Detection and quantitative estimation of proteins by different methods

BCH303 [Practical]

Protein quantification:

- The accurate quantitation of protein content is a critical step in protein analysis.

- Importance of protein quantification ?

- Depending on the **accuracy required** and the **amount and purity** of the protein available:

➔ different methods are appropriate for determining protein concentration.

Different methods of protein quantification:

- **Methods:**

1. Direct assay: measure the absorbance at 280 nm.

2. Colorimetric/fluorescent and reagent-based protein assay: Protein is added to the reagent, producing a color change or increased fluorescence in proportion to the amount added.

- The most commonly used reagent-based techniques involve:
  - Biuret test.
  - Bradford test.
  - Bicinchoninic acid assay (BCA assay).
  - Lowry test.

Choosing the compatible method:

- **Best or ideal method ? WHY?**
- Each method has its **advantages** and **disadvantages**.
- How to choose the appropriate method?

➔ Compatibility with the sample.
➔ Availability.
➔ Interfering substances .
➔ Accuracy.
➔ Sensitivity.
➔ Time.
➔ ….

Choosing the compatible method:

Method	Sensitivity	Time	Reagent	Interferences	Disadvantages and comments
Biuret	Low 1-20 mg	Moderate 20-30min	Alkaline copper sulphate	Zwitterionic buffers, Some amino acids	Similar color with all proteins. Destructive to protein samples.
Lowry	High ~ 5 µg	Slow 40-60min	Cu^{+2} Folin–Ciocalteau	Ammonium sulphate, glycine, Zwitterionic, buffers, Mercaptans	Time-consuming. Color varies with proteins. Destructive to protein samples.
Bradford	High ~ 1 µg	Rapid 15 min	Coomassie Brilliant Blue G-250	Strongly basic Buffers, detergents TritonX-100, SDS	Stable color, which varies with proteins. Reagent commercially available. Destruction to protein samples. Discoloration of glassware.
BCA	High ~ 1 µg	Slow 60 min	Cu^{2+}, bicinchoninic acid	EDTA, DTT, Ammonium sulphate	Compatible with detergents. Reagents commercially available. Destructive to Protein samples.
Spectroph -otometric (A_{280})	Moderate 50-100 µg	Rapid	-	Purines, pyrimidines, Nucleic acids	Useful for monitoring column eluents. Nucleic acid absorption can be corrected. None-destructive to protein samples. Varies with proteins.

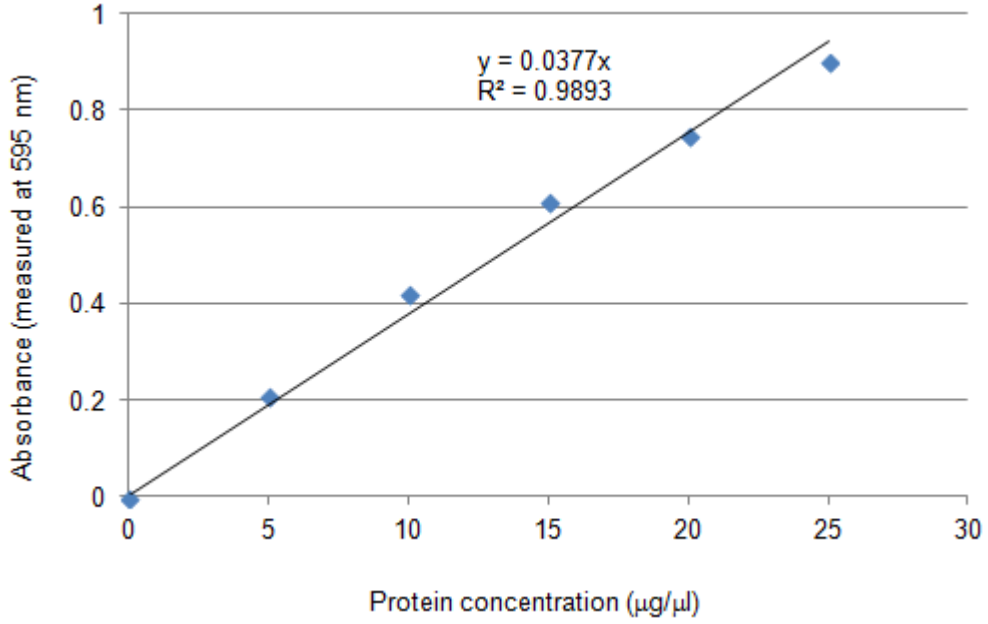
Criteria for choosing an assay:

