

Quantitative Proteins Estimation by Biuret Method

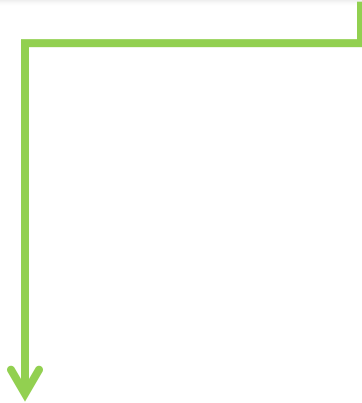
BCH302 [Practical]

Importance of determining concentration of protein :

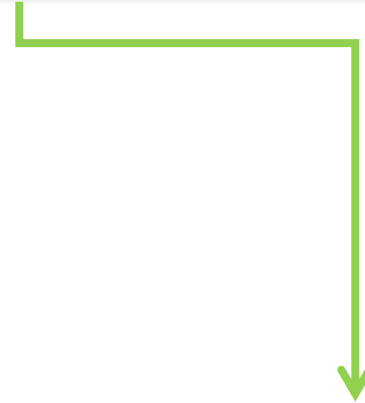
- **Quantitative assays** → determine the concentration of a substance.
- Protein assays are one of the most widely used methods in life science research.
- Estimation of protein concentration is necessary in protein purification, cell biology, molecular biology and other research applications.
- Is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



Specificity and Sensitivity of a method



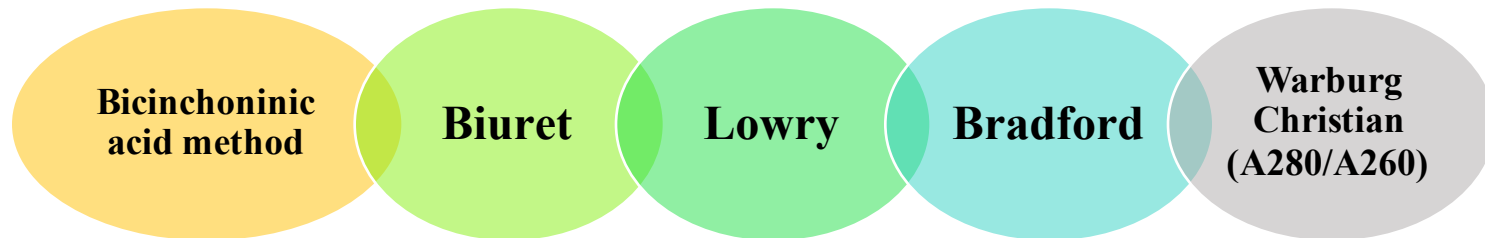
Sensitivity of an assay is a measure of how little of the analyte the method can detect.



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.

