



Practical Biochemistry

Lecture 5 :

Qualitative Tests of Amino acids and Proteins

Prepared by :

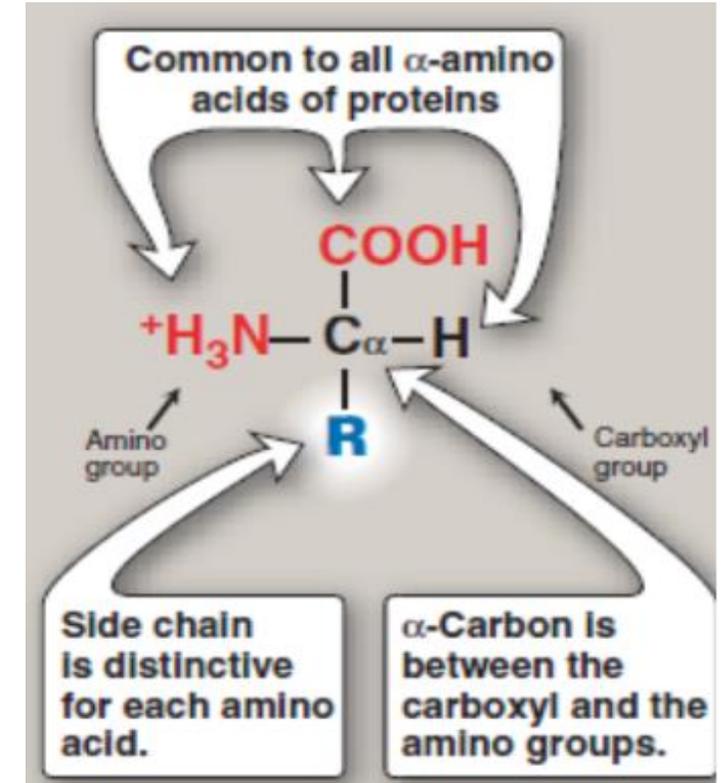
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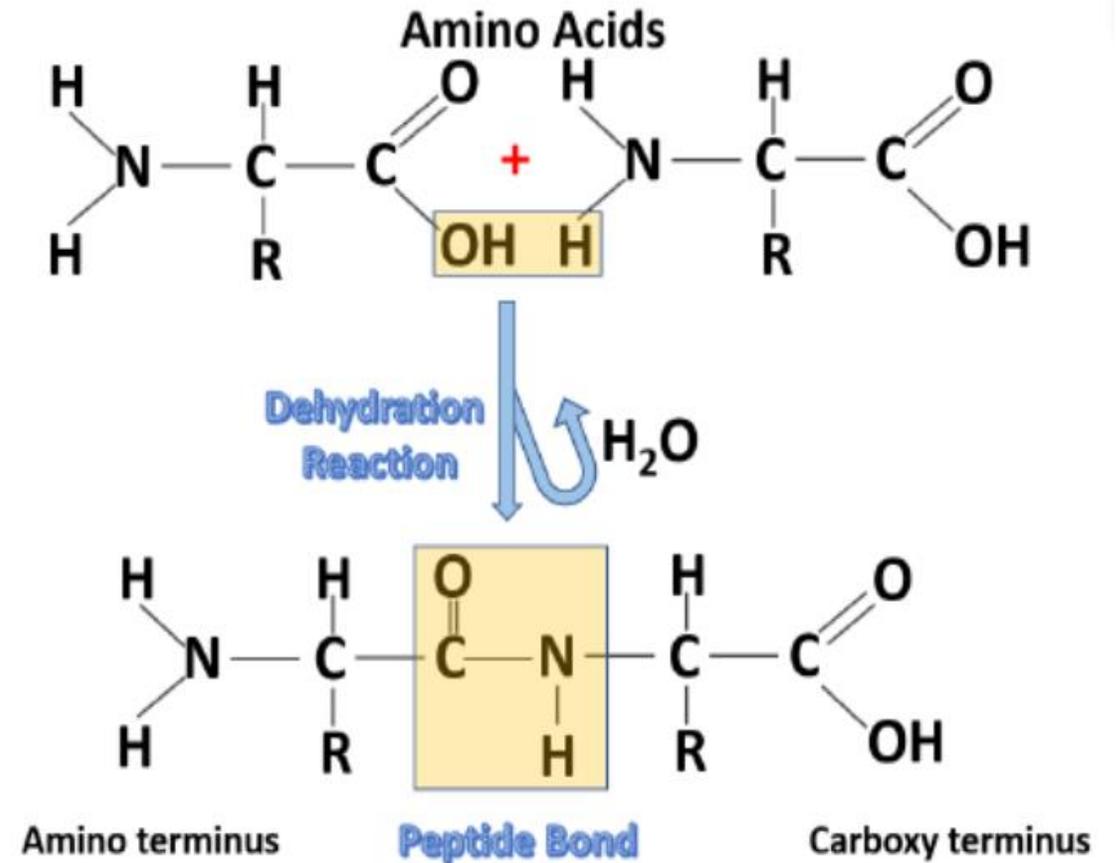
Introduction