- Therefore, successful use of protein assays involves selecting the method that is:

  - **Most compatible with the samples to be analysed, choosing an appropriate assay standard, and understanding and controlling the particular assumptions and limitations that remain.**

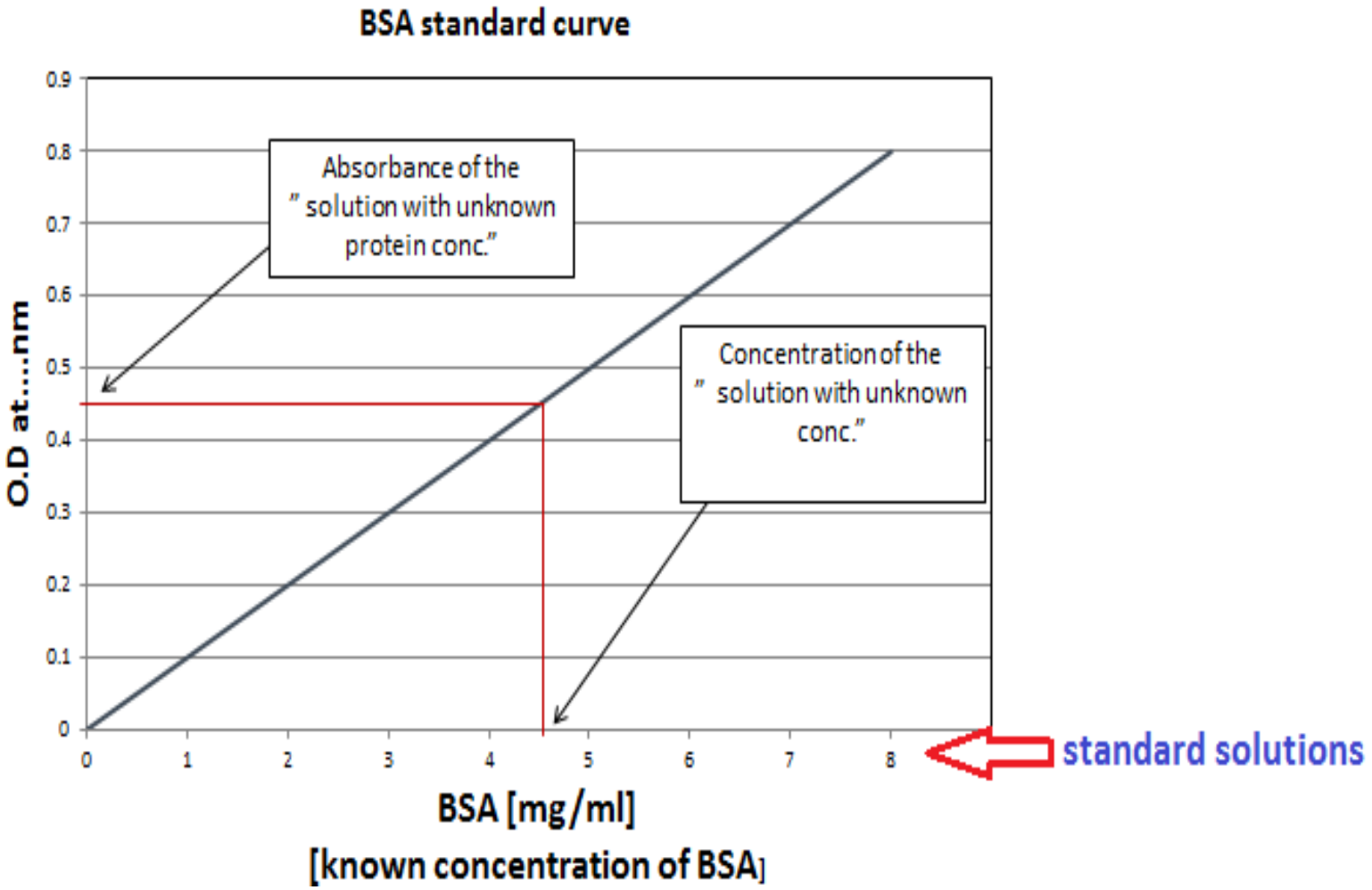
Determination of protein concentration:

