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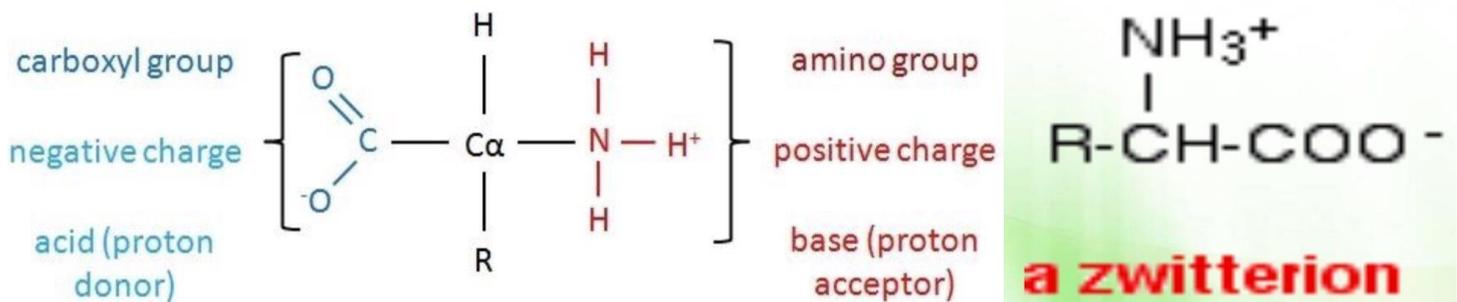
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Ionization Of Amino Acid

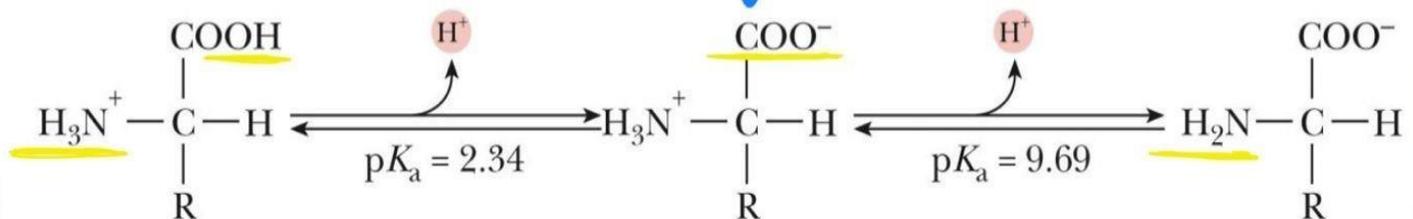
You've probably noticed that the carboxyl functional group can be represented as (COO⁻) rather than (COOH), this is due to deprotonation and thus we have a "deprotonated format" (acidic). The negative charge formed on the group is due to the loss of a proton.

Whereas the amino functional group (NH₂) can be represented as (NH₃⁺), this is due to protonation and hence we have a "protonated format" (basic). The positive charge formed on the group is due to the gain of a proton.

Therefore, under suitable physiological conditions (normal PH of the body's cells) the whole molecule excluding the R chain will have a net charge of zero because of its oppositely charged groups, this is called a **zwitterion**.



because electrically neutral → **Isoelectric zwitterion**

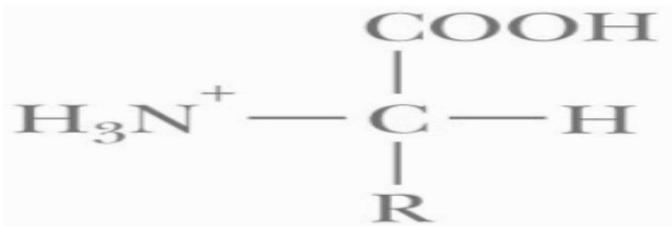


For example, if we take this general amino acid and put it under suitable physiological conditions (PH approximately 7.4 in blood and around 6.9 to 7 in other cells depending on the cell type) we can call it an isoelectric zwitterion

According to the **Henderson Hasselbalch equation** any acid loses its proton after reaching its pKa value. The pKa value of the carboxyl functional group is approximately 2, so at the PH of body cells it loses its proton, and once the pKa

