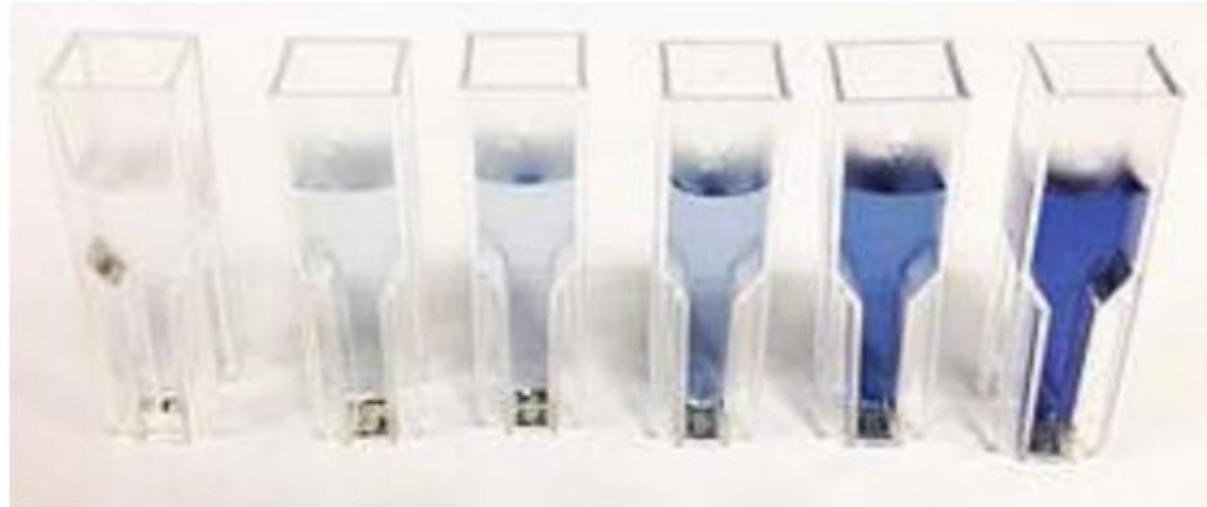


Quantitative Proteins Estimation by Lowry method



Protein Estimation

Qualitative

Quantitative

refers to descriptions or distinctions based on some quality or characteristic. It can be a form of analysis that yields the **identity** of a compound (Determine if specific substance is there or not, by color or some other quality).

refers to a type of information based in quantities or else quantifiable data (determination of unknown **protein concentration** in a sample using standard curve obtained, eg. using lowry method).



Importance of determining concentration of protein

-Quantitative assays Determine the concentration of a substance.

- Protein assays are one of the most widely used methods in life science research. Estimation of protein concentration is necessary in protein, cell biology, molecular biology and other research applications.
- Is necessary before processing protein samples for isolation, separation and analysis.

Specificity and Sensitivity of the method



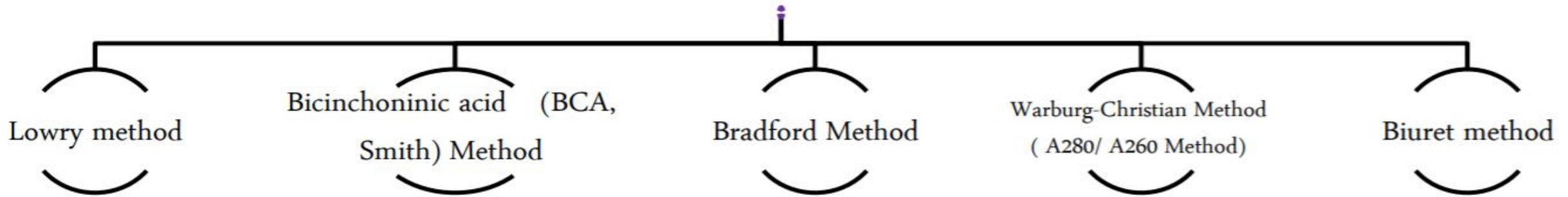
Sensitivity of an assay is a measure of how little of the analyte the method can detect.



Specificity of an assay relates to how good the assay is in discriminating between the requested analyte and interfering substances



Most familiar spectrophotometric methods for Determination of proteins concentration:



The factors that you should consider in choosing a method:

- Sensitivity
- The presence of interfering substance
- Time available of the assay

Introduction:

Lowry's assay for total protein estimation is one of the most commonly used colorimetric assays.

The Biochemist Oliver H. Lowry developed the reagent in the 1940s. His publication on the same in the year 1951 was highly cited and has been used in protein labs.