- Protein concentration is determined by reference to a standard curve consisting of known concentrations of **a purified reference protein.**



- **Next lab.**
- Typically, standard curves are constructed using at least two replicates for each point on the curve.

Determination of unknown concentration by standard curve:



Practical part

Experiment 1 : Qualitative detection of proteins by biuret test.

Objective:

- To detect the presence of a protein or peptides using biuret test.

Principle:

- In this reaction, peptide bonds in the proteins and peptides treated with an **alkaline solution** of dilute **copper sulphate $CuSO_4$** (biuret reagent) forming a purple coloured complex.
- The colour density is proportional to the amount of proteins present.
- Two or more peptide bonds.
- Name?

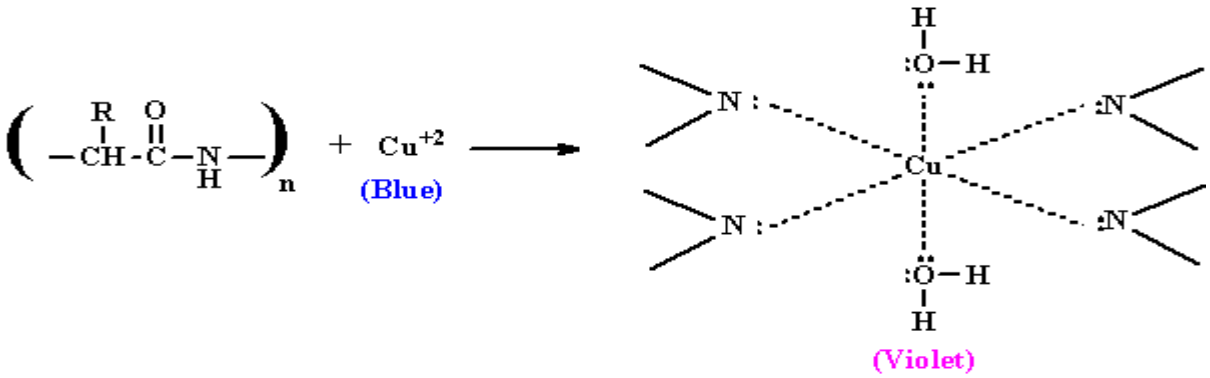


Figure 1. The formation of biuret complex in biuret reaction.

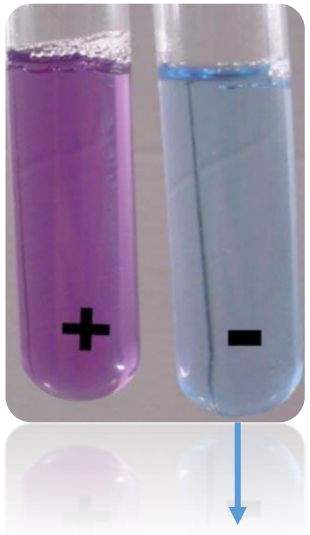
Experiment 1 : Qualitative detection of proteins by biuret test.

Method:

1. Label three test tubes as **A and B.**
2. In tube **A**: add 1 ml of animal crude extract.
3. In tube **B**: add 1 ml of water.
4. Add 1 ml of biuret reagent to all tubes and mix well.

Results:

Tube	Observation
Animal crude extract	
Water	



Blue color is the biuret reagent color

Experiment 2 : Quantitative estimation of proteins by Lowry assay.

