Why are Protein structures important ?

- Hormones (Ex: Insulin)
- Enzymes: Catalyzes all the chemical reactions in the body
- Movement (Ex: Actin and Myosin in muscles)
- Structural proteins (Ex: Collagen)
- Binding and transfer of nutrients (Ex: Hemoglobin, Transferrin etc)
- Antibodies of immune system

Proteins are made up of what?

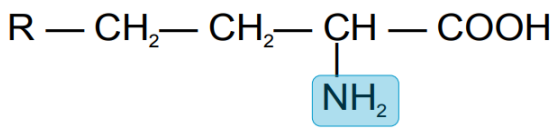
Proteins are the polymers of amino acids.

Proteins are biopolymers also called polypeptides with amino acids as monomeric units

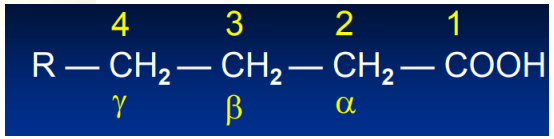
Amino acids

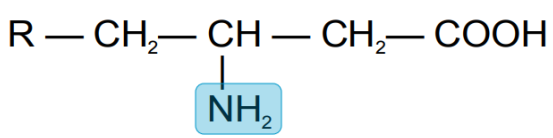
Amino acids are the group of compounds which contains.....

- An amino group ($-NH_2$) and
- Carboxylic group ($-COOH$).



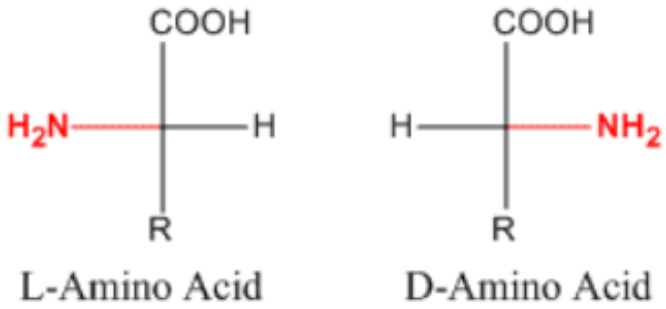
α-Amino acid (2-amino acid)





β-Amino acid (3-amino acid)