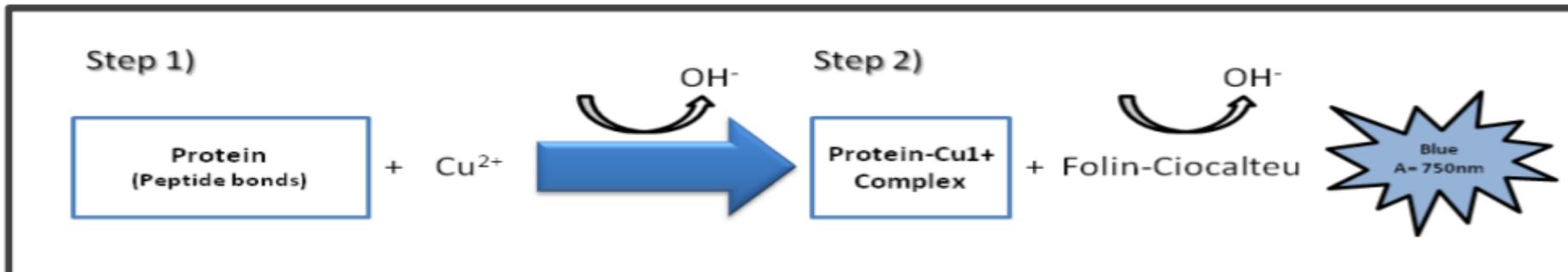
Objectives

- To determine the concentration of proteins by Lowry's method.
- To prepare the required lab reagents.
- To construct standard graph on paper.
- To develop standard graph

Lowery Method

Is based on two chemical reactions:

- **The first reaction** is the reduction of copper ions under alkaline conditions, which forms a complex with peptide bonds .
- **The second reaction** is the reduction of Folin-Ciocalteu reagent by the copper-peptide bond complex, which subsequently causes a color change of the solution into blue with an absorption in the range of 650 to 750 nm detectable with a spectrophotometer.



Principle:

- The principle of this method is based on **two reactions leading to colour complex formation**.
- Firstly, the **Biuret reaction** in which Cu^{2+} of the reaction mixture reacts with the peptide bond of proteins under alkaline conditions resulting in their reduction to cuprous ions (Cu^+),
- and **Lowry's reaction** - in which the **Folin Ciocalteu reagent**, which contains **phosphomolybdic complex** which is a mixture of **sodium tungstate, sodium molybdate and phosphate**, along with **copper sulphate solution** and the protein forms a **blue purple colour** which can be assessed by measuring the absorbance at 650-700nm.





Protein
(peptide bonds)



Cu^{2+}

OH^-



Protein - Cu
Complex



OH^-



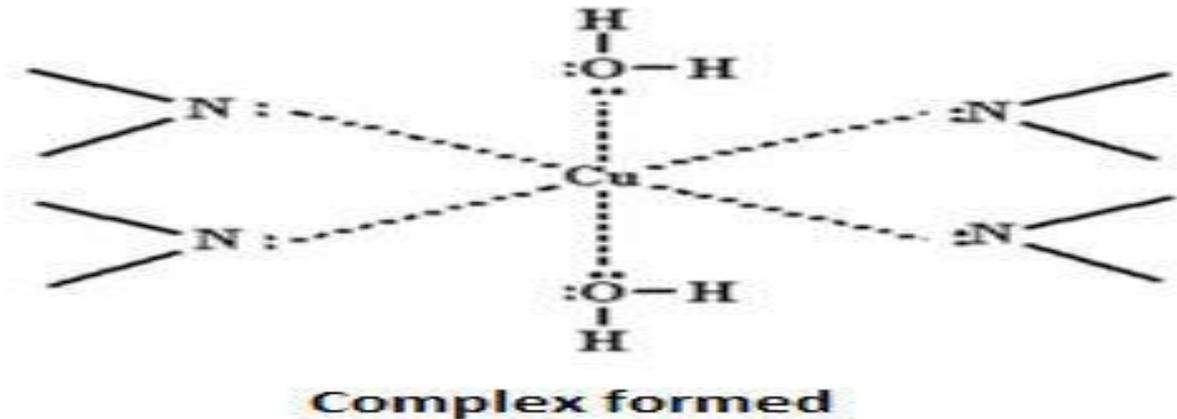
Folin-Ciocalteu
reagent



Blue
Color

Principle:

- The phenolic group of the amino acid (tyrosine and tryptophan) residues will produce a blue purple colour due to the reduction of phosphomolybdotungstate to hetero-polymolybdenum blue by the copper catalysed oxidation of the amino acids and its intensity depends on the amount of these aromatic amino acids present.
- The blue purple colour formed thus differ from protein to protein.
- The blue purple color is formed of aromatic amino acids tryptophan and tyrosine. The reaction is pH dependent and works best in alkaline conditions with pH between 9 and 10.5



Advantages

- It is a sensitive assay which requires no digestion of protein.
- It is 10 or 20 times more sensitive as compared with ultraviolet absorption at 280 nm.
- It is more specific and less interrupted by turbidity,
- It is significantly more sensitive than the ninhydrin reaction and biuret reaction.
- It is simple to perform and can be easily used on small scale in the labs.

Disadvantages

- The amount of colour developed differs from protein to protein,
- It is less constant than the biuret reaction, but more constant than the absorption at 280 nm.
- The color is not exactly proportional to concentration.

Applications:

- It is used in measurement of protein during enzyme fractionations, mixed tissue proteins, measurement of very small absolute amounts of protein, or highly diluted protein and analyses of large numbers of similar protein samples.

