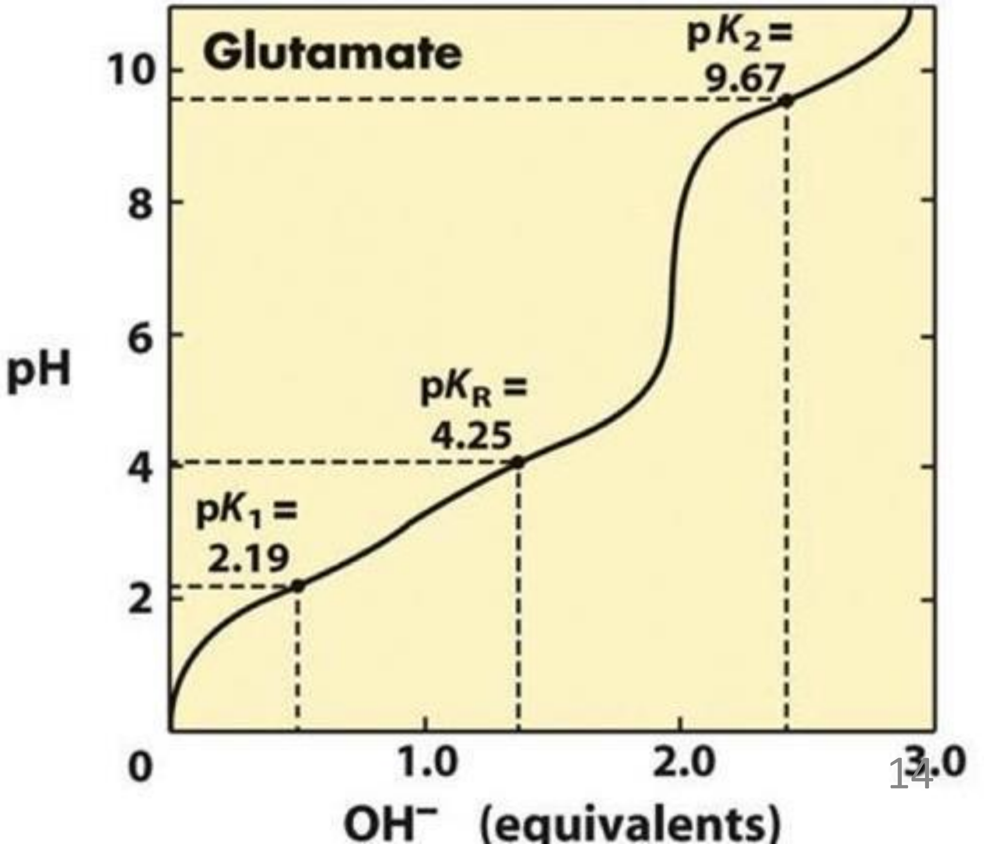
50% protonated
50% unprotonated

Amino group charge:
Protonated=+1
Unprotonated=no charge
Carboxylic group charge:
Protonated=no charge
Unprotonated=-1

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

- pKa of COOH =2
- pKa of NH3=9
- pKa of Glutamate =4

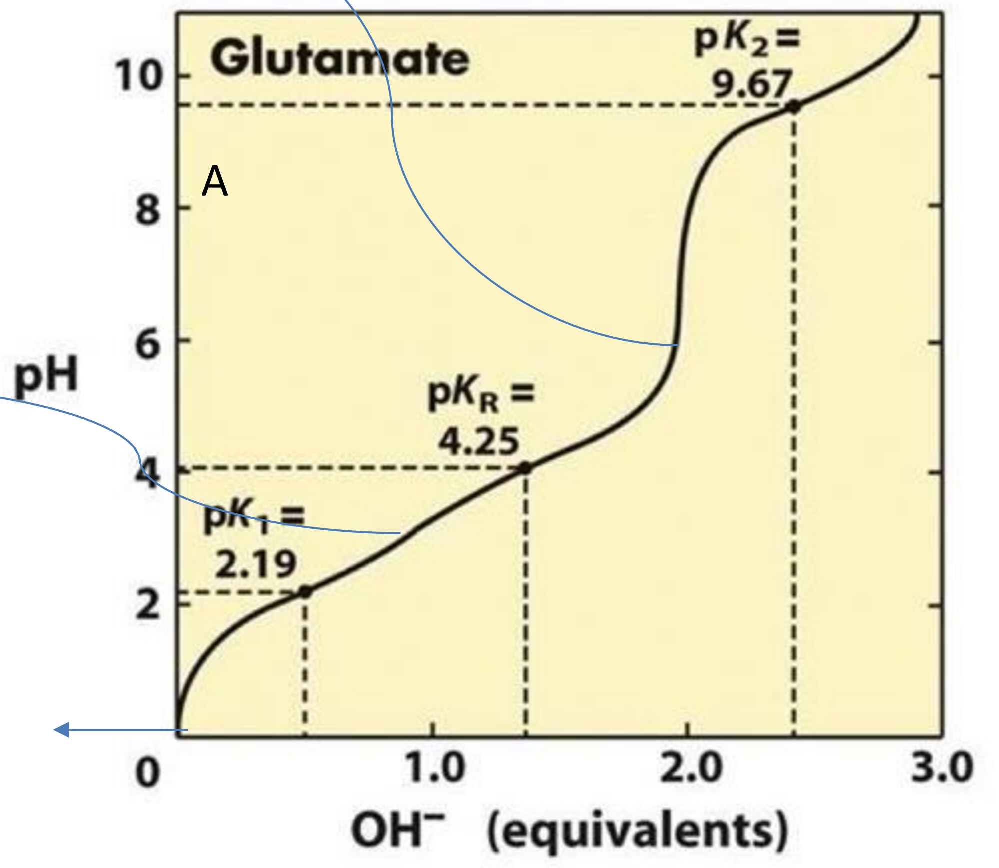
Two buffering capacities ,for the R group and the carboxylic group, and between them the isoelectric point.