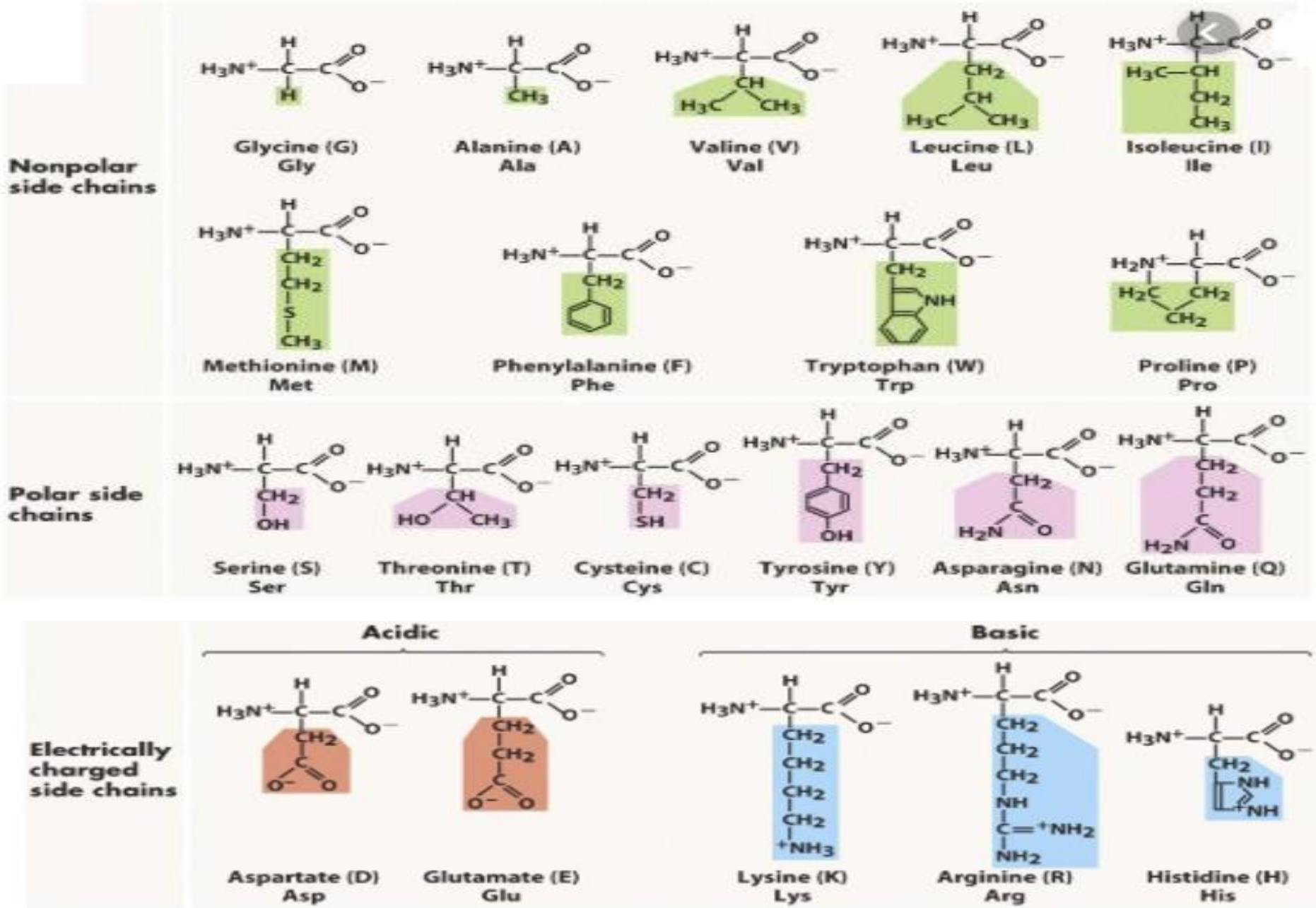
Proteins are made up of only α-Amino acid



Nature selects L-isomer over the D-isomer

Only 20 amino acids are used to synthesize proteins. These are known as standard amino acids

Classification of amino acids based on side chain R



Essential / non essential amino acids

1. Proteins are synthesized from 20 standard amino acids

2. All the standard amino acids are equally important for protein synthesis

3. However, some of these amino acids can be synthesized in our body

4. Some of the standard amino acids cannot be synthesized by human beings

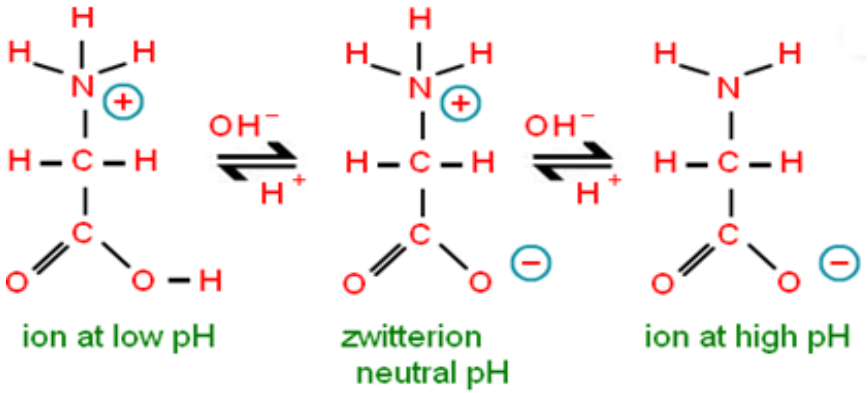
5. If these are not provided in diet, protein synthesis will be impaired

6. These amino acids are known as essential amino acids

7. Valine, leucine, isoleucine, threonine, methionine, lysine, phenylalanine, tryptophan, arginine and histidine are known as ***essential amino acids*** *. Glycine, alanine, serine, cysteine, aspartate, glutamate, asparagine, glutamine, tyrosine and proline are* ***non-essential amino acids****.*

Amino acids & Zwitterions

Amino acids in solution at neutral pH exists predominantly as dipolar ions, called zwitterions. In dipolar form, amino group is protonated and the carboxyl group is deprotonated.