- Amino acids are the building blocks for proteins.
- Although more than 300 different amino acids have been described in nature, only 20 are commonly found as constituents of mammalian proteins.
- All amino acids found in proteins have this basic structure, differing only in the structure of the R-group or the side chain.



Proteins

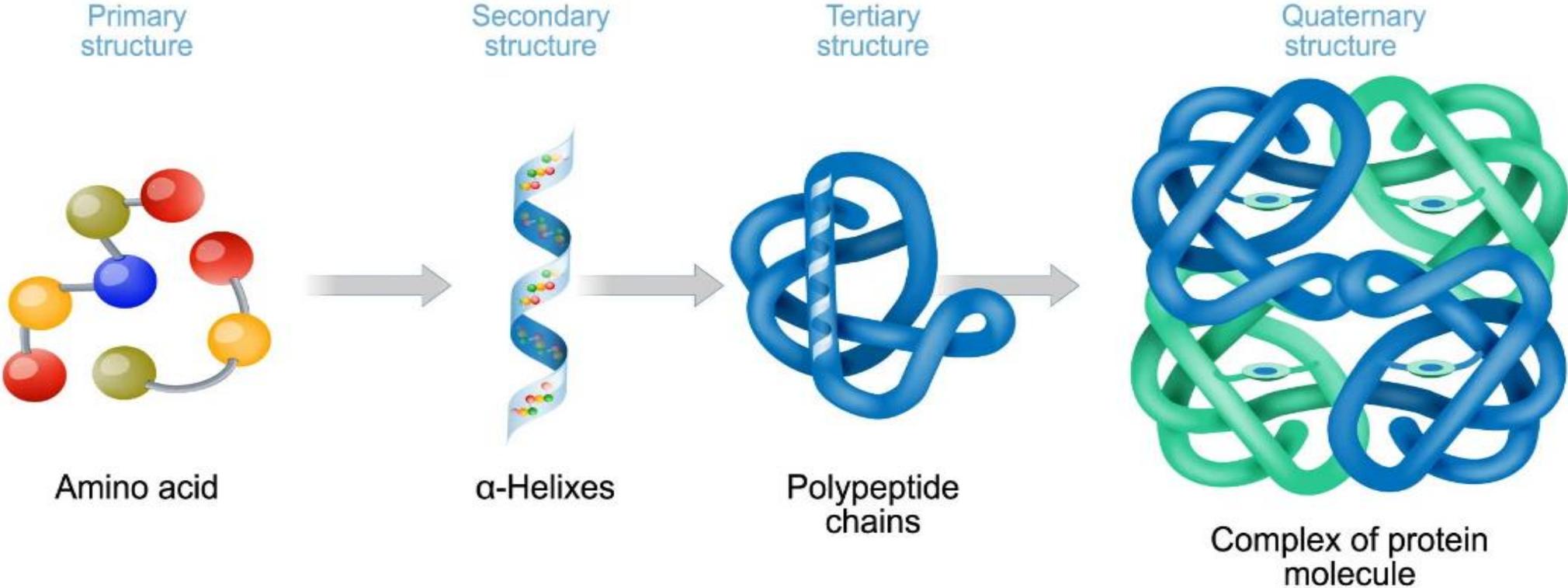
- **Proteins** are organic compounds.
- They are made of **amino acids** arranged in a linear chain.
- The amino acids in a polypeptide are joined together by the **peptide bonds**