Also:

At pH=6, R group is predominantly unprotonated , carboxylic group is unprotonated , amino group is protonated (we're still under pKa for amino group -which is 9- and since pH is less than it's pKa , it will be protonated.
The net charge =-1

At pH=3, carboxylic group is mainly unprotonated, we're out it's buffering range , the amino group is protonated , the R group is still protonated because we're at pH less than it's pKa.
The total net charge is zero , so we're in the zwitteronic form.



Low pH and no addition of a strong base, all groups are protonated , the net charge is +1

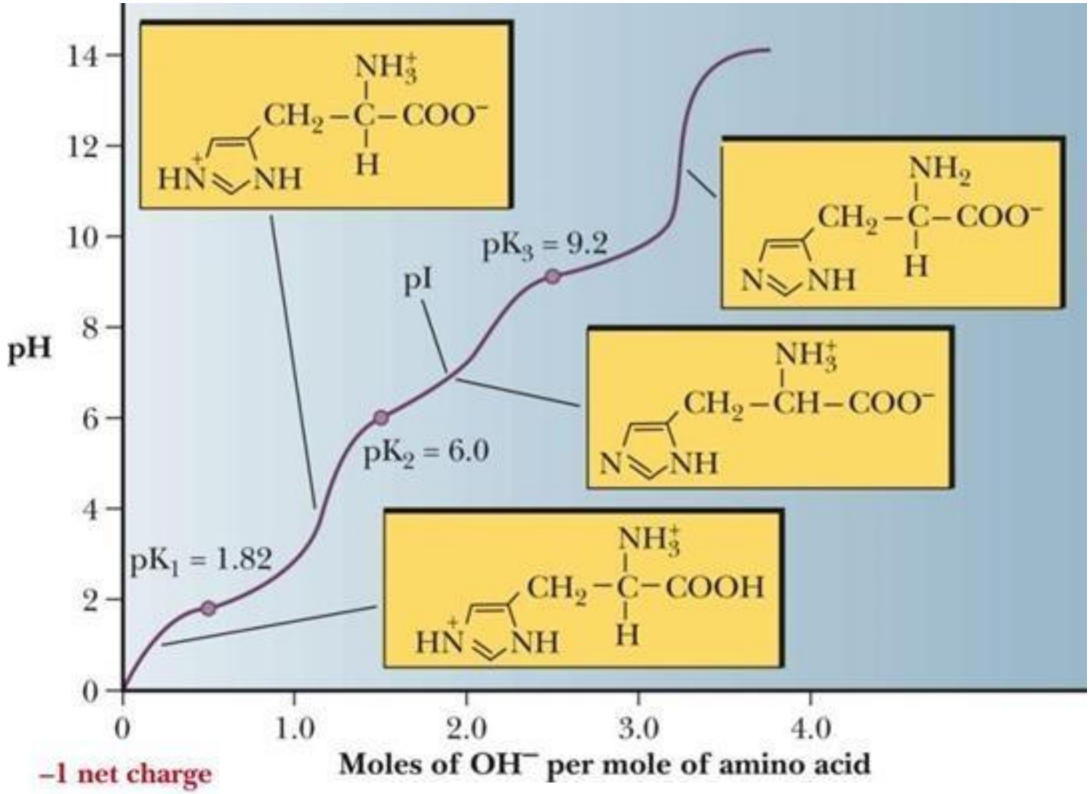
Histidine

I it's a very important amino acid because the pKa of its groups is close to the physiological pH. Its really important in buffering the blood , cells, etc.

pKa values of the groups are changeable according to the environment.