*Amino acids can act as acids as well bases so they are **amphoteric** in nature.*

Isoelectric point

Isoelectric point is the pH at which the amino acid does not migrate in the electric field.

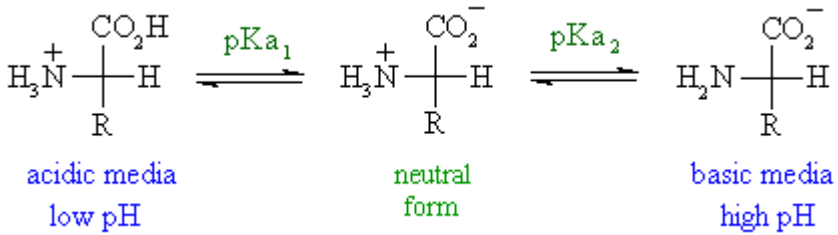
pH at which amino acid is neutral or zwitterionic form is maximum.

Isoelectric point (pI) can be calculated using the formula, pI = (pKa1 + pKa2)/ 2 for amino acids without ionizable side chain (Ex-Glycine). But in case of ionizable side chain, we take the average whose charge predominates.

Calculation of isoelectric point

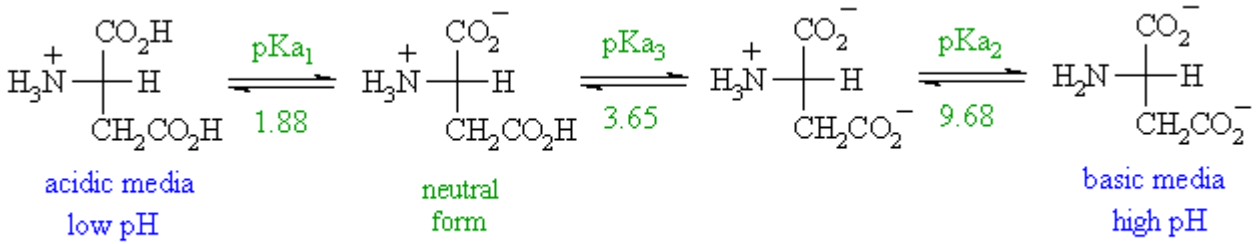
Three cases are possible :

1) For neutral side chains: pI = 1/2 (pKa1 + pKa2)



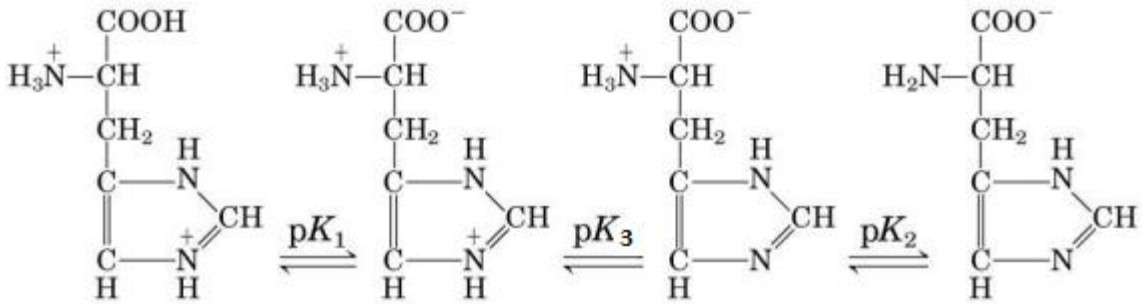
Ex. Glycine, pKa1= 2.34 and **pKa2 = 9.6, pI = 5.97**.

2) For acid side chains: **pI = 1/2 (pKa1 + pKa3).**



Ex. Aspartic acid, pKa1= 1.88, pKa2 = 9.68 and pKa3 =3.65, pI = 2.77

3) For basic side chains: **pI = 1/2 (pKa2 + pKa3)**.



Ex. Histidine, pKa1= 1.82, pKa3= 6.0 and pKa2 =9.17, pI = 7.59

Table of pK_a and pI values for the 20 α-amino acids.