Levels of Protein Structure:

1. **Primary Structure:** The sequence of amino acids in a protein is called the primary structure of the protein. Many genetic diseases result in proteins with abnormal amino acid sequences, which cause improper folding and loss or impairment of normal function.
2. **Secondary Structure:** Which produced from hydrogen bonding between the NH and CO In the main chains of Aminoacids.
3. **Tertiary Structure:** Which produced from four Kinds of interactions between the in the side chains of Aminoacids.
4. **Quaternary Structure:** Which is produced from nonpolar interactions between two protein subunits.

Levels of Protein Structure:



General Qualitative Reactions of Proteins

In regular analytical laboratory procedures, examinations of amino acids and proteins can be categorized into two main areas of focus.

A. Precipitation Reactions of Proteins

B. Color Reactions of Proteins

Precipitation of Proteins

The stability of a protein in a solution relies on its electric charge and how well it interacts with water molecules.

If something counteracts the charge or reduces the presence of water, it leads to the protein clumping together and settling out of the solution

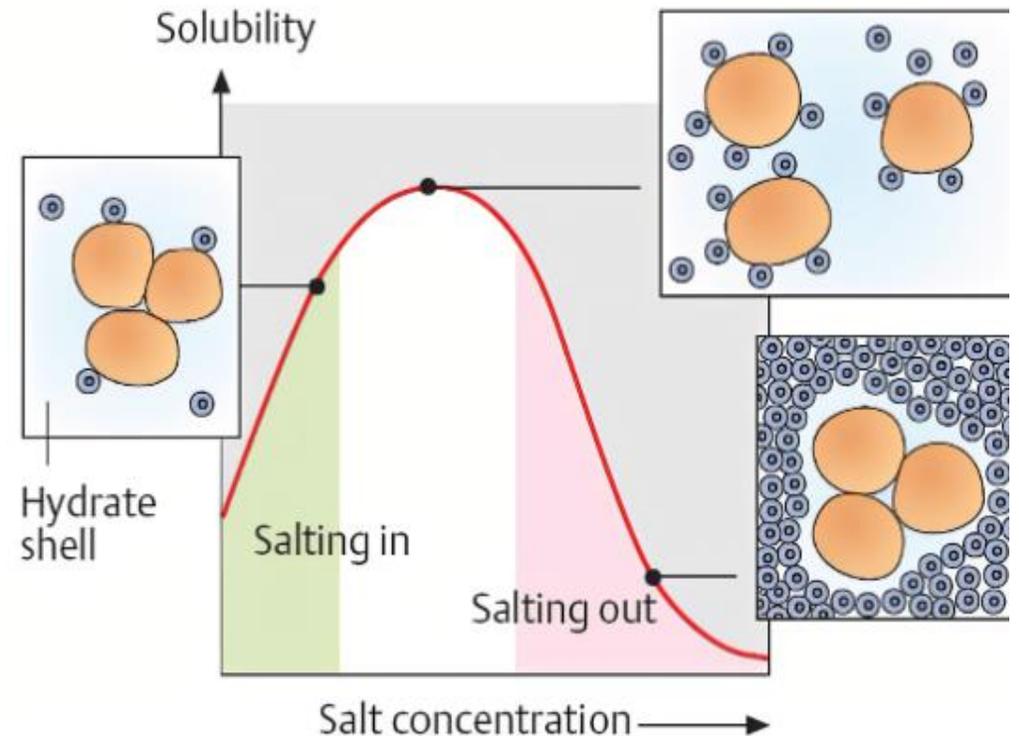
The methods used for precipitation of proteins are:

