AL-Mustaqbal University College

Medical laboratory Techniques Department

First Lecture (Determination of Total Protein)

Assistant lecturer M. Sc. Hawraa Saad Al-Kawaz

Proteins

Proteins are large biomolecule or macromolecules consisting of one or more long chains of amino acid residues.

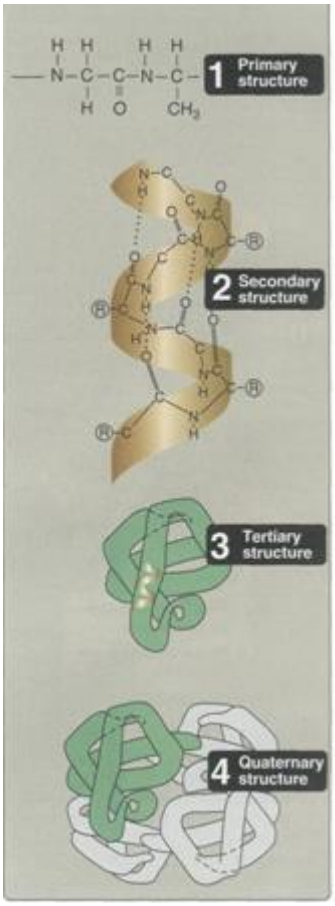
A linear chain of amino acid residue is called a polypeptide.

A protein contains at least one long polypeptide.

The twenty amino acids commonly found in proteins are joined together by peptide bonds.

Functional proteins have four levels of structural organization:

1- Primary structure: the linear structure of amino acid in the poly peptide chain.
2- Secondary structure: hydrogen bonds between peptide group chains in an alpha-helix or beta sheet.
3- Tertiary structure: three dimension structure of alpha helixes and beta helixes folded.
4- Quaternary structure: three-dimential structure of multiple polypepties and how they fit together.



Note: Understanding the primary structure of proteins is important because many genetic diseases results in protein with abnormal amino acid sequences which cause improper folding and loss or impairment of normal function.

General reactions of proteins

1- The biuret test for peptide bonds.

Is a chemical test used for detecting the presence of peptide bonds.

Cupric ions chelate with peptide bonds of proteins in alkaline medium to produce a pink or violet color. The intensity of the color is proportional to the peptide bonds.

Advantage: the biuret method is simple one step process and is the most widely used method for plasma protein estimation.

Disadvantage: sensitivity of the method is less and it is unsuitable for estimation of proteins in small quantity.

2- **Denaturation of proteins by heat and extreme pH.**

3- **Precipitation by heavy metal ions.**

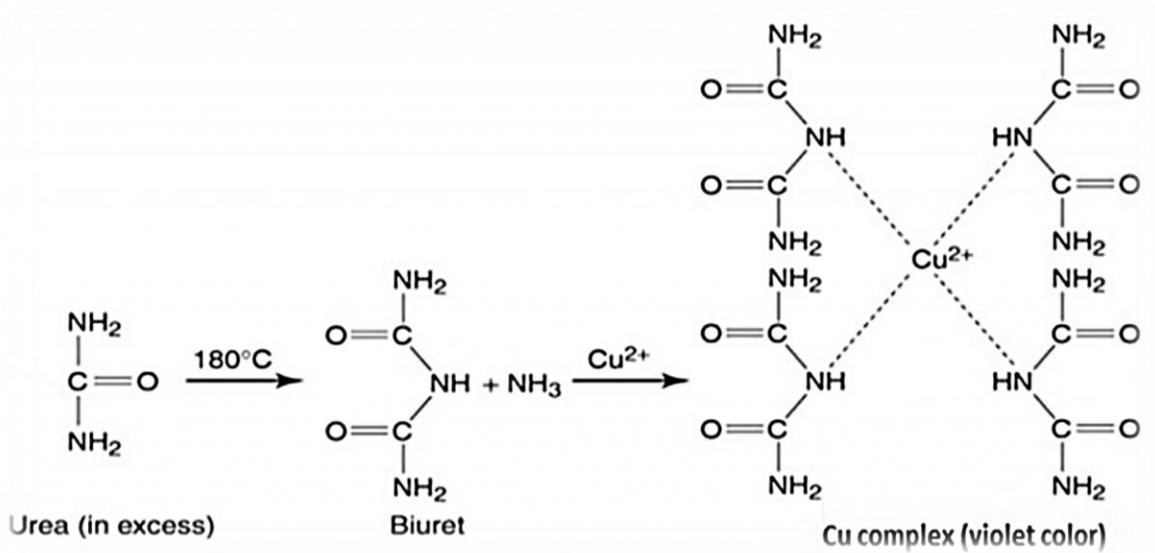
In alkaline medium proteins have net negative charge or anions.

4- **Precipitation by acidic agents.**

Determination of Total Protein by Biuret Method

1. Principle

The Biuret approach focused on the complexation of cupric ions in the protein's peptide bonds to functional groups. To form a Cu^{2+}-protein complex and produce a violet-colored chelate product as shown in Scheme (2-1) that was measured at 540 nm by absorption spectroscopy, two peptide bonds or longer were required.



Scheme (2-1): Biuret Reagent Reacts with an Alkaline Solution of $CuSO_4$ to Form a Violet Chelate Compound

2. Preparation of Working Reagent

By adding 3 ml of R2 to a container of R1, the working reagent prepared, and this reagent was stable for 6 months.

3. Procedure

Three tubes were prepared and the procedure carried out as in the following Table

Table Procedure Used for Determination of Total Protein

Tubes	Blank	Sample	Standard
Working Reagent	1 mL	1 mL	1 mL
Reagent 3 (Standard)			20 µL
Sample		20 µL	
All tubes were mixed and incubated for 2 min. at room temperature then the absorbance was reading at 540 nm against the blank			

4. Calculation

The result was calculated as follows:

$$\textbf{Concentration of Total Protein(g/L)} = \frac{\textbf{Abs. of Sample}}{\textbf{Abs. of Standard}} \textbf{*Concentration of Standar}$$

Concentration of Standard = 50 g/L