

Amino proton count
on each will be
determined



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Amino acids consist of carboxylic acids (acidic) and amines (basic). Acids contain a hydrogen atom which is a proton with one electron, technically an H ion is just a proton, and hence an acid is a proton donor. As well as, bases are proton acceptors. Moreover, there are two options for amines, they can either be neutral (COOH) or lose a proton (COO⁻). The proton count on each will be determined by the acidity of the solution and that amino acids can only be neutral or zwitterion (overall neutral but have formal charges across the molecule).

<http://study.com/academy/lesson/zwitterion-structure-function.html>

A zwitterion is sometimes called dipolar ions. This is due to having a negative end (anion) and a positive end (cation).

At a neutral pH, amino acids exist in their zwitterion form (dipolar form). This means that the amino acids contain a positive charge and a negative charge.

a) At the low pH of 0/1 the carboxylic group is protonated. At this acidic pH, amino acids are positively charged species. b) As the pH increased, the carboxylic acid group loses its hydrogen to form a zwitterion.

Ion exchange chromatography: c) The zwitterion form usually persists until around a pH of 9. At this basic pH, the low H⁺ causes the amino acids to lose its hydrogen therefore forming a negatively charged species. Do you know how to write the structure of an amino acid? Can you draw and explain the structure of a zwitter ion and how is it affected by acidic and basic pH? Can you explain two techniques which separate amino acids on basis of their

ionisation at different pH? Ion exchange chromatography: This method can be used to purify mixture of proteins based on their net charge. So ion exchange

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chromatography separates charged molecules and polar molecules.

Hydrophobic molecules cannot be separated by this method. There are two different phases known as the stationary phase and the mobile phase.

The interaction between these two phases varies for different molecules. One again ion exchange chromatography separates ions or polar or charged molecules. In this case of ion exchange chromatography, we run a column, meaning it's a structure made with polymers which creates chamber. Inside that chamber we add the stationary phase. An amino acid has different charged properties based on the pH of the solution it is in. pH dictates whether the amine and carboxyl group in an amino acid will be protonated or deprotonated. Overall, charge differences are affected by pH.

This is because increasing pH in general tends to deprotonate functional groups and makes the net charge on an amino acid more negative. However, if pH is decreased then it protonates functional groups and make the net charge more positive. Ion exchange chromatography can separate amino acids on the basis of their net charge. You have column filled with resin beads that are either negatively charged or positively charged. As a result, amino acids will stick to the column with certain timings based on their charges. On the diagram above, charged molecules attached to the beads hold onto the amino acids to be separated.

The ion exchange chromatography can be classified into two types which are the anion exchange chromatography and the cation exchange chromatography. This method is based on the reversible electrostatic interactions of proteins with the separation matrix. Mechanism of protein

separation has two types which are PH based binding and salt based binding. The differences between the net surface charges on the solute molecules, is what the PH based binding dependson. As you can see above, the value which is the PH underneath p_i , the protein will gain itself a net positive charge.

It will also bind reversibly to the width of the surface of a cation exchange resin which is negatively charged group based on the PH. Note that At PH value below the p_i , the protein will have a net positive charge and will tend to bind reversibly to the width of the surface of a cation exchange resin that is one that has negatively charged groups at the PH.

(1) [http://technologyinscience.blogspot.co.uk/2011/09/ion-exchange-chromatography-principle.html#.Wl-qlq5I_IU\(1\)](http://technologyinscience.blogspot.co.uk/2011/09/ion-exchange-chromatography-principle.html#.Wl-qlq5I_IU(1))