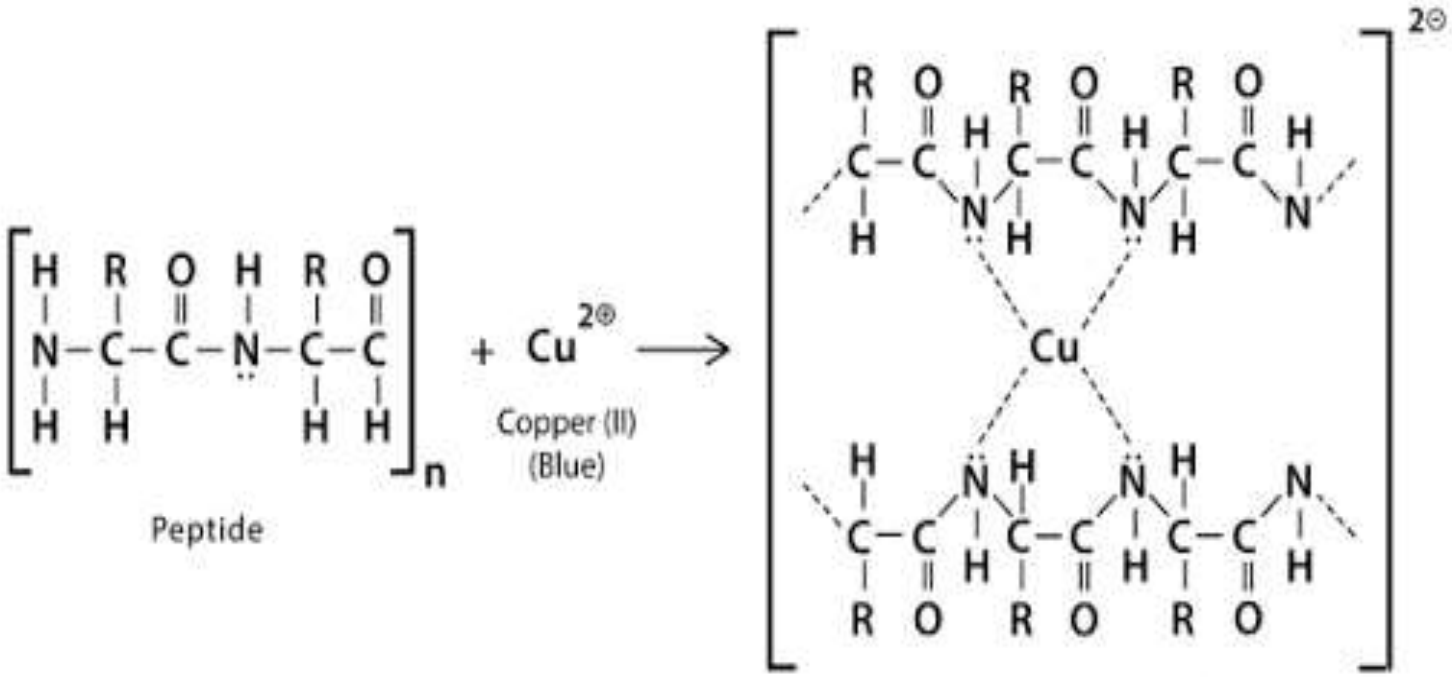
Estimation of Proteins & Amino acids

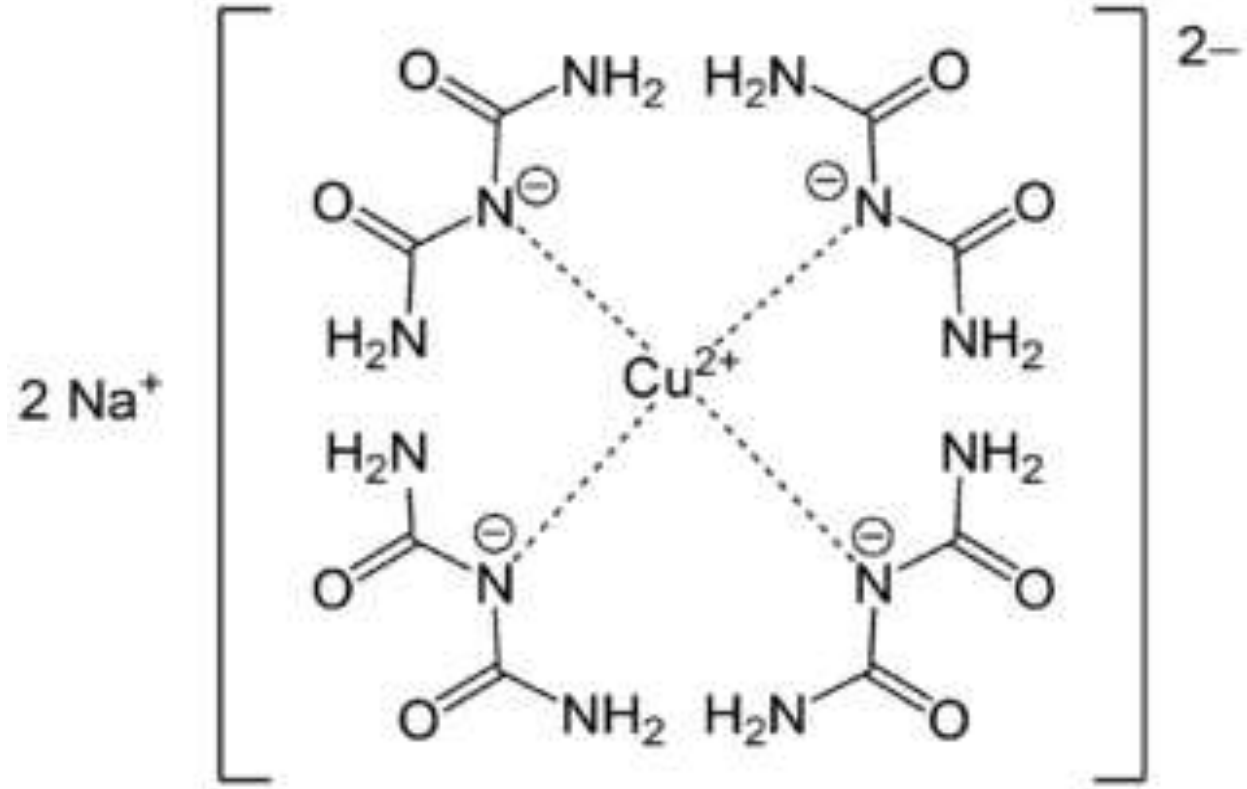
Biuret & Ninhydrin Tests

Biuret Test

- In strong alkaline solution, a protein, having atleast two peptide bonds, develops a purple or violet or pinkish-violet colour with a dilute solution of copper sulphate
- This is due to the coordination of Cu^{2+} ion with unshared electron pairs of peptide nitrogen forming a coloured coordination complex
- When Biuret reagent (2ml) is added to a protein solution (1ml), a purple or light violet colour develops which is due to the formation of a complex between –CO-NH- group of protein and copper(II) ion
- However, amino acids do not react with Biuret reagent as they lack peptide bonds

Biuret Test



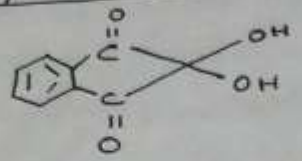

2 Na+
O
NH2
H2N
O
O
N
N
O
H2N
NH2
Cu2+
H2N
NH2
O
N
N
O
O
NH2
H2N
O
2–

Ninhydrin Test

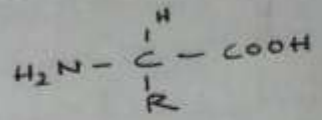
- Ninhydrin reagent gives positive test with both amino acids and proteins by forming a complex called Rheumann's purple
- Formation of Rheumann's purple is due to the condensation of two molecules of ninhydrin with one molecule of ammonia from amino acid. The alpha amino group is the reactive group
- When ninhydrin reagent (1ml) is added to protein or amino acid solution (2ml) and boiled in water bath for 2 minutes, a violet/purple colour develops due to the formation of Rheumann's purple complex

Ninhydrin Reaction

A.

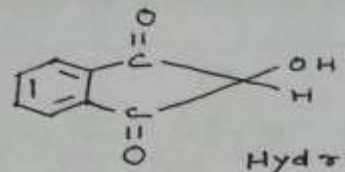


+



Ninhydrin

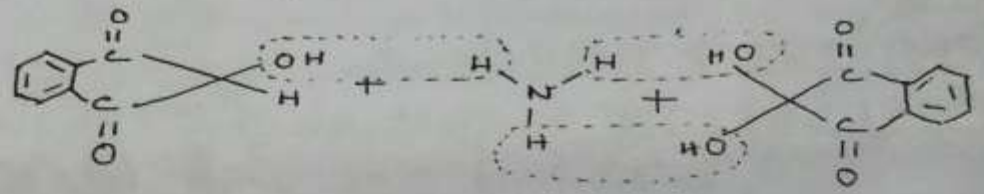
Amino acid



+ $R-CH(H)=O + NH_3\uparrow + CO_2\uparrow$

Hydrindantin