- **Salting out**
- **Isoelectric pH** : All proteins are least soluble at their isoelectric pH (PI) and can then be precipitated.
- **Heavy positive or negative ions**
- **Organic solvents**: Organic solvent like alcohol, dehydrates and precipitates the proteins.

Salting out method

Neutral salts such as **ammonium sulfate** or **sodium sulfate** are added to a protein solution, the addition may **precipitate a protein from its solution**.

- **Mineral ions** attract **water molecules** and consequently **remove the shell of hydration** (solvation layer) from around protein molecules.
- Since water layer around protein particles is removed, the protein is precipitated. This is called *salting out*.



Precipitation by heavy positive or negative ions

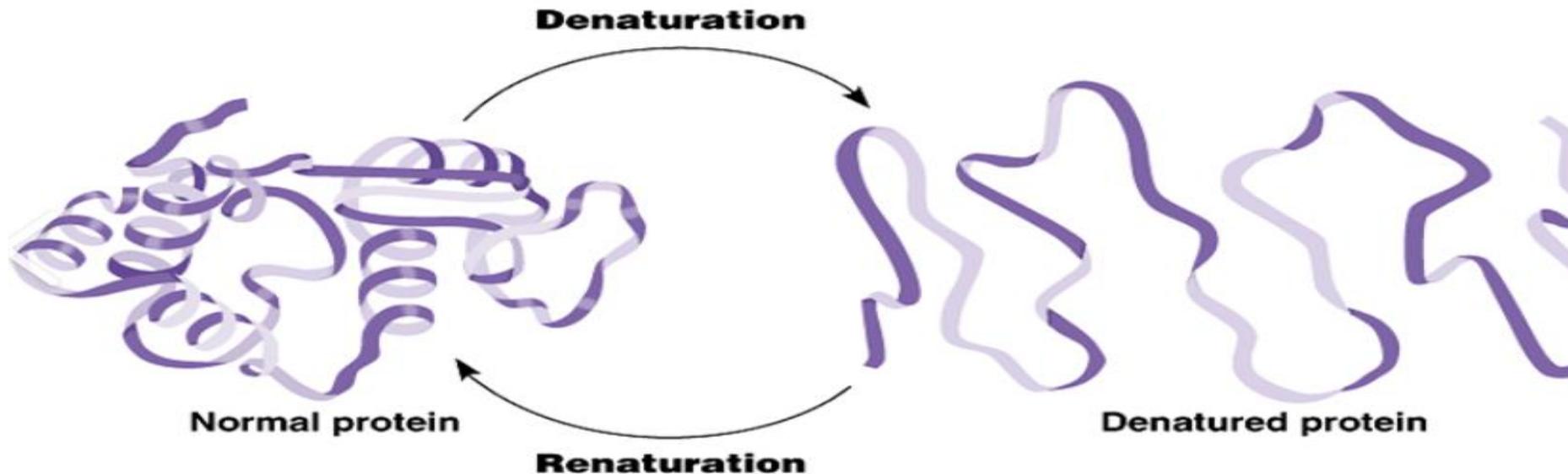
- On the **acidic side** of its PI, a protein remains as **cation (+vely charged)** and may then be precipitated by neutralizing the charge on protein by **adding anion (-vely charged) or alkaloidal reagents** like *tungstate, trichloro acetate or picrate*.
- On the **alkaline side** of its PI, a protein exists as an **anion** and may then be **precipitated as metal proteinates** by heavy metal ions **like zinc, lead, mercury, etc.**

Denaturation

Denaturation is a process where the higher-order structures of proteins (secondary, tertiary, and quaternary) are disrupted, but the basic structure (primary, held together by peptide bonds) remains intact.

