

AL-RASHEED PHARMACY

Dpt.
2021

3rd year 1st semester

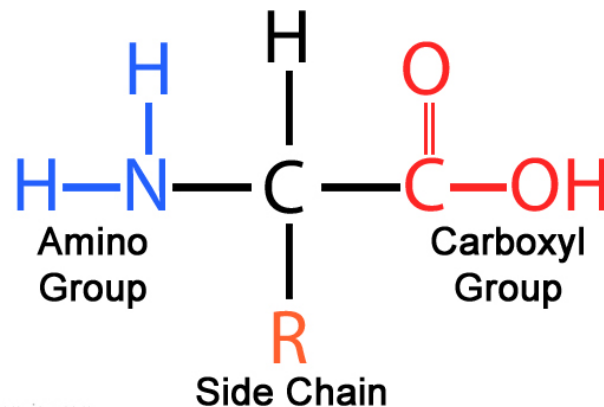
Biochemistry

Lab 5
Protein

Introduction

Proteins are polymers of amino acids. Each amino acid contains a central carbon, a hydrogen, a carboxyl group, an amino group, and a variable R group.

Amino Acid Structure



Introduction

Proteins have a variety of function in cells. Major functions include acting as enzymes, receptors, transport molecules, regulatory proteins for gene expression, and so on.

Color reaction of protein and
amino acid

BIURET TEST

Definition

Biuret test is a chemical test used to determine the presence of peptide bond in a substance. A peptide bond is formed when two amino acids are connected through the amino and carbonyl groups. Protein composes of many amino acids connected to each other through peptide bonds.

Objectives of Biuret Test

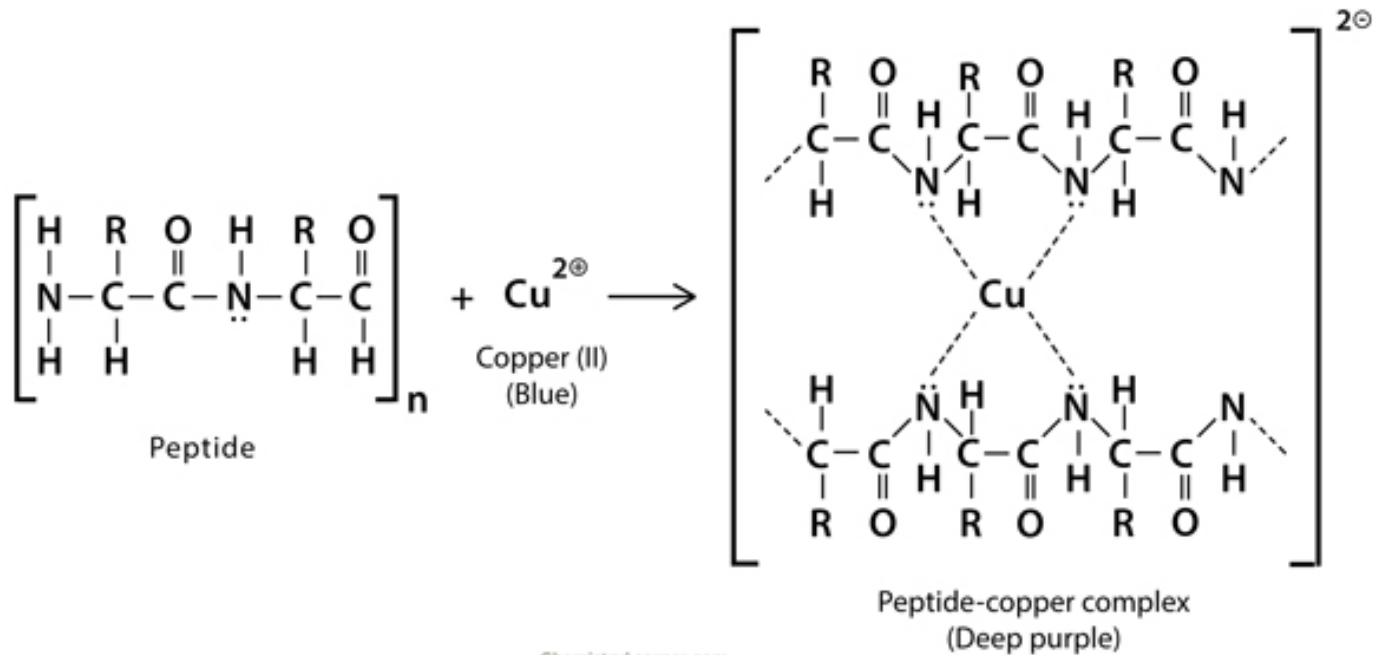
Biuret test is used to determine the presence of protein in a sample.

Principle

Biuret test is based on the reaction of the cupric ions Cu^{2+} with peptide bonds in an alkaline solution. These ions react with the nitrogen of the peptide bond to form a purple or violet colored complex. In alkaline medium, cupric hydroxide is generated from copper sulfate of biuret reagent that helps in chelating the peptide bond with cupric ions to give violet or purple color.

principle

Biuret Test



Preparation of Biuret Reagent

The Biuret reagent is a solution that is made of sodium hydroxide (NaOH) , hydrated copper (II) sulfate, and potassium sodium tartrate. The latter is added to chelate and thus stabilize the cupric ions.

