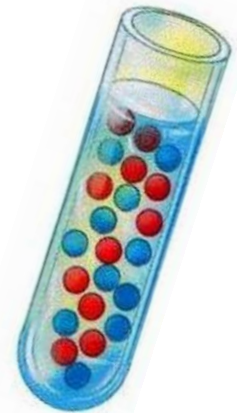


Quantitative Proteins Estimation by Lowry Method

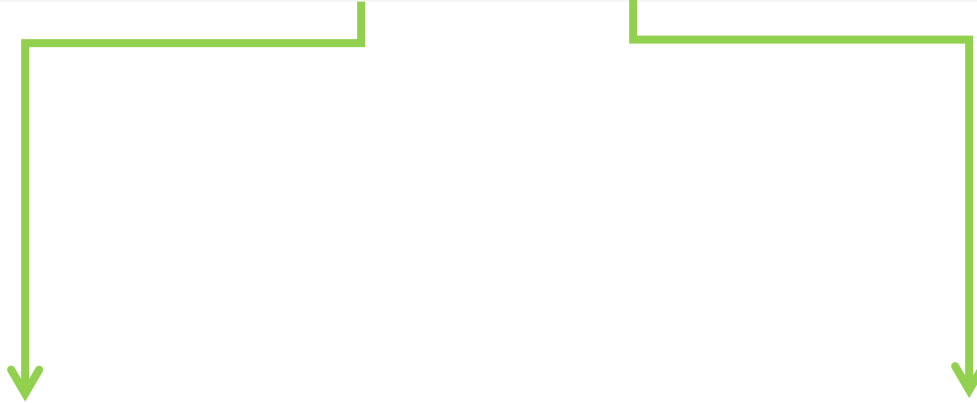
BCH302 [Practical]

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 purification, cell biology, molecular biology and other research applications.
- Is necessary before processing protein samples for isolation, separation and analysis.



Specificity and Sensitivity of a method:

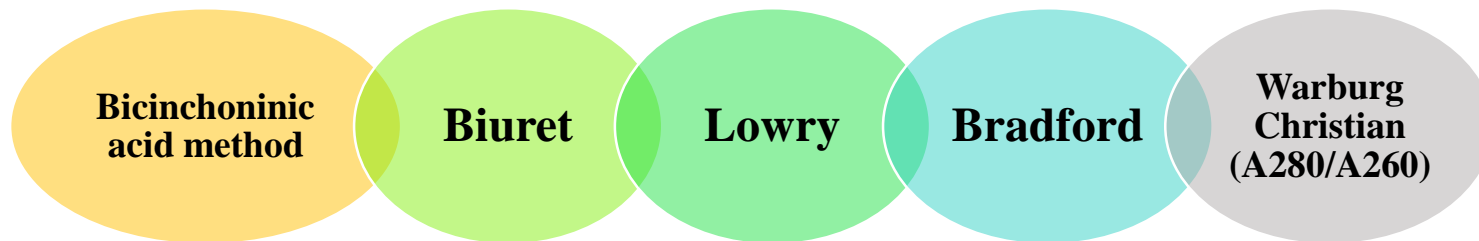


Sensitivity of an assay is a measure of how little of the analyte the method can detect.

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 :

- There are a wide variety of protein assays available, but each assay has its own advantages and limitations.