This can be caused by either physical factors like high temperatures (exceeding 60°C), or chemical compounds such as acids, bases, heavy metal ions, urea, detergents, or specific organic solvents.

Typically, denaturation is irreversible. However, if the denaturing agent is mild and applied for a short duration, the protein can sometimes regain its original structure and properties through a process called renaturation.



Clinical Notes

Protein precipitation by heavy metals is used for its **disinfectant properties** in external applications.

- **Silver nitrate (AgNO₃)** is employed to safeguard newborn infants from gonorrhoea infections in the eyes. It is also used in treating infections in the nose and throat, as well as in cauterizing wounds.
- **Mercury salts**, like Mercurochrome or Merthiolate, react with sulfhydryl groups, leading to protein precipitation. This action helps prevent infections in wounds.
- precipitation of proteins by heavy metals is utilized for the household treatment of accidental ingestion of heavy metals. **The protein, egg white or milk** acts as an antidote and binds with the heavy metals and prevents absorption of heavy metals.

Color Reactions of amino acids and proteins

- There are number of test to detect the presence of amino acid
- This is largely depend on the natural of side chain.

Qualitative Tests:

1. Ninhydrin test: for α -L amino acids
2. biuret test: for Peptides and Proteins

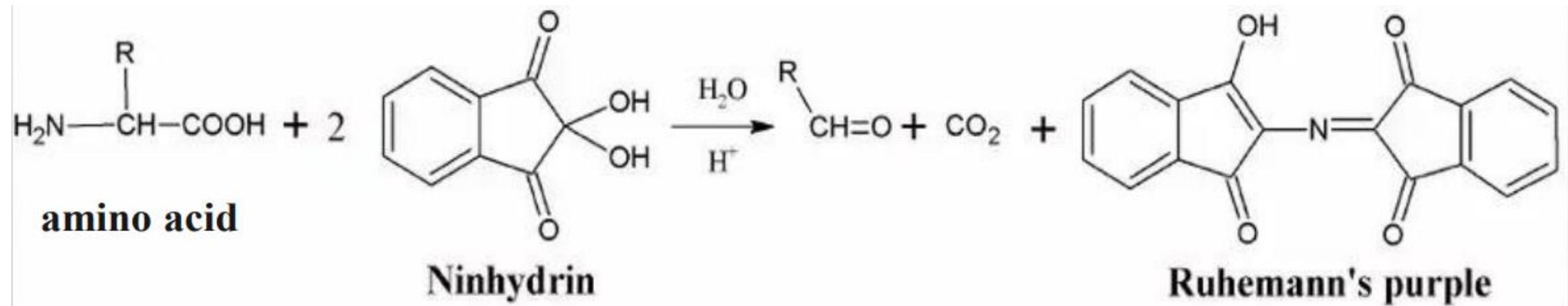
Ninhydrin test

Ninhydrin test is chemical test used to determine whether the sample contains amines or α -amino acids.

- A test solution of the analyte is mixed with ninhydrin, a powerful oxidizing agent, react with all α -amino acids to give **blue** or **purple**-colored compound.
- The reaction is also given by primary, secondary amines ,amino containing compounds.
- The imino acids, proline and hydroxyproline ,also react with ninhydrin , but in this case a **yellow** color is formed.