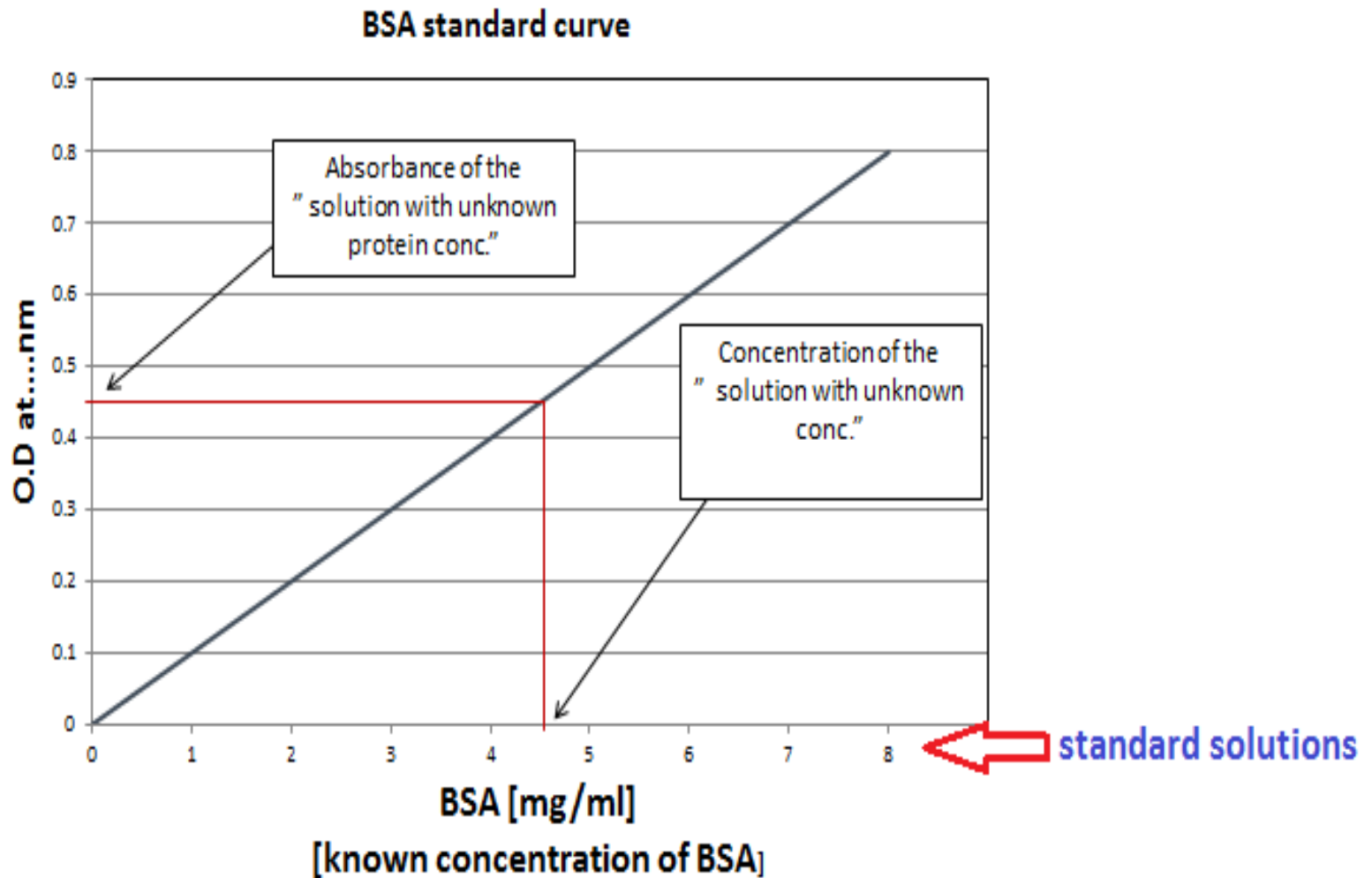


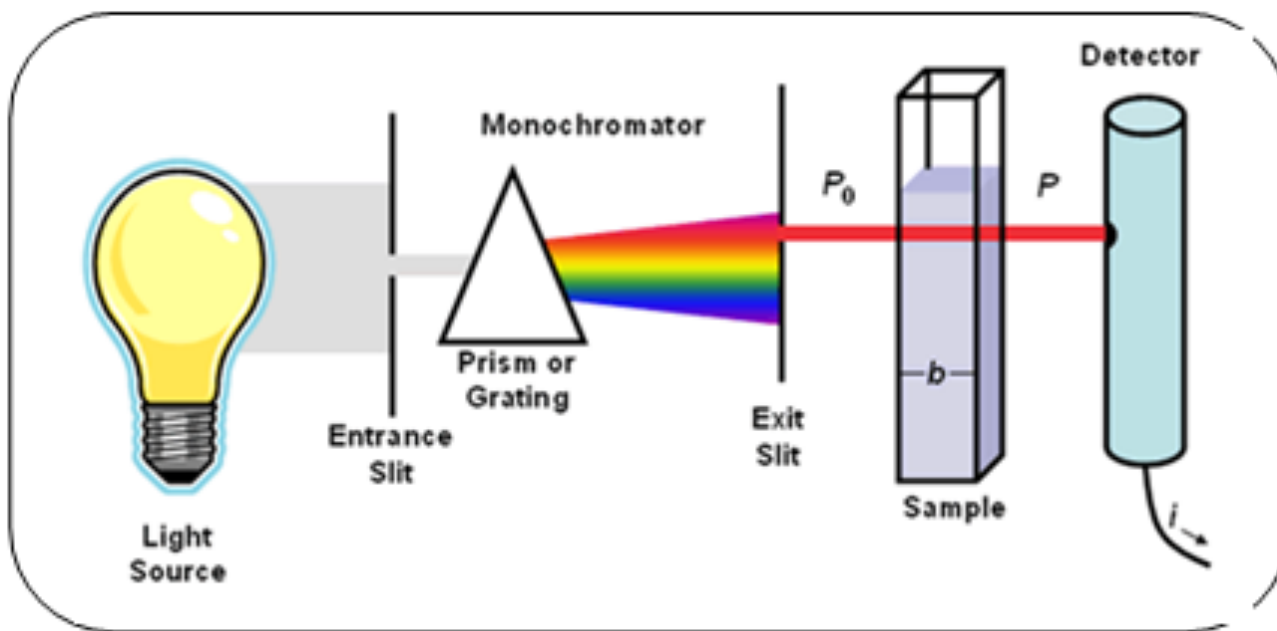
- **The factors that you should consider in choosing a method:**
 - Sensitivity.
 - The presence of interfering substance.
 - Time available of the assay.

