Medical Laboratory Techniques Department

Practical Biochemistry

SECOND STAGE \ SECOND COURSE

Lab 5-6

Proteins

Assist Lecturer

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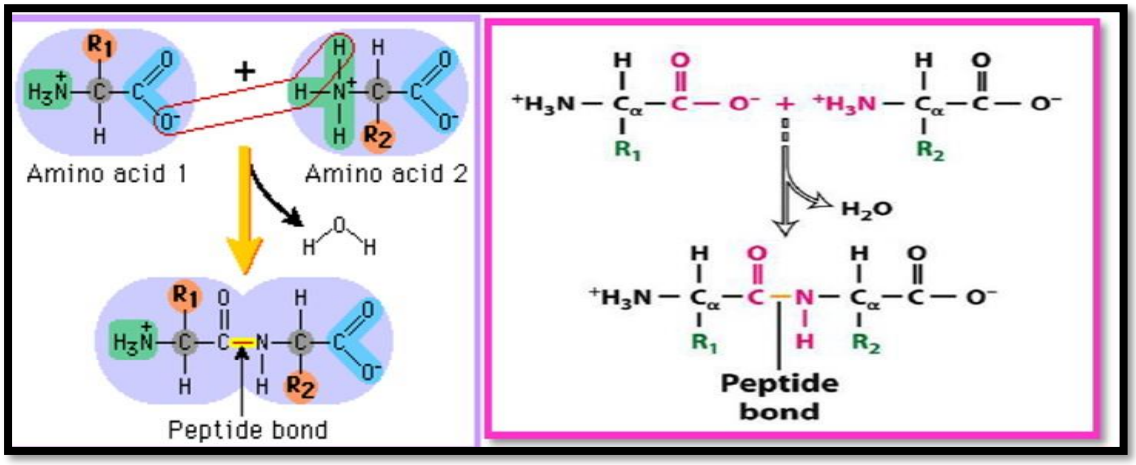
Proteins Vehicles membership Related Weights Molecular big And she phrase About Chains From Acids Amino Associated with Together Some With links Peptide.

Play Proteins A role Important in a Body The object District Where intervention in a Installation Many From Materials Biological Specialized Such as Objects Countermeasures And Enzymes And Some Hormones as such help in a Transfer Liquidity Nervousness And control in a Expression Genetic And she Component The primary For tissues Live.

Consists of Protein From Series From Acids Amino Associated with With Some of them With links Peptidic And In which Linked Collection Carboxyl in a Acid Ameen With Collection The Secretary in a Acid Ameen else With Removal part Water.



Different Proteins About Some of them Some in a Build it The chemist Accordingly For several Factors:

1- Number And type Acids Amino Component For her chains Peptide.
2- Order And continues Acids Amino.
3- Engagement Protein With Molecules Other Non Proteinuria.

Shapes Constructivism For protein:

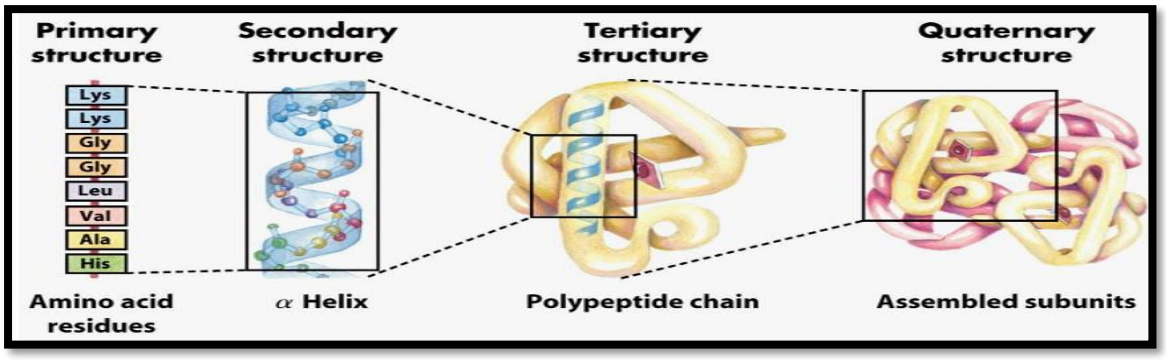
Take chains Peptide Component For protein Shapes Space Resulting About Circumvent That chains Given four Compositions Constructive.