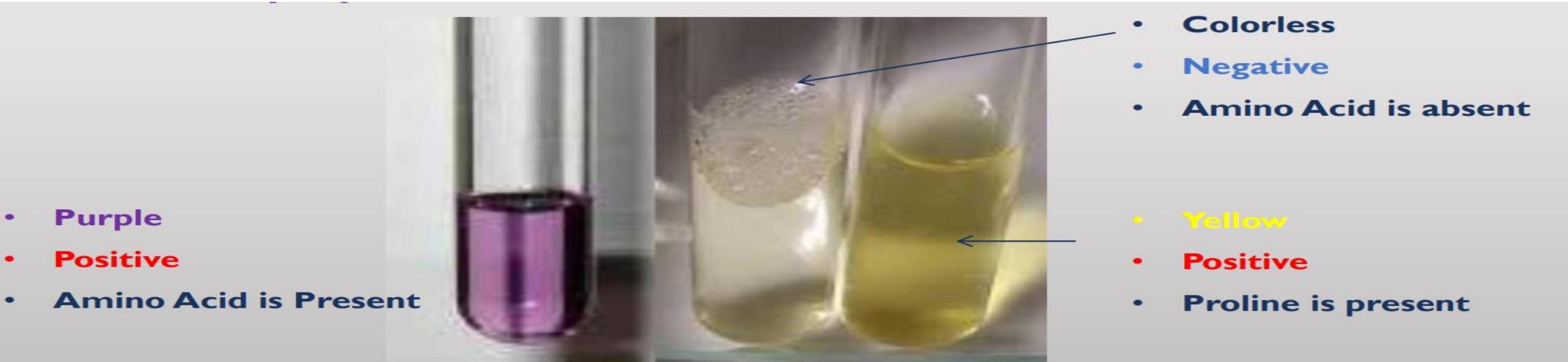
Ninhydrin's Test Principle and Reaction

- Ninhydrin degrades amino acids into aldehyde, ammonia, and CO₂ through a series of reactions; the net result is ninhydrin in a partially reduced form hydrindantin.
- Ninhydrin then condenses with ammonia and hydrindantin to produce an intensely blue or purple pigment, sometimes called **Ruhemann's purple**:



Experimental procedure

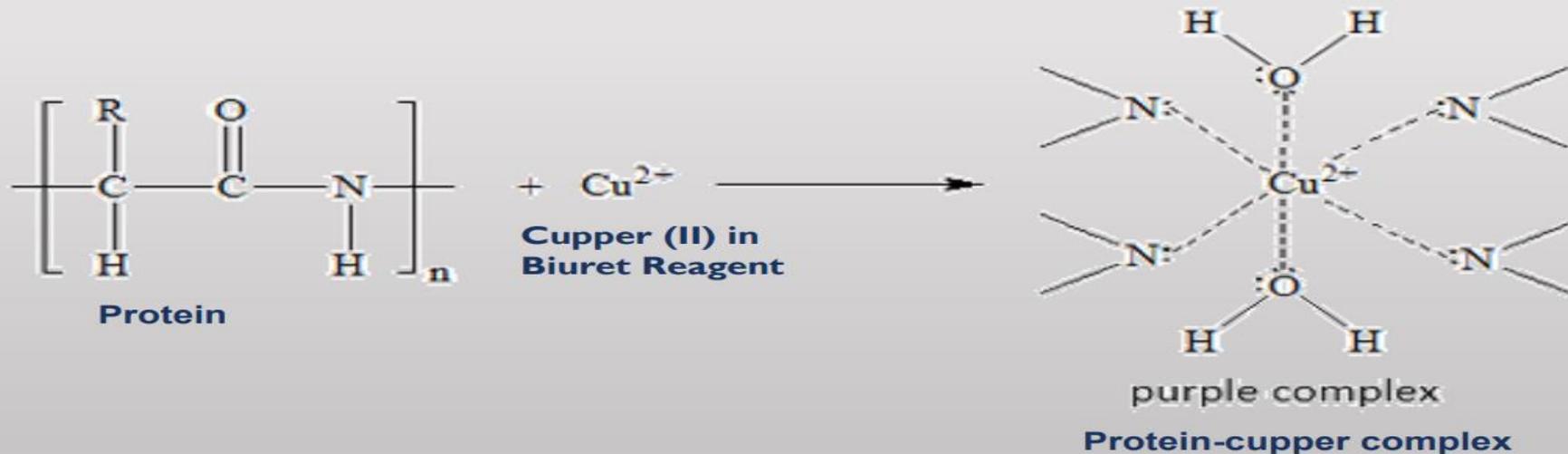
- 1- Into **three clean test tubes** pipet **1 ml** of each the following solutions: **2% egg albumin, 0.5% glycine and water** (one solution per tube).
- 2- Add **3 drop of 0.2 % ninhydrin solution**.
- 3- **Mix the contents** of each tube and **heat in a boiling water bath for 2 min**.
- 4- **Allow to cool and observe the blue color formed**.