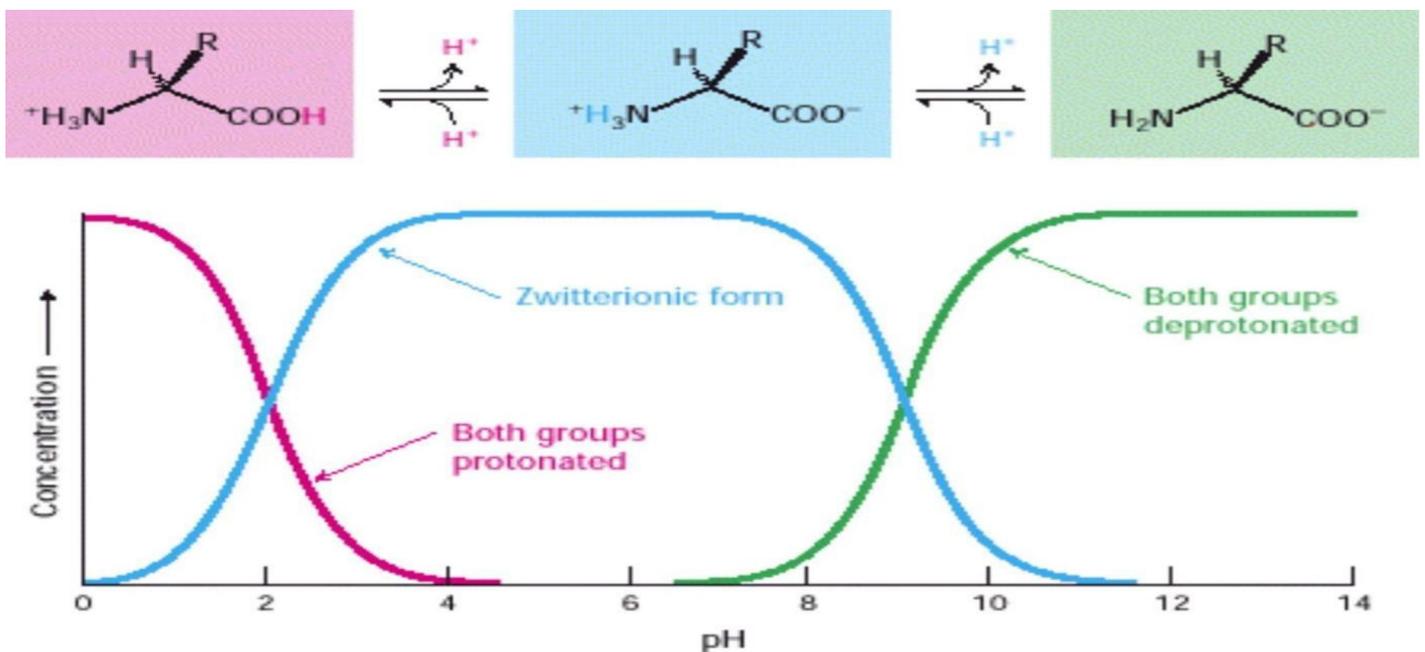
value of NH_3^+ (9.69) is reached, deprotonation also occurs. Resulting in a molecular net charge of (-1).

Now, if we were to put this amino acid outside of our blood or cellular system and have a PH of 1, which molecular format would you expect to form?



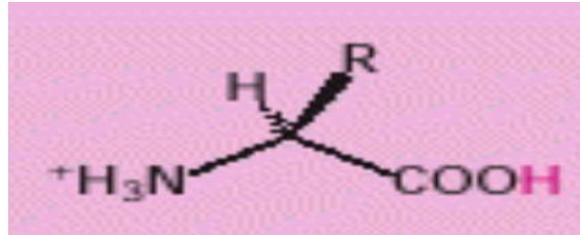
The format above would form inevitably, since the PH (1) is too low for any of the groups to undergo deprotonation, subsequently both groups would maintain their protons and remain protonated with a total net molecular charge of (+1).

Hence, depending on the surrounding environment these amino acids will alternate between their different charge states, but when suitable physiological conditions are met the amino acid is in the zwitterionic form with a total net molecular charge of zero.

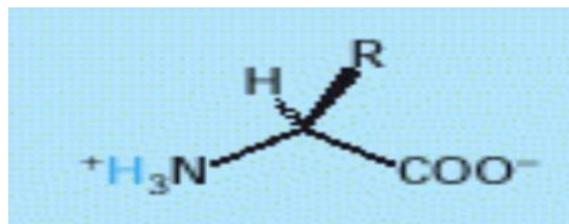


Now this figure shows the changes of concentration of different amino acid forms whenever we alter the PH surrounding them.