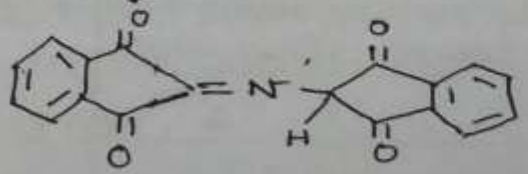
B.



Hydrindantin

Ammonia

Ninhydrin



Purple complex

Qualitative Estimation of Unknown samples containing Proteins & Amino acids

- At first, physical characteristics namely, nature, texture, colour, odour of both the samples and their solubility in water are noted.

- Then both the samples are allowed to react with Biuret & Ninhydrin reagents

Sample 1

Biuret Test

↓

Negative result;

Light blue colour of Biuret

Reagent remains unchanged

↓

May be the sample is an amino Acid

Ninhydrin Test

↓

Positive result;

Purple colour develops

↓

It may be either a protein or an amino acid

Sample 2

Biuret Test

↓

Positive result;

Purple colour develops

↓

May be the sample is a protein

Ninhydrin Test

↓

Positive result;

Violet colour develops

↓

It may be either a protein or an amino acid

Conclusion

- Sample 1: It gives positive result in Ninhydrin test. Therefore, the sample is either a protein or an amino acid because, both protein and amino acid form Rheumann's purple complex due to the condensation of Ninhydrin and ammonia

  However, it gives negative result in Biuret test. Amino acids cannot react with Biuret reagent due to lack of peptide linkage

  Therefore, Sample 1 is an amino acid

- Sample 2: It gives positive result with both Ninhydrin and Biuret reagents. Positive result in Ninhydrin test indicates that the sample may be a protein or an amino acid

  Proteins in alkaline solution give purple colour with dilute copper sulphate (II) solution due to the formation of a purple complex between –CO-NH- group of protein and copper ion.

  Therefore, sample 2 is a protein