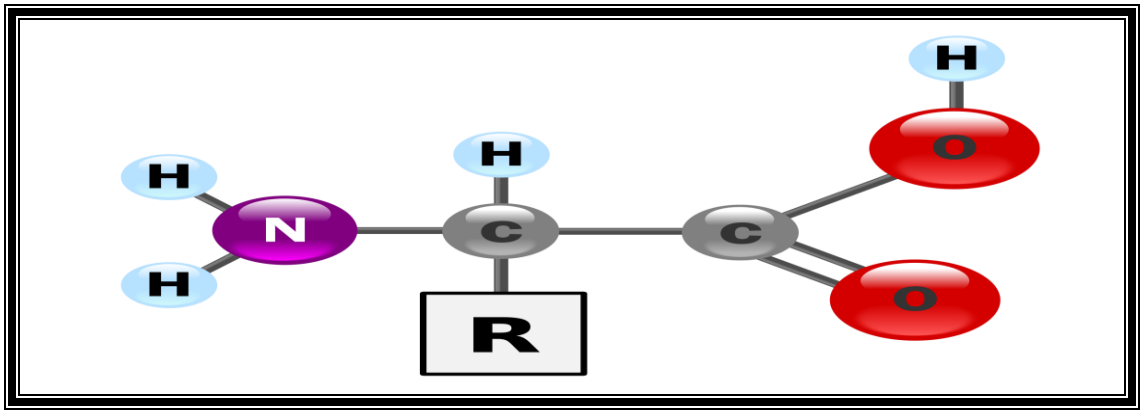
Color Test For Proteins And Amino Acids

Proteins are polymers or macromolecules, the building units or monomers of which are the alpha amino acids. An amino acid contains both a carboxyl group and an amino group, both of which are attached to the alpha carbon atom of the acid.



Amino acid structure

Beta – amino acids and gamma-amino acids also occur in nature but not as components of proteins. With the exception of glycine, all α- amino acids are asymmetric, i.e., four different groups are bonded to the α- carbon atom, so are optically active. Also an α- amino acid can be L-isomers or D- isomer. In natural proteins of higher organisms, only the L-isomer of one or more of approximately 20 amino acids are present.

When an amino group and a carboxyl group of two amino acids combine the bond is called the peptide bond and the constituent amino acids are termed amino acids residues.

A peptide consists of two or more amino acid residues linked by peptide bonds. Peptides of more than ten amino acid residues are termed polypeptides. With the increase in molecular weight the proteins will form. The dividing line between large polypeptides and small proteins is usually taken to be between MW 8000 and 10000.

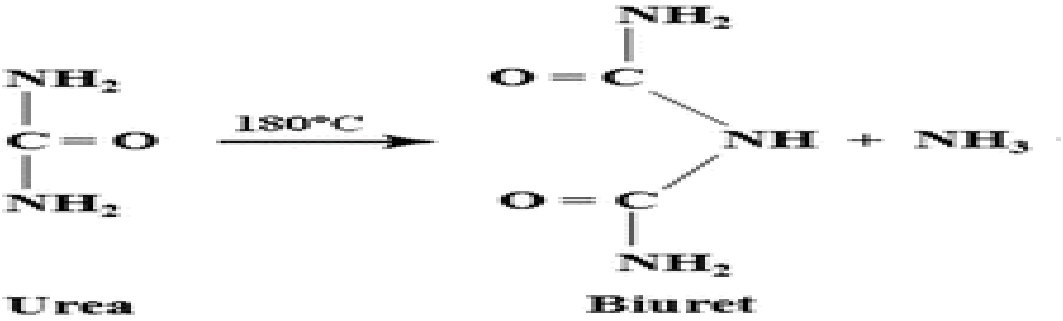
Proteins and amino acids can be analyses qualitatively and quantitatively. Different proteins and amino acids may be separated by chromatography or electrophoresis before individual testing.

Principles of the color test:

1- Biuret test:

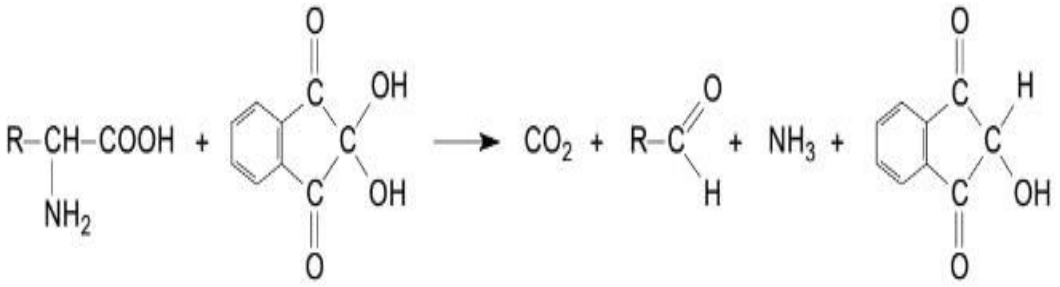
Compounds containing two or more peptide bonds react with cu (II) ions in alkaline solution to form a purple (pink to violet) color. the color is due to coordination complex formation or chelation of the cu (II) ion and the carbonyl oxygen and amid nitrogen atoms of the peptide bond . At least two peptide bonds (tri- peptide) are required for a positive test.