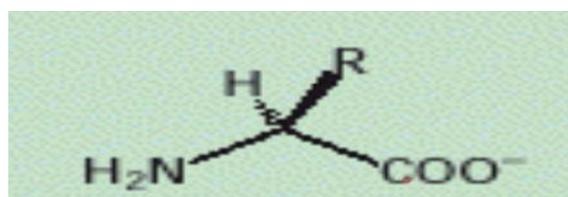
At very low PH levels we have a high concentration of the format where both functional groups are protonated.



With the increase of PH levels, the concentration of the double protonated format would start to decrease gradually on account of the increase of the zwitterionic form, in which both groups are charged due to the loss of the carboxyl's proton (at this moment all the molecules become zwitterions).



Accordingly, the PH levels keep rising until the pKa value is approximately 9, resulting in an increase in the concentration of the double deprotonated format (possesses a net molecular charge of -1), whereas the concentration of zwitterions would decrease.

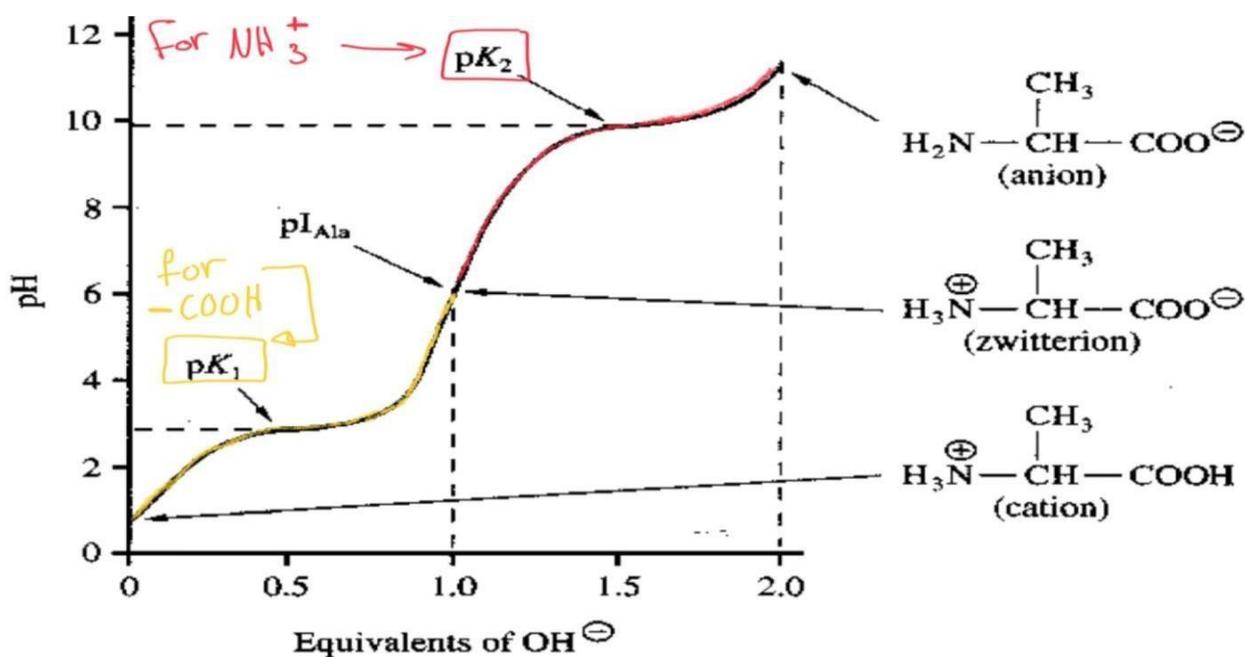


Titration Curve of Alanine

Alanine as an amino acid only has a methyl group (CH₃) as an R group, so this R group doesn't have an ionizable functional group, but as a whole molecule it has a carboxyl and amino group on its backbone (two ionizable groups on the backbone, but not in the R chain).

Notice that we have two titration curves after one another for each functional group; the first curve is for the carboxyl group because it's relatively stronger as an acid than the (NH₃⁺) group as a conjugate acid of NH₂, making the second curve for the amino group.

The pK_a value for a carboxyl group is lower than that for an amino group, which means it's a stronger acid while an amino group (the conjugate acid of NH₂) is the weaker acid because it has the higher PH value.



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

Starting at a very low PH we begin to add the equivalents of OH⁻ where both groups are protonated, maintaining a net positive charge thus preventing the formation of a zwitterion as we move along. In titration as we add the basic OH⁻

we are losing acidity, thereby increasing the PH, resulting in the deprotonation of the molecules; this is immediate in the carboxyl group as it loses its proton regularly and is soon followed by the amino group as it reaches its pKa value.

