

Qualitative analysis of proteins

1-Ninhydrine test : general test for proteins .

- ninhydrine consists of 585 amino acids that bonded together by peptide bond .- this test is common in all amino acids because its acts in the basis of protein not amino acids .

- ninhydrine is the strong oxidizing agent that reacts with amino acids of the protein and produces purple complex .

Procedure :

1-using two test tubes , the first one we adding albumin and the second one adding fructose .

2-we add 5 drops of ninhydrine for both test tubes.

3-put them into the water bath for 5 mins

4- determine wether the purple complex is formed or not .



Biuret Test: this test is applied to indicate peptide bond within protein .

Amino acids are building blocks of the proteins that are linked together by peptide bonds .

-Reagents in this test are CuSO_4 and NaOH .

- CuSO_4 (Chelating agent) is the source of Cu^{++}

- NaOH is to raise the Ph of the medium and reach the solution to alkaline level . the aim of this alkaline solution is for the reaction to occurs.

-when peptide bonds are present in the alkaline solution , Cu^{++} ions will form coordination complex with four nitrogen atoms from peptide bond.

-As the number of peptide bond increase , the more intense the change .

-This test gives positive result when 2 or more than 2 peptide bond available.
If you do this test for a single amino acid , it will give you negative result .

Procedure :

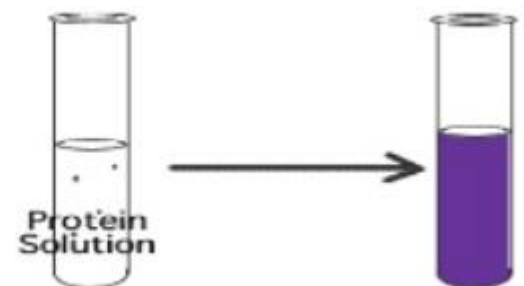
Add 30 drops oof albumin into test tube .

Then add 5 drops of CuSO_4

And then 10 drops of NaOH .



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- **Xanthoproteic test** : Test for aromatic amino acid .

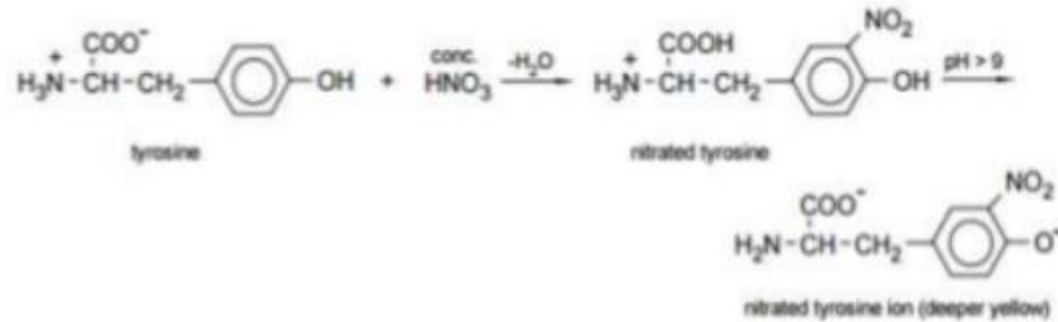
Aromatic amino acids are L-tyrosin , Tryptophan and Phenyl Alanine .

-Reagents used are HNO₃ and NaOH .

-HNO₃ is used to for nitration to react with the ring .

-If the reaction occurred the xanthoproteic acid or nitrogen derivate is formed which it has yellow color.

-Then NaOH is used to produce a salt (Orange color salt).

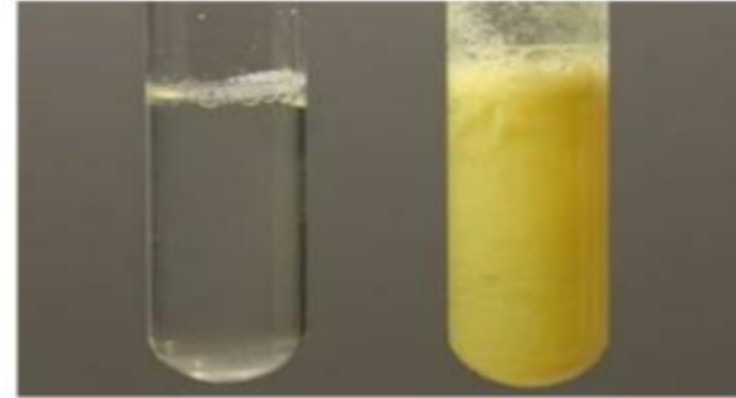


Procedure:

Add 30 drops of albumin into test tube .

Add 5 drops of nitric acid then heat and shake (in order for the solution not to coagulate.)

Finally add 20 drops of NaOH.



Millons test

Objective:

- to detect amino acid containing phenol group (hydroxyl group attached to benzene ring) ie. Tyrosine

Principle of Millon's test:

Compounds containing hydroxybenzene radical react with Millon's reagent to form red complexes. The only amino acid having hydroxybenzene ring is tyrosine. Thus, this test is specific for the amino acid tyrosine and the protein containing this amino acid. Tyrosine when reacted with acidified mercuric sulphate solution gives yellow precipitate of mercury-amino acid complex. On addition of sodium nitrate solution and heating, the yellow complex of mercury-amino acid complex converts to mercury phenolate which is in red color.

Result interpretation:

- **Positive Millon's test:** Brick red color (Tyrosine and phenol solution)
- **Negative Millon's test:** no red color (arginine)

Reagents:

- test solution: 1 % arginine, 1 % tyrosine, phenol solution
- Millon's reagent (Acidified mercuric sulphate)
- 1 % sodium nitrite

Procedure of Millon's test:

1. Take 1ml test solution in dry test tube.
2. Similarly, take 1ml distilled water in another test tube as control.
3. Add 1ml of Millon's reagent and mix well.
4. Boil gently for 1 minute.
5. Cool under tap water.
6. Now add 5 drops of 1 % sodium nitrite.
7. Heat the solution slightly.
8. Look for the development of brick red precipitate.