1. Primary structure: Installation Constructive Initial crosses About sequence And continues Acids Amino Associated with With Some of them Some have links Peptide.

2. Secondary structure: Produces About formation Links Hydrogen between Groups Terminal R group For acids Amino With Some of them Some Than Cause in a Circumvent And twisting Series Peptide Made up As for Form The plate alpha helix or the shape Spiral staircase B-sheet Folded

3. Tertiary structure: Produces About formation Links Hydrogen between Groups Terminal R groupFor acids Amino Far About Some of them Made up the shape Trio Dimensions.

4. Quaternary structure: And in it Linked Units Different or Are similar From chains Peptide (subunits)With Some of them Some To be the shape Quadruple Dimensions For protein. Example part Hemoglobin The Formator From four Units Linked together.



And proteins in a This Composition Become able On performance Careers Biological. And like Proteins in a Its characteristics Physical And chemical That Properties Which Is characterized by It out Acids Amino Component to her. So Let the proteins Property Amphoteric in a Its interaction With Acids So bear shipment positive While With the rules We find It Is gaining shipment Negative. Therefore The Its movement in a the field Electrophoresis Depends On Values pH To the middle. The series begins Peptide Component For proteins Party The amino Free Proteins It ends Party Carboxylic.

Quantitative Proteins Estimation

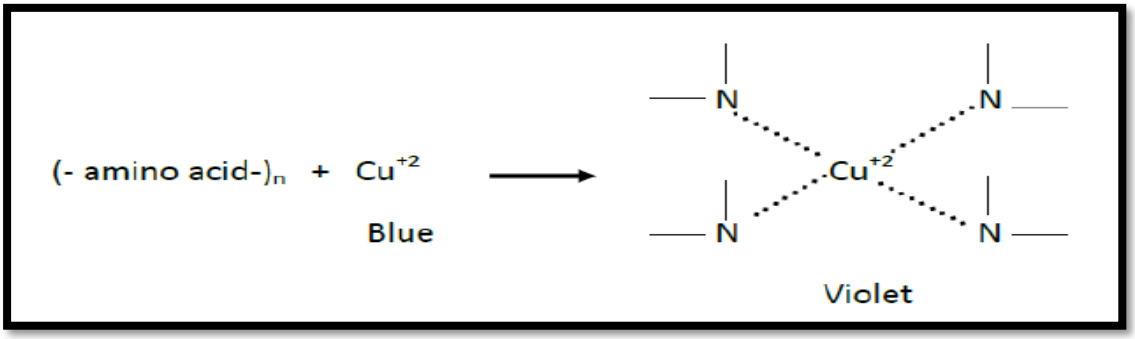
Determination of Proteins using Biuret Protein Assay

Aim:

To determine the presence of Proteins in the given sample using Biuret test

Principle :

This test is used to detect the presence of protein because this test is specific for the peptide bond therefore substances containing not less than two peptide linkages give this test. When proteins are treated with an alkaline solution of dilute copper sulfate a complex with violet color is formed. The intensity of the color formed is directly proportional to the amount of protein present in the sample therefore this test is used to determine the concentration of proteins quantitatively.



Reagent:

A formula for biuret reagent is (per liter final volume): 9 gm Sodium potassium tartrate, 3 gm Copper sulfate, 5 gm Potassium iodide, dissolved in order in 400 mL 0.2 M NaOH before bringing to final volume.

Procedure:

1. In clean dry test tube add 2 ml of 5% albumin solution (protein).
2. In the second test tube add 2 ml of 5% argenine solution (amino acid).
3. For each test tube add 1 ml of biuret reagent and mix well.
4. Observe the formation of violet color for protein (positive result) and no color change for amino acid (negative result).