- pI = ~7.5 (The imidazole group can be uncharged or positively charged near neutral pH).

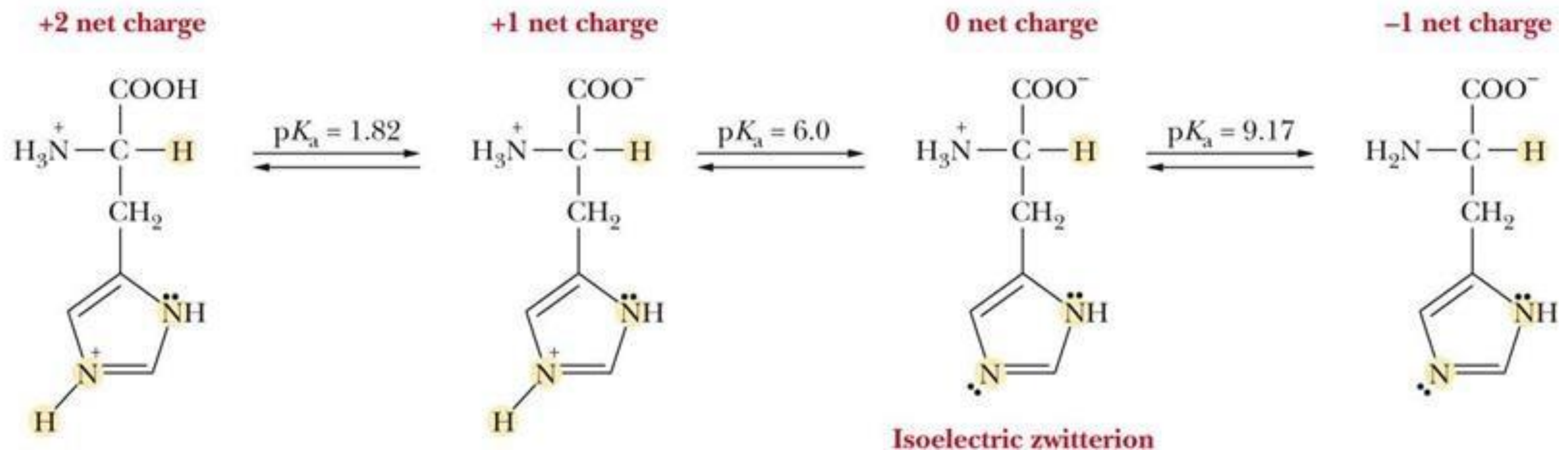
Note that the pKa's of the side chains are different when amino acids are part of proteins.



protonated NH3+ (+1)
protonated R groups (+1)
protonated COOH (no charge)

pH=4
protonated NH3+ (+1)
unprotonated COOH (-1)
protonated R groups (+1)

pH=7.5
protonated NH3+ (+1)
unprotonated COOH (-1)
unprotonated R group (no charge)
Zwitterionic form



pKa of the R group is 6 , we have 3 buffering ranges , the isoelectric point is 7.5

Questions

- Draw the titration curve of histidine.
- What is the ratio of conjugate base/acid of glutamate at pH 4.5?
- What is the total charge of lysine at pH 7?

What do you need to know?

- The names of amino acids
- The special structural features of amino acids
- Their abbreviations or designations
- The uncommon amino acids, their precursor and function (if any)
- The pKa of groups
  - not exact numbers, but which ones are acidic, basic, or near neutral

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2	Slide 8 , in the first point Slide 6	* so the carboxyl group (positive charge) and amino group (no charge) so total charge is +1	* so the carboxyl group (no charge) and amino group (positive charge) so total charge is +1 * Pka= 9 they have two buffering capacity
V2 → V3			
V3 → V4			

Additional Resources:

1. • Marks' Basic Medical Biochemistry

وتضيق دنيانا فنحسب أننا
سنموت يأسا أو نموت نحيبا

وإذا بلطف الله يهطل فجأة
يربي من اليبس الفتات قلوبا

قل للذي ملأ التشاؤمُ قلبه
ومضى يضيق حولنا الآفاقا

سر السعادةِ حسن ظنك بالذي
خلق الحياةَ وقسَّم الأرزاقا.