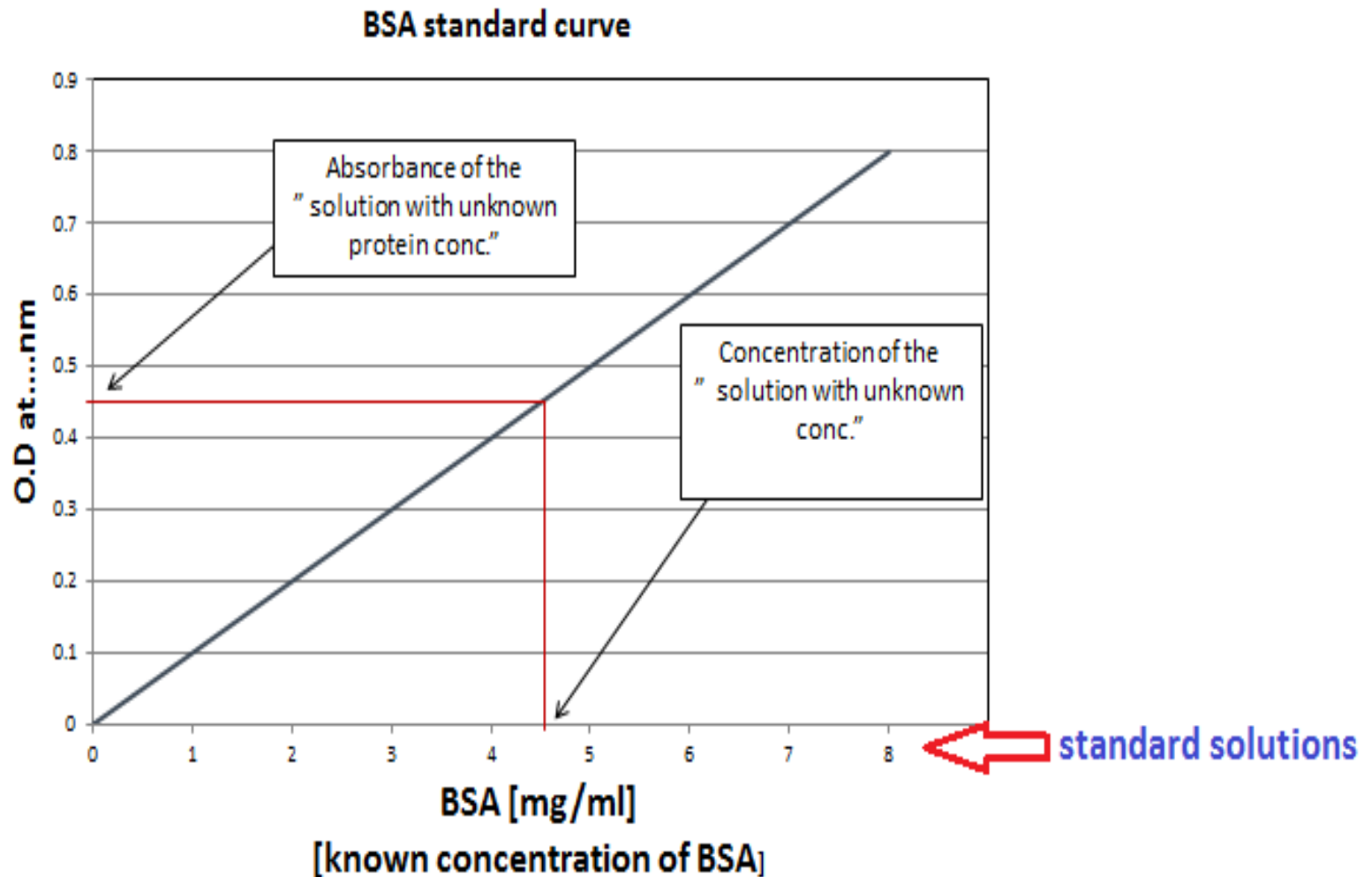


- **The factors that you should consider in choosing a method:**
 - Sensitivity.
 - The presence of interfering substance.
 - Time available of the assay.

Standard curve:

- It is a graph that shows the relationship between different **known concentrations** of a substance and the **absorbance at a specific wave length**.
- Standard curve are most commonly used to determine the **concentration of a unknown substance**, using serial dilution of solutions of known concentrations[**standard solution**], such as (Bovine serum Albumin) BSA and casein.
- There is a **linear relationship** between absorbance and concentration.

Determination of unknown concentration by standard curve:



Practical part

Objectives:

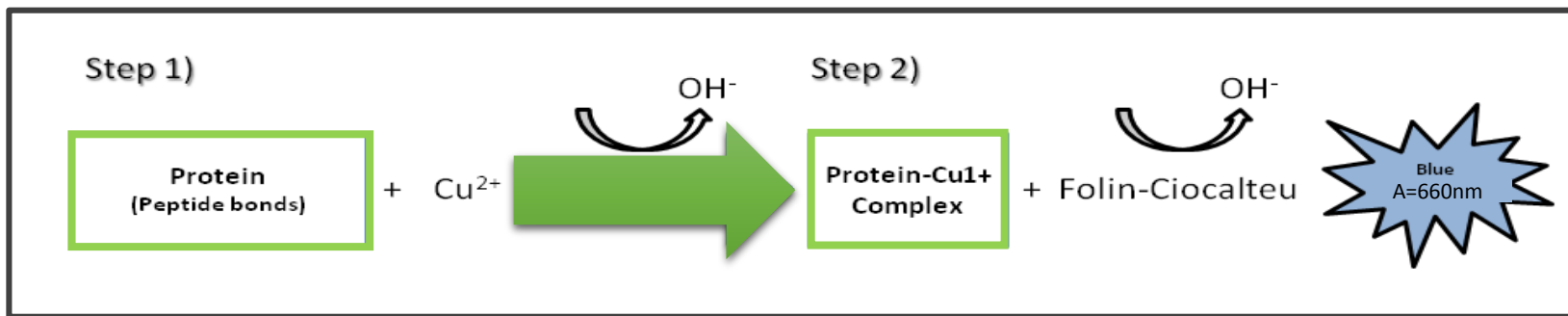
- Determination of an unknown concentration for protein sample by lowry method.
- Getting familiar with standard curve.