Biuret test

This test is specific for the **peptide bond**. Substances containing not less than two peptide linkages give this test.

- ❖ **Objective:** detect the presence of **peptides or proteins in a sample**.
 - ❖ **Principle:** When proteins and peptides (i.e peptide bonds) treated with an **alkaline solution of dilute copper sulfate (CuSO₄)** a **violet color** is formed . A positive test is indicated by the formation of a **violet color**.
 - ❖ **Note:** the color density is proportional to amount of proteins present.
- * **biuret reagent is alkaline copper sulfate solution.**



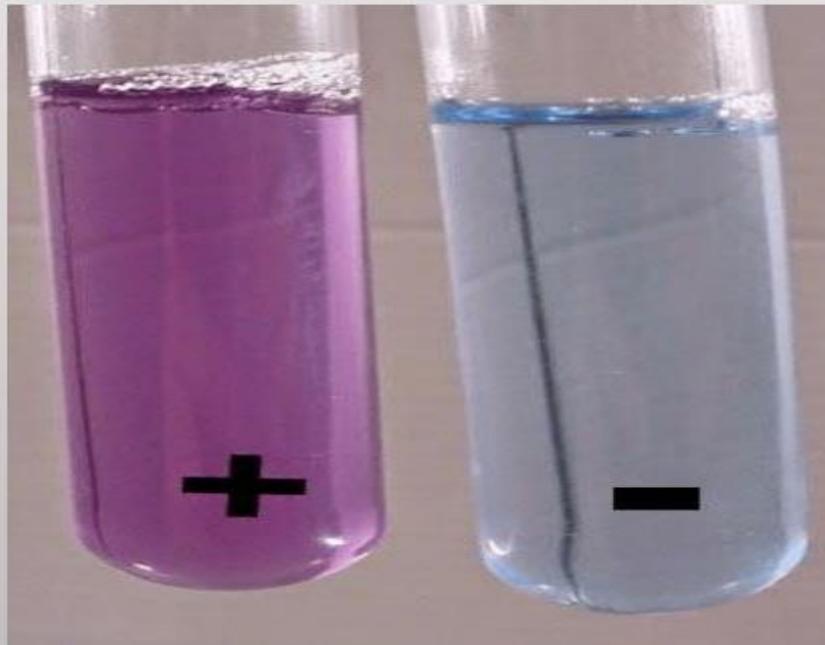
Experimental procedure

– Into three separate test tubes, add 2 ml of the following solution: 2% egg albumin, 0.5 % alanine (or any amino acid), and H₂O.

2 – Add 2 ml of 10 % NaOH into each of the four tubes and mix the contents.

4 – Add 4 to 6 drops of 0.5 % CuSO₄ into each tube.

- **Violet**
- **Positive**
- **Protein present**



- **Blue**
- **Negative**
- **Protein is absent**

Lab Questions

- 1- What is the purpose of using the salting out technique in protein purification? Describe the principle behind how it works.**
- 2- Do you think free amino acids will give a positive result with Biuret reaction? why?**
- 3- Why proline gives a yellow color for ninhydrine test?**