After the titration of the carboxyl group concludes, we end up in zwitterionic form, thus triggering the start of the second curve, due to the need of titrating both groups of the molecule here, the doubling of the quantity of basic OH⁻ (equivalents) is essential. After reaching the pKa value of the amino group gradually (approx. 9) it begins to lose its proton, forming a negative charge on the molecule (anion).

The PH at which the zwitterion forms is the PH at the end of the first titration, the midpoint of the whole titration process when we have one equivalent out of two used, this point is called the pi or isoelectric point.

We can calculate pi when we have two pKa values by taking the numerical average of these two values.

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

Ionization of Side Chains

Nine of the 20 amino acids have ionizable side chains (functional groups on their R chains):

1. **Tyrosine, serine, and threonine** have OH groups on their side chains and thus can donate protons.
2. **Cystine** contains a thiol group and can donate a proton as well.
3. **Arginine, lysine, and histidine** are three amino acids with basic side chains hence they can accept protons.
4. **Aspartic and glutamic acids** can donate protons due to the presence of carboxyl groups in their side chains.

These amino acids have additional pKa values for their side chains other than the pKa values for the (-COOH) and (-NH₃⁺) in their backbones.

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

Now this is the third pKa value; for side chains, the only amino acid that is really close to the physiological PH is Histidine and that's because; it gives the proteins the property or function to act like buffer systems inside the cells as well as extracellular fluids, and can alternate between the protonation and deprotonation statues (difficult for other amino acids).

For example, cysteine, even though it has an ionizable side chain can oxidize in an oxidizing environment and form a disulfide bridge with another cysteine, making it part of a reaction, thus the proton is unavailable for donation because it's part of the disulfide bridge.

If we were to consider that the pKa of -NH₂ = 9, and -COOH =2 for all amino acids. We could compare them with the pKa value of the side chain, for example aspartic and glutamic acids have (-COOH) groups in their side chains, with pKa values that are approximately 4, whereas the pKa values of (-COOH) in their backbones are approximately less by 2. (حسب الي افترضناها قبل شوي)

This means (-COOH) in the backbone is relatively a stronger acid.

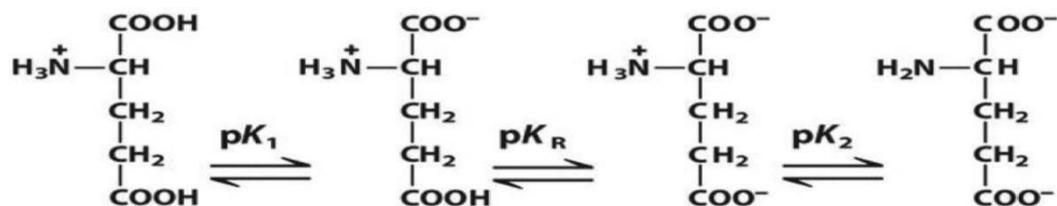
If we were to compare the amino groups for Lysine and Arginine, the amino groups in their side chains would have very high pKa values, whereas the amino group of the backbone would have a pKa of approximately 9, hence making it a stronger acid relative to the amino groups in the side chains.

Calculation of pI of amino acids with ionizable R groups (third pKa

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 ... so 1) you need to look where the zwitterionic form of the amino acid is found 2) take the average of the pKa's around that molecule (the pKa after and the pKa before).

In this case, the total charge on the groups with similar charge must equal one (+1) so that it can be balanced by the one (-1) opposite charge present on the molecule.

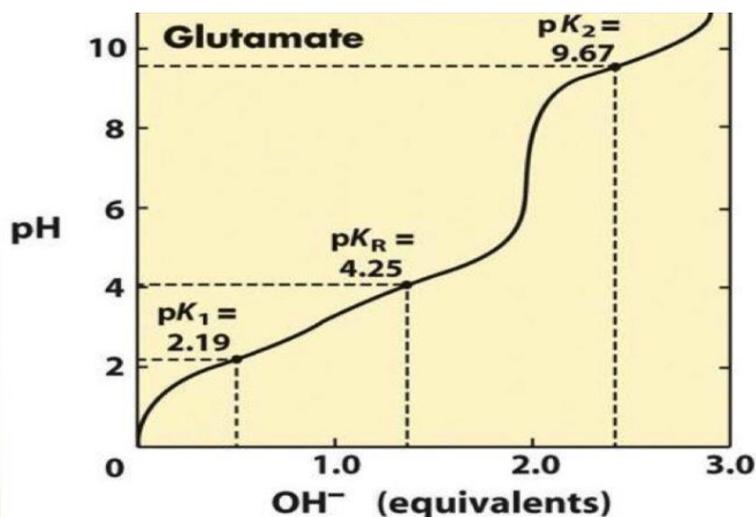
let us take an example (Glutamate):