ESTIMATION OF PROTEIN BY LOWRY'S METHOD

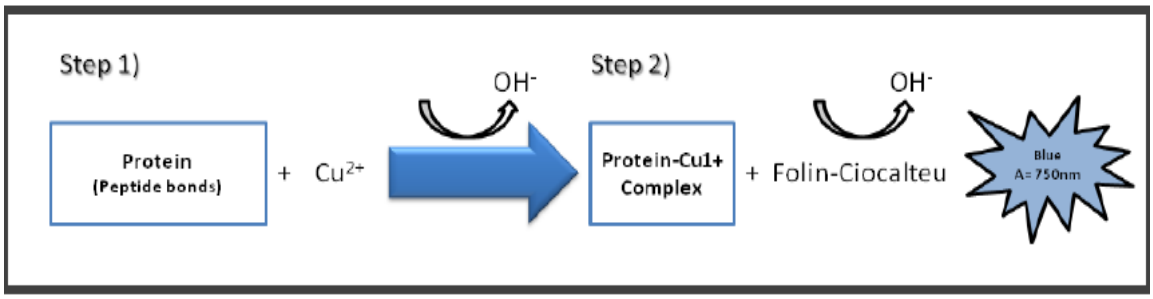
Aim:

To estimate the protein using Lowry's method.

- It is one of the common methods due to its ease and speed of procedure, as well as its high sensitivity. It is used In the determination of diluted proteins when their concentration is low.
- The Lowry method is considered a development and derivative of the Biuret method for detecting proteins.

Principle:

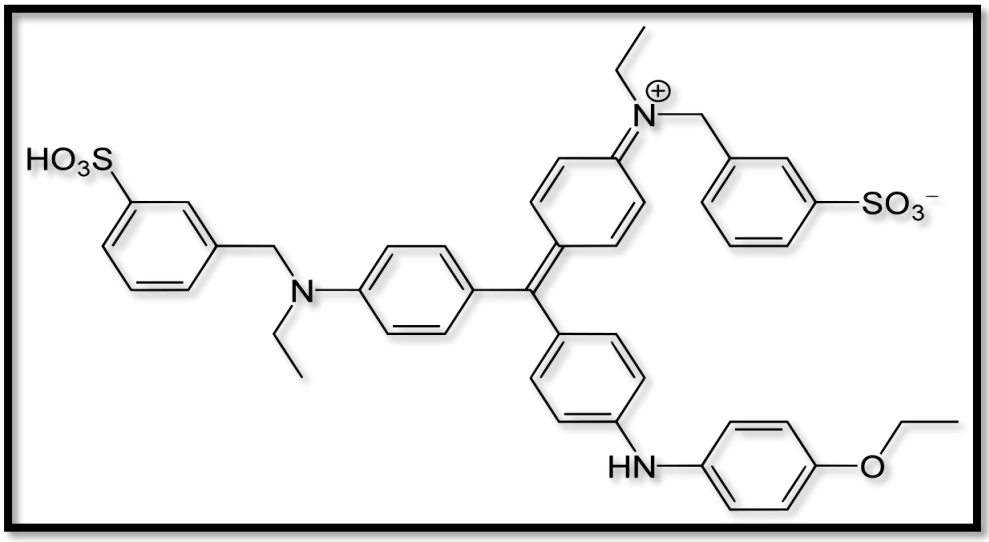
When a protein is treated with a solution of copper sulfate in a basic medium, the cupric ion forms a complex with the peptide bond in the protein It is called a biuret complex, and this complex reduces Folen's solution (which consists of complex salts of tungsten phospholipids) to give the color Blue, the optical absorption of which can be measured at a wavelength of 750nm



Determination of Protein Concentration by Bradford Assay (Coomassie Dye-based Protein Assay)

Principle :The Bradford Protein Assay is a quick, inexpensive and simple way to determine protein concentration This method is based on the fact that the Coomassie Brilliant Blue G-250 (CBBG) dye exists in red color, which turns into a blue chemical form (in acidic media), that is, when it binds with the protein to be measured in the sample.

This method is characterized by its sensitivity to low-protein concentrations



Coomassie Brilliant Blue

Advantages of Bradford Assay

1) It is the fastest and easiest to perform of all protein assays.
2) It is performed at room temperature and no special equipment is required.
3) It is not specific for any particular protein.