The name of this test comes from the compound biuret, which due to structural similarity to peptide bond also gives a typically positive reaction.

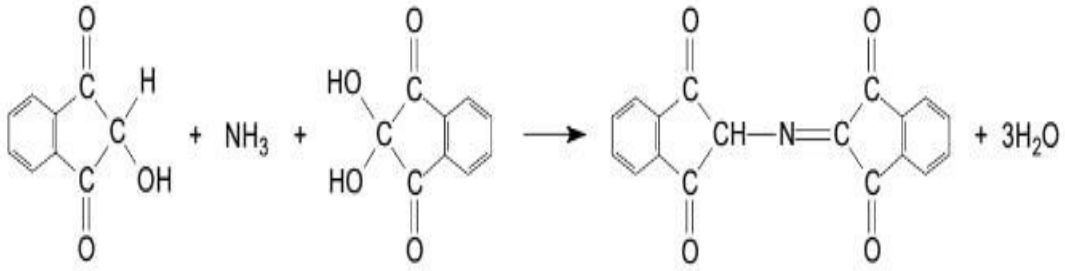


2- Ninhydrin test:

Ninhydrin is a powerful oxidizing agent which causes oxidative decarboxylation of alpha – amino acids producing CO_2, NH_3 and an aldehyde. The liberated ammonia reacts with two equivalents of ninhydrin to produce a blue or a purple colored product.



Ninydrin Reduced ninydrin



Reduced ninhydrin Ninhydrin Violet-blue condensation product

The reaction depends on presence of free amino group so Proline and hydroxyproline which lack a free amino group yield a yellow color with ninhydrin. Peptides and proteins, owing to their free terminal amino groups yield a positive test.

3- **Xanthoproteic test**:

Nitration of the aromatic ring of an amino acid by hot concentrated nitric acid produces a yellow color so tyrosine and tryptophan give a positive test.

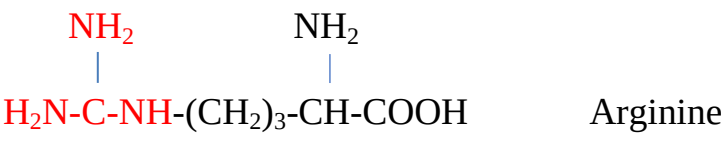
Under the conditions of the test, phenylalanine doesn't produce the color. However, if one adds a small amount of concentrated sulfuric acid together with the nitric acid one will obtain a positive result.

4- **Hopkins-cole test for tryptophane**:

Tryptophan, due to its indole ring, condenses with the aldehyde glyoxylic acid (CHO-COOH) in presence of concentrated H_2SO_4 to produce a purple to blue color.

5- **Sakaguchi test for arginine**:

The only amino acid containing the guanidine group is arginine and this reacts with alpha-naphthol and an oxidizing agent such as bromine to give a red color.



Arginine

Guanidine group

This is a sensitive test for free and comnined arginine.

Procedure:

1- Biuret test:

Samples: albumin, tryptophan.

Mix 1 ml of each sample with (**1 ml**) of 10% NaOH and (**0.5 ml**) of 1% $CuSO_4$. Observe the color produced.

2- Ninhydrin test:

Samples: albumin, tryptophan, Proline.

A- Mix **1 ml** of each sample with **1 ml** of 0.1% aqueous ninhydrin.

B- Heat the tubes in boiling water for 3-4 minutes and observe the color after standing for few minutes.

3- Xanthoproteic test:

Samples: albumin, tryptophan, Proline.

A- Mix **1 ml** of each sample with **1 ml** of concentrated HNO_3 .

B- Heat for 1-2 minutes in boiling water, observe any change in color.

4- Hopkins-cole test:

Samples: albumin, tryptophan,arginine.

A- Mix **1 ml** of each sample with **1 ml** of Hopkins-Cole reagent.

Mix thoroughly.

B- Carefully add **1 ml** of concentrated H_2SO_4 along the side of the tube so that the two liquids from separate layers.

C- Notice the ring at the junction.

5- Sakaguchi test:

Samples: Arginine, albumin.

A- Mix **2ml** of each sample with **0.5ml** of 10% NaOH and **0.5 ml** of α-naphthol solution.

B- After 3 minutes add 2-4 drops of NaOBr and observe the color change.

Materials and reagents:

1- 1% solution of alanine, arginine, tryptophan, albumin, gelatin.

2- Reagents for sakaguchi test :

10% NaOH , 0.02 % α- naphthol

Sodium hypobromite(2 grams Br_2 in **100 ml** of 5% NaOH).

3- Reagent for Hopkins-Cole test :

10 gm. of powdered magnesium are covered with shaking by some distilled water. **250 ml** of saturated oxalic acid are adding slowly with cooling under tap water. Filtrate to remove the insoluble magnesium oxalate. Acidify the filtrate with acetic acid to prevent partial precipitation of the magnesium on long standing and make up to a liter with distilled water. This solution contains only the magnesium salt of glyoxylic acid.