At a very low PH we all of the groups are protonated, once PH is increased by adding equivalents (bases), ionizable groups will start to donate their protons, the first group to donate its proton is the carboxyl group of the backbone because it's stronger as an acid than the carboxyl group of the side chain, thus it becomes negatively charged, whereas the amino group is positively charged already because it maintains its proton, hence in the second molecule the net charge is zero, giving us a zwitterionic form of glutamate.

Increasing PH by adding more base would allow the deprotonation of the second acid (the side chain carboxyl group) because it is stronger as an acid than the amino group, the net charge of the resulting molecule is (-1).

With continuation of adding the base and increasing PH the last proton is lost from the amino group, giving the last molecule a net charge of (-2).

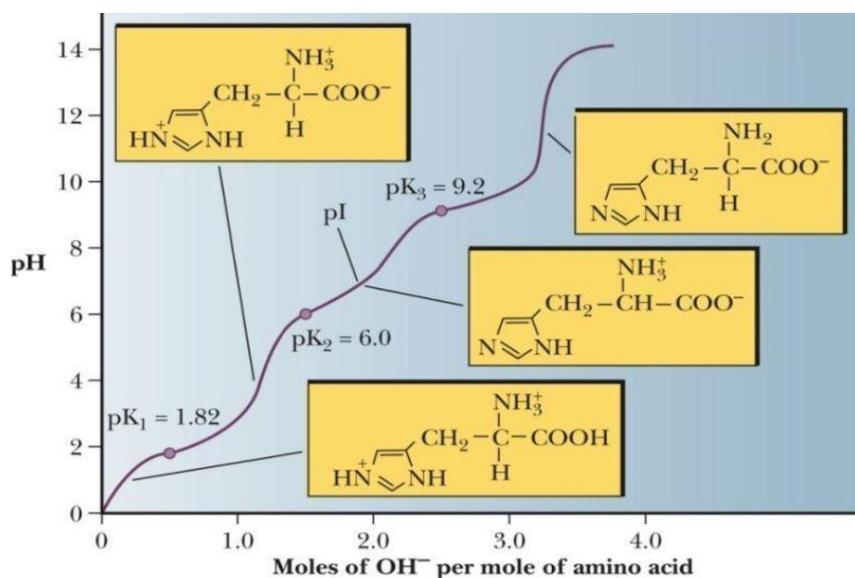


Now to calculate the isoelectric point of Glutamate, the pKa's of the two carboxyl groups are averaged: we take pKa1's value and pKaR's value and find their average (pKa2 has nothing to do with the isoelectric point).

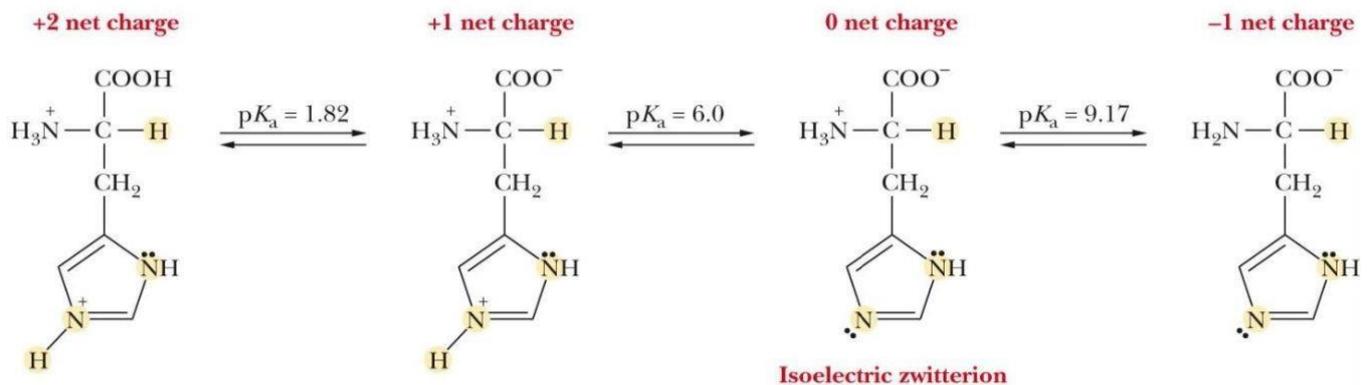
Zwitterionic form يعني الأرقام التي بالزبط قبل وبعد ال

As you can see the titration curve is done in three stages because we have three ionizable functional groups.