Lowry method:

Principle:

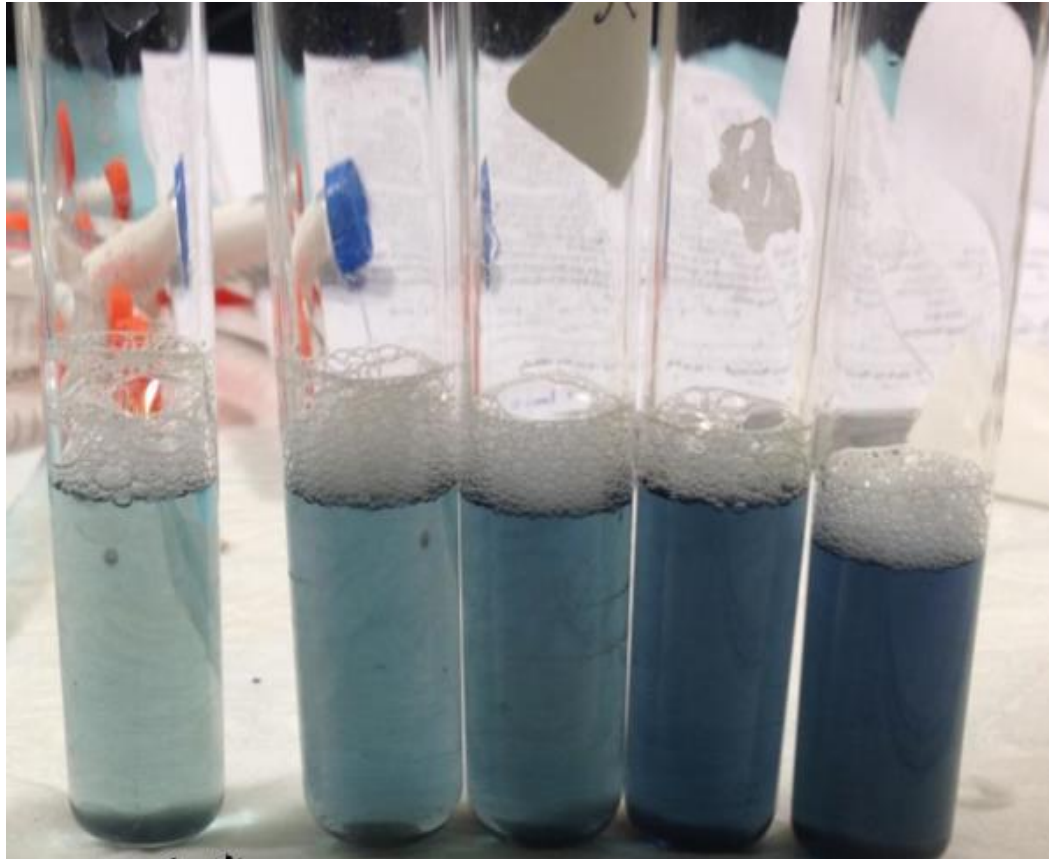
- It is based on two chemical reactions:

- 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.



- The advantages of this assay are its **sensitivity**, and most importantly **accuracy**.
- However, it requires more time than other assays (slow method).

From lower to higher concentration



There is a linear relationship between blue color developed and concentration.

Experiment 1 : Estimation of protein concentration by Lowry method

Method:

- Set up 7 tubes as follows:

Tube	Water (ml)	Bovine serum albumin Standard Concentration [100mg/dl]	sample [unknown concentration]	Reagent C (include copper and alkaline reagent)	Mix and let stand at room temperature for 15 min	Folin-Ciocalteu reagent
Blank	1 ml	-	-	3ml		0.3 ml (Add this reagent to one tube at a time and immediately after adding it mix well)
A	0.8 ml	0.2 ml	-			
B	0.6 ml	0.4 ml	-			
C	0.4 ml	0.6 lm	-			
D	0.2 ml	0.8 ml	-			
E	-	1.0 ml	-			
F	-	-	1.0 ml			

- Let the tubes stand at room temperature for 45 min.
- Read absorbance at 660 nm against the blank.

Experiment 1 : Estimation of protein concentration by Lowry method

Results:

Tube	Albumin concentration (mg/dl) [X- axis]	Absorbance at 660nm [Y-axis]
A	$C_1 \times V_1 = C_2 \times V_2$ $100 \times 0.2 = ? \times 1$ $? = 20$	
B		
C		
D		
E		
F	?	

- Plot a standard curve for absorbance at 660 nm against Albumine std. concentration (mg/dl).
- From the standard curve find out the unknown protein concentration.