Lowry protein Assay method:

- a) Solution A: 2% (w/v) sodium carbonate in 0.1 M sodium hydroxide.
- b) Solution B: 1% (w/v) copper sulphate
- c) Solution C: 2% (w/v) sodium potassium tartrate.
- d) Solution D: Copper reagent- Mix 0.5 volume of solution B, 0.5 volume of solution C and 50 volumes of solution A.
- e) Solution E: Folin-Ciocalteu reagent is diluted to 1M acid according to the supplier's instruction.

• Lowry Method to estimate protein:

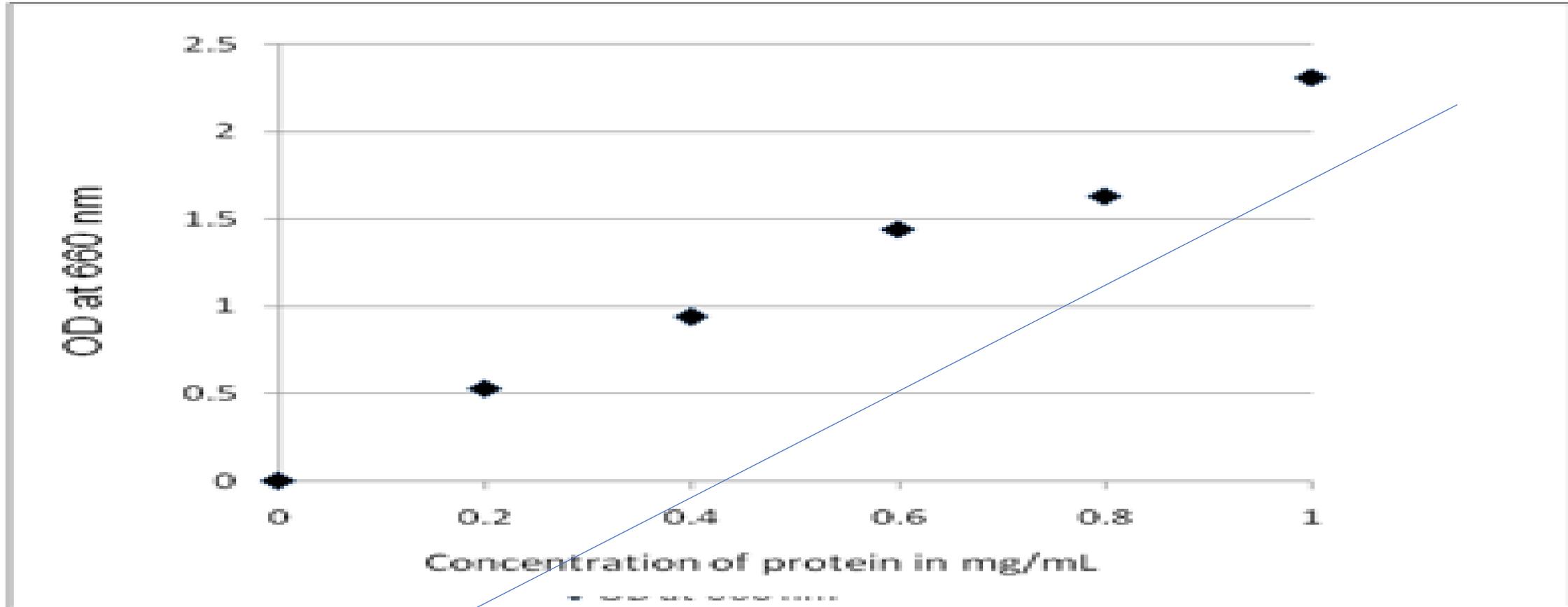
- 1) To **1 mL of the test solution**, add **5mL of solution of Solution D** (Copper reagent), mix thoroughly by vortexing and stand at room temperature for **10 min**.
- 2) Add **0.5 mL of solution E** (Folin-Ciocalteu reagent), mix rapidly, and incubate for **30 min** at room temp.
- 3) Measure the absorbance at **600nm** against reagent blank not containing protein.
- 4) The concentration is estimated by referring to a standard curve obtained at the same time using known concentrate of bovine serum.

Reagents for Lowry method:

- **Folin-Ciocalteu reagent:** This is commercially available and has to be diluted with equal volume of water just before use.
- **Standard protein solution:** Dissolve 10mg of BSA (as it is easily available, cheap and with improved purity) in 100ml of distilled water in a volumetric flask. (for concentration-100 $\mu\text{g/ml}$)

Graph:

Note (Plot a standard graph of Concentration of protein v/s OD at 660)



- To determine the total protein concentration using **graph paper** plot.
- 1. Plot the values of protein concentrations on X axis and the values of absorbance on Y axis of a graph paper.
- 2. Construct a straight line through the points representing the values of absorbance drawn on the paper.
- 3. From the absorbance value of the Unknown Protein drop a perpendicular on the X axis and find the protein concentration for the unknown.

PROTEIN ESTIMATION BY LOWRY METHOD

Calculations:

A) A of sample / A of Standard \times Conc of Standard = ----- $\mu\text{g/ml}$

A) Calculate by Graph using the standard curve = ----- $\mu\text{g/ml}$

Result: The concentration of protein in the given unknown sample is
_____ mg/ml .