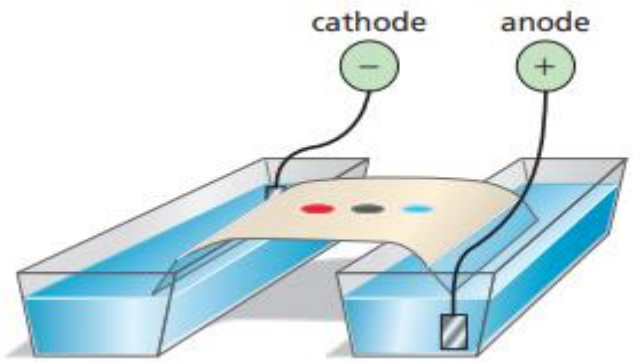
Amino acid	pKa_1	pKa_2	pKa_3	pI
Glycine	2.34	9.60	---	5.97
Alanine	2.34	9.69	---	6.00
Valine	2.32	9.62	---	5.96
Leucine	2.36	9.60	---	5.98
Isoleucine	2.36	9.60	---	6.02
Methionine	2.28	9.21	---	5.74
Proline	1.99	10.60	---	6.30
Phenylalanine	1.83	9.13	---	5.48
Tryptophan	2.83	9.39	---	5.89
Asparagine	2.02	8.80	---	5.41
Glutamine	2.17	9.13	---	5.65
Serine	2.21	9.15	---	5.68
Threonine	2.09	9.10	---	5.60
Tyrosine	2.20	9.11	---	5.66
Cysteine	1.96	8.18	---	5.07
Aspartic acid	1.88	9.60	3.65	2.77
Glutamic acid	2.19	9.67	4.25	3.22
Lysine	2.18	8.95	10.53	9.74
Arginine	2.17	9.04	12.48	10.76
Histidine	1.82	9.17	6.00	7.59

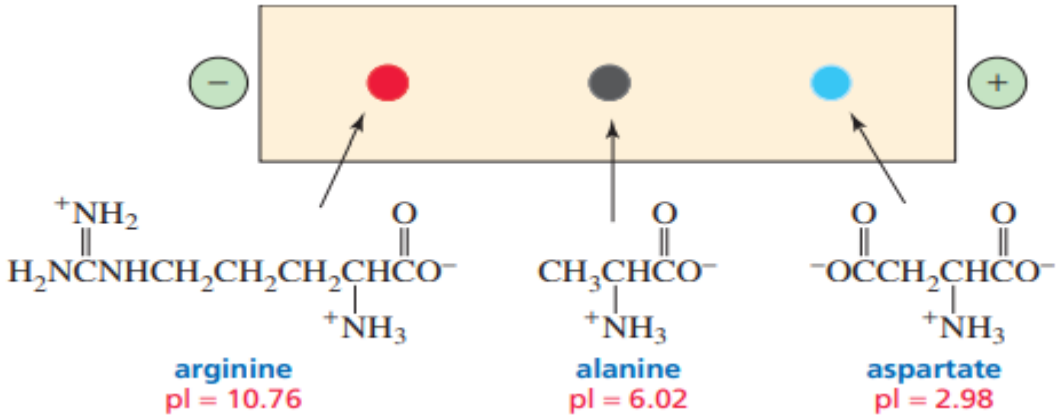
pKa_1= α-carboxyl group, pK_{a2} = α-ammonium ion, and pK_{a3} = side chain group.