Procedure

- Take 2 clean and dry test tubes.
- Add 1-2 ml of the test solution, egg albumin and deionized water in the respective test tubes.
- Add 1-2 ml of Biuret reagent to all the test tubes.
- Shake well and allow the mixtures to stand for 5 minutes.
- Observe for any color change.

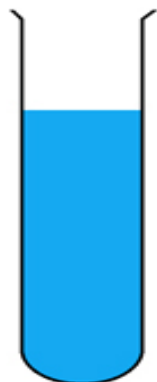
Observation

The solution will turn violet or purple.

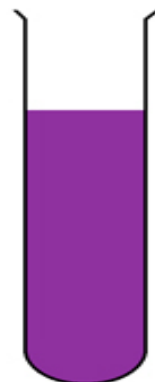
Results

The violet color indicates the presence of protein(peptide linkage) in the solution.

Biuret Test Result



Blue => Negative
(Proteins are absent)



Deep purple => Positive
(Proteins are present)



Ninhydrin test

Ninhydrin test

Definition

- Ninhydrin test is a chemical test performed to detect the presence of α -Amino acid in sample except proline.

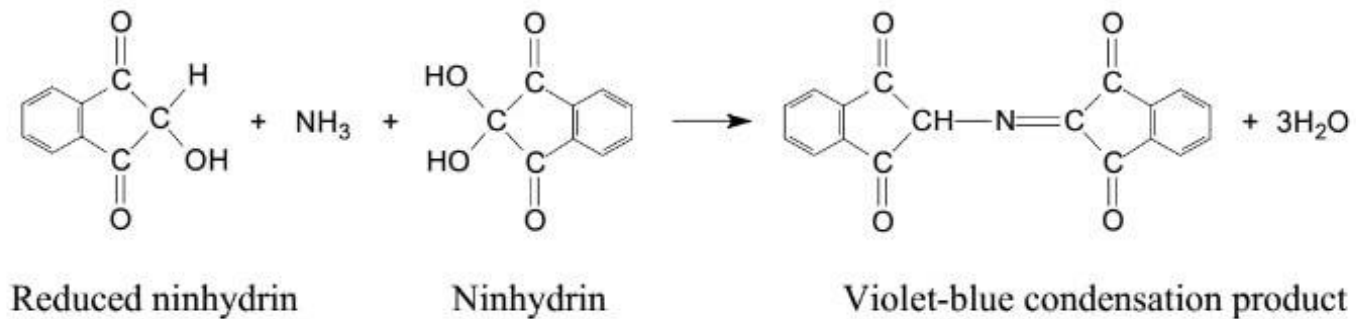
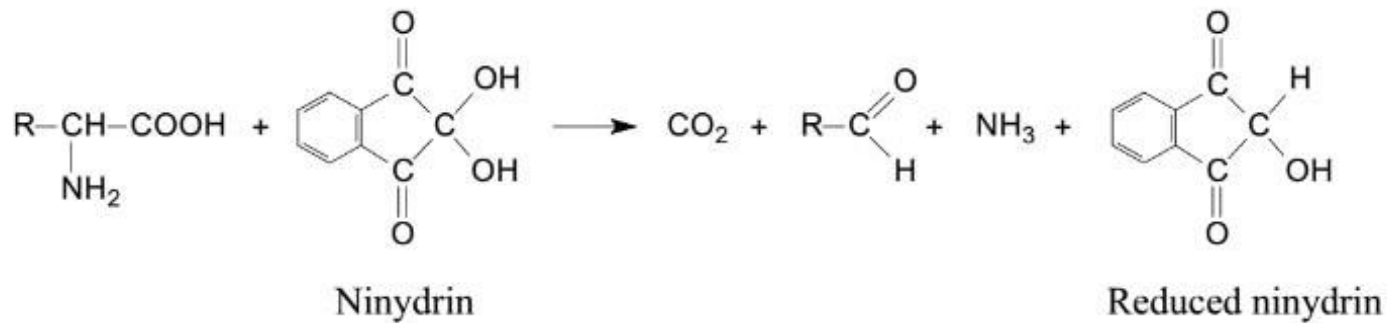
Objectives of Ninhydrin Test

- To detect the presence of amino groups in the test solution.
- To distinguish carbohydrates from amino acids.

Principle

Ninhydrin is a powerful oxidizing agent that causes oxidative decarboxylation of the alpha amino acids forming an aldehyde, ammonia, and Hydrindantin (reduced form of Ninhydrin). This Hydrindantin reacts with ammonia and another Ninhydrin molecule to form a bluish-purple colored complex.

Principle



Procedure of Ninhydrin Test

- Take 1 ml of standard protein solution in one test tube and 1 ml of the test sample in another dry test tube.
- Add a few drops of ninhydrin reagent to both the test tubes.
- Place the test tubes in the water bath for 5 minutes and then allow cooling to room temperature.
- Observe the formation of color and note down the result.

Observation

Bluish-purple color will be formed in the solution.

Results

The formation of bluish-purple color indicates the presence of free alpha amino acids in the solution.

Ninhydrin Test- Definition, Principle, Procedure, Result, Uses



**Negative
Ninhydrin Test**

**Amino acid
Absent**



**Positive
Ninhydrin Test**

Amino acid Present

**Purple-colored
complex present**



Proline present

END

THANK YOU FOR
LISTINING

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