Now let's have another example the Histidine with the imidazole group



The pKa value of this group is approximately 6, whereas the pKa value of the carboxyl group is approximately 2 and the amino group is approximately 9 as we mentioned before, so again the curve is going to be done in three stages (stage one for the carboxyl, stage two for the amino group of the side chain, stage three for the amino group of the backbone).



At a very low PH molecules with ionizable groups are protonated with a net charge of +2, when the PH is increased by adding more base; the carboxyl group donates its proton, giving the carboxyl group a negative charge (-1), making the net charge of the molecule (+1), the PH continually increases until we reach the pKa of the second group, which is in the side chain, so it loses its proton making the net charge of the molecule zero, again we keep increasing the PH during titration until the last group (the amino group of the backbone) loses its proton giving the molecule a net charge of (-1).

we take pKa values just before and after the zwitterionic form and find their average so $6 + 9.17$ over $2 = I_p$ (isoelectric point).

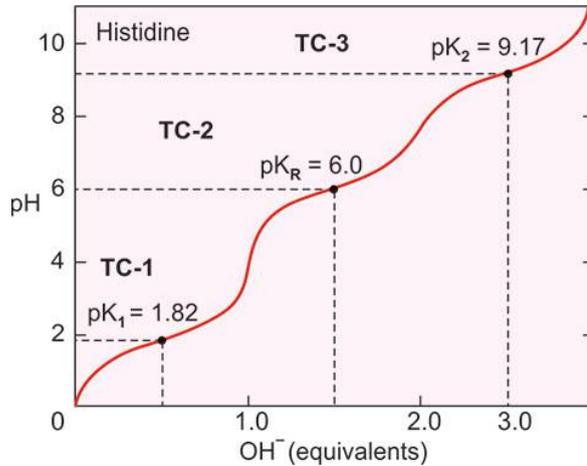
I can determine the I_p on the curve by taking the midpoint between the two curves after the second curve is done and the third curve would start so this midpoint is the I_p where we added two of the equivalence for this titration.

NOTES: هاد حكته الدوك بآخر المحاضرة عن الأشياء الّتي مطلوب منا حفظها:

You need to know the names of the 20 amino acids present in our proteins, know their structure features, identify the structures of them (you need to distinguish them), you don't need to memorize them but know what is special about their side chains, for example serine has an OH in its side chain with no benzene ring, which ones are polar or nonpolar, but in some few examples like to be able to distinguish glutamic acid and aspartic acid u need to know the number of carbons because the difference between them is just one carbon, u need to know their three letter abbreviation, know examples on uncommon amino acids (precursor, function .. but without details), know the pKa values of the groups in order to be able to draw the titration curve, identify the charges on the molecules ..., and distinguish between the side chains whether they are acidic, basic or neutral.

Questions

1. Draw the titration curve of **Histidine** :

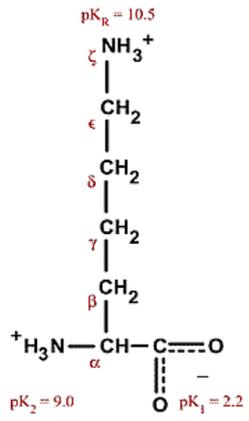


2. What is the ratio of conjugate base/acid of glutamate at PH 4.5 ?

Answer → $\text{PH} = \text{pK}_a + \log \left(\frac{[\text{conjugate base}]}{[\text{acid}]} \right)$
 $4.5 = 4.25 + \log \left(\frac{[\text{conjugate base}]}{[\text{acid}]} \right)$
 $0.25 = \log \left(\frac{[\text{conjugate base}]}{[\text{acid}]} \right)$
 $\left(\frac{[\text{conjugate base}]}{[\text{acid}]} \right) = 1.78$

3. What is the total charge of (Lysine) at PH 7 ?

Answer → +1



When we reach PH=7 we pass PH=2.2 (so we pass the pK_a of the carboxyl group)
 ... carboxyl group will be deprotonated
 ... now we have : -1 from COO⁻ and +2 from the two NH₃⁺
 ... so total charge = +1