Separation of Amino Acids

1. Electrophoresis

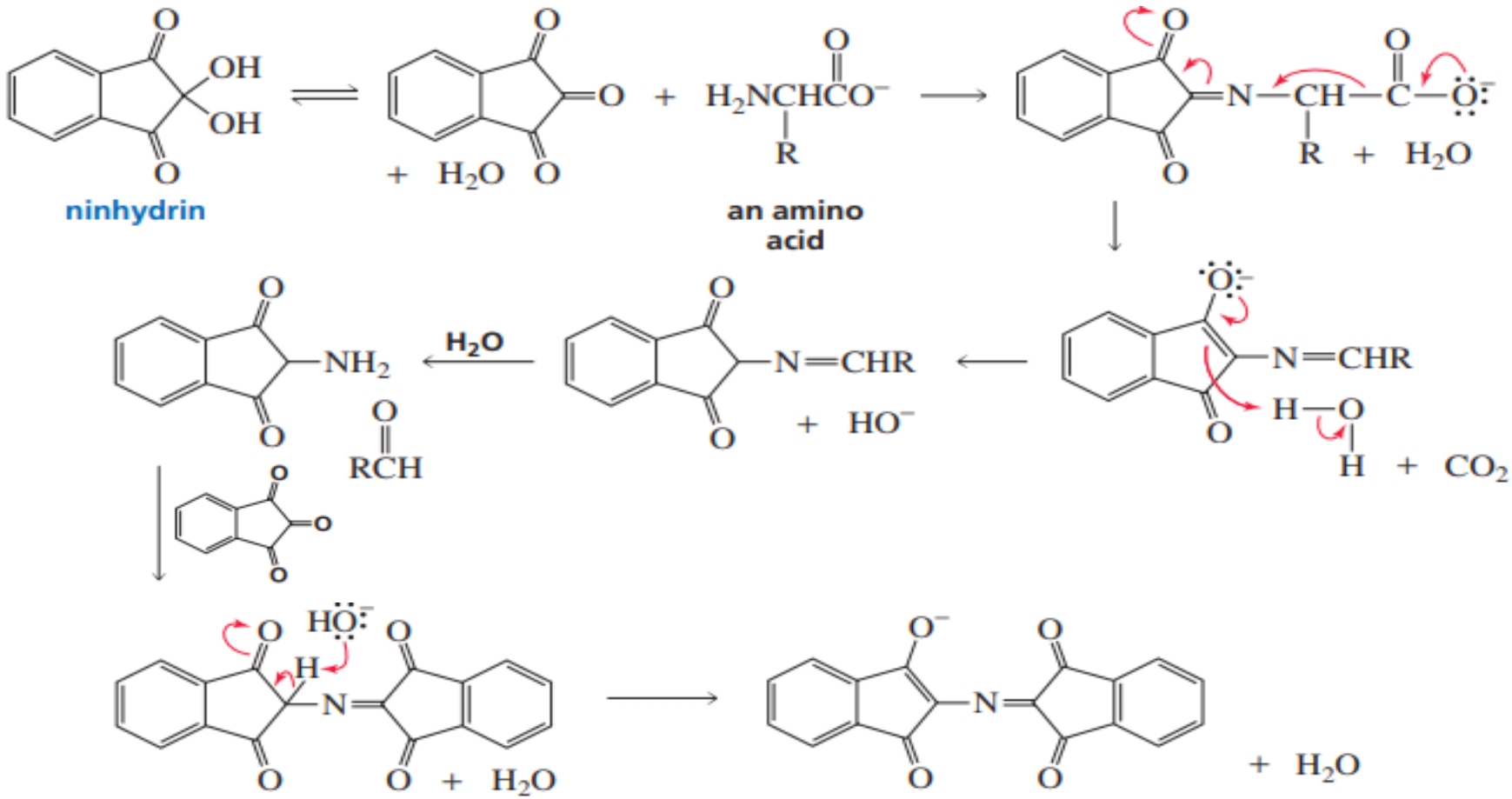
Electrophoresis separates amino acids on the basis of their pI values.





A few drops of a solution of an amino acid mixture are applied to the middle of a piece of filter paper or to a gel. When the paper or the gel is placed in a buffered solution between two electrodes and an electric field is applied, an amino acid with a pI greater than the pH of the solution will have an overall positive charge and will migrate toward the cathode (the negative electrode). The farther the amino acid's pI is from the pH of the buffer, the more positive the amino acid will be and the farther it will migrate toward the cathode in a given amount of time. An amino acid with a pI less than the pH of the buffer will have an overall negative charge and will migrate toward the anode (the positive electrode). If two molecules have the same charge, the larger one will move more slowly during electrophoresis because the same charge has to move a greater mass.

Since amino acids are colorless, how can we detect that they have been separated? When amino acids are heated with ninhydrin, they form a colored product. After electrophoretic separation of the amino acids, the filter paper is sprayed with ***ninhydrin*** *and dried in a warm oven. Most amino acids form a purple product. The number of different kinds of amino acids in the mixture is determined by the number of colored spots on the filter paper.*



mechanism for the reaction of an amino acid with ninhydrin to form a colored product