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

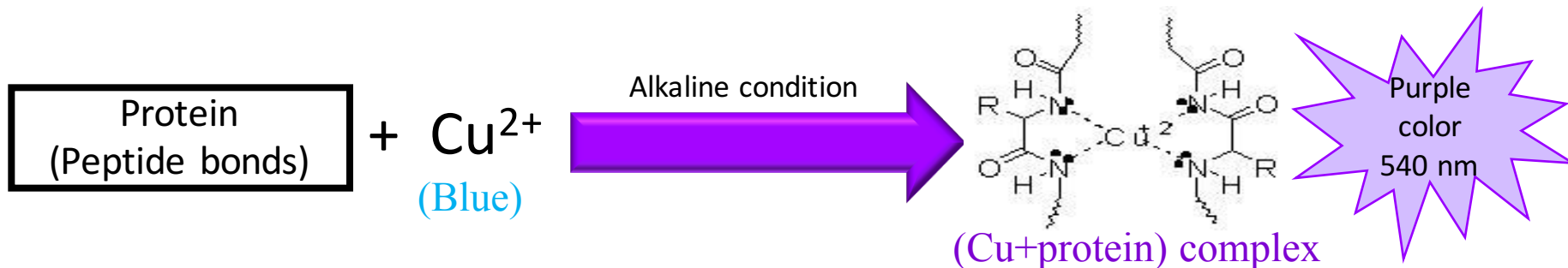
Objectives:

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

Biuret method:

Principle:

- Biuret method is based on **copper ions (Copper sulphate)** binding to peptide bonds of protein under alkaline condition to give a **violet (purple) colour**.
- The intensity of the color resulting from the (Cu+protein) complex is **linearly proportional** to the **concentration** of protein present in the solution.
- Maximum absorbance at 540 nm.



- The advantage of this assay that it **don't take a lot of time**.
- The disadvantage that it is **not as sensitive as other methods** – requires higher amounts of protein.

From lower to higher concentration



There is a **linear relationship** between purple color developed and concentration.

Experiment 1 : Estimation of protein concentration by Biuret method

Method:

- Set up 8 tubes as follows:

Tube	Water (ml)	Bovine serum albumin (BSA) Standard Concentration (5 g/L) (ml)	Sample [unknown concentration] (ml)	Biuret reagent
Blank	2	-	-	3 ml
A	1.6	0.4	-	
B	1.2	0.8	-	
C	1	1	-	
D	0.8	1.2	-	
E	0.6	1.4	-	
F	0.4	1.6	-	
Unknown sample	-	-	2	

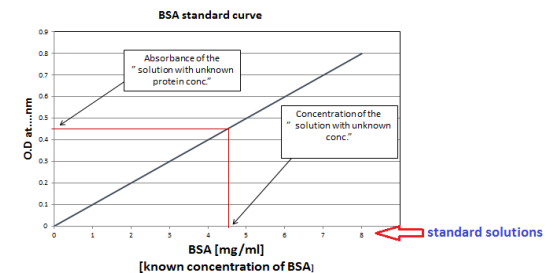
- Let the tubes stand at room temperature for 10 min.
- Read absorbance at 540 nm against the blank.

Experiment 1 : Estimation of protein concentration by Biuret method

Results:

Tube	Protein concentration (5 g/L) [X- axis]	Absorbance at 540 nm [Y- axis]
A	$C_1 \times V_1 = C_2 \times V_2$ $5 \times 0.4 = ? \times 2$ $\rightarrow ? = 1$	
B		
C		
D		
E		
F		
Unknown sample	

- Plot a standard curve for absorbance at 540 nm against Albumine std. concentration (g/L).
- From the standard curve find out the unknown protein concentration.



Question...

If your unknown sample had an absorbance higher than the highest absorbance recorded by standard, how will you determine its concentration correctly?