Objective:

- To determine the concentration of extracted protein by Lowry assay.

Principle:

- Replaced by the more sensitive methods.
- The method is based on two chemical reactions.
- The resultant strong blue colour is partly dependent on the tyrosine and tryptophan content of the protein sample.

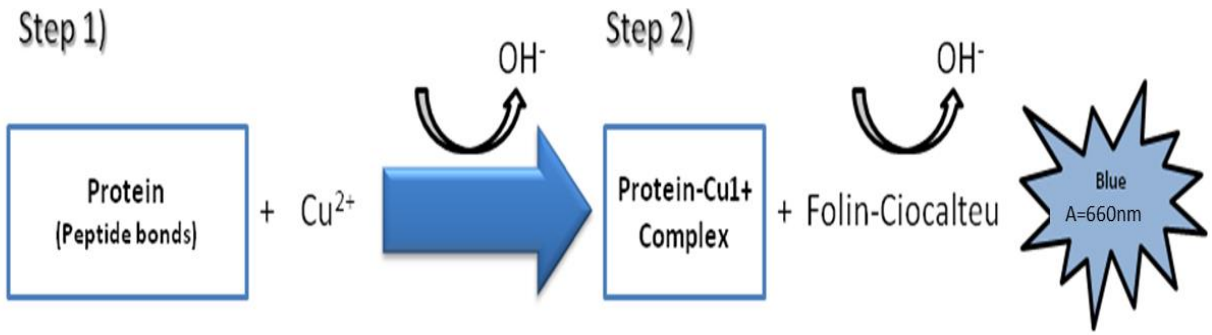


Figure 1. Series of reaction on Lowry method.

Experiment 3 : Quantitative estimation of proteins by biuret assay.

Objective:

- To determine the concentration of extracted protein by biuret assay.

Principle:

- Biuret method is based on copper ions Cu2+ binding to peptide bonds of protein under alkaline condition to give a violet colour that have a **maximum absorbance at 540 nm.**
- The intensity of the color, and hence the absorption at 540 nm, ***is directly proportional to the protein concentration, according to the Beer–Lambert law.***

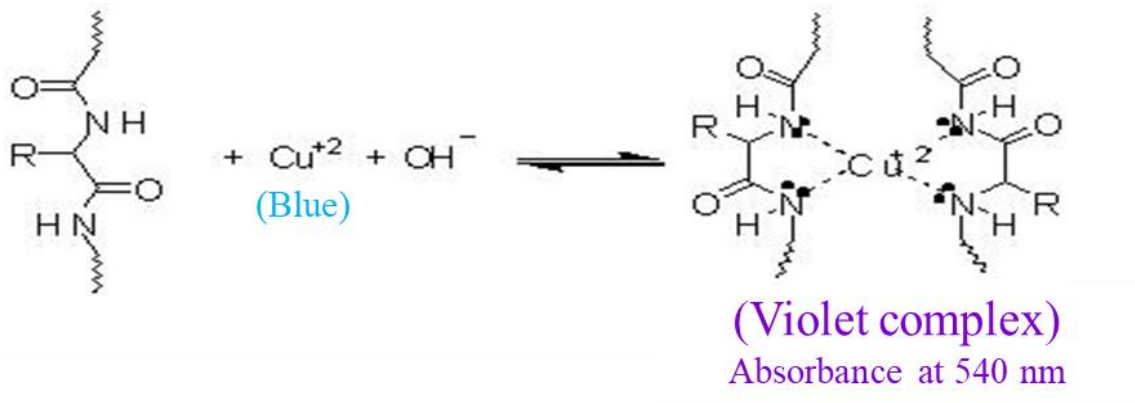


Figure 1. The formation of biuret complex in biuret reaction

From lower to higher concentration

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

Experiment 2 : Quantitative estimation of proteins by biuret assay.

Results:

Table 1. Concentration of standard BSA solution and their absorbance at 540 nm.

Test tube	Protein concentration (g/L) [X- axis]	Absorbance at 540 nm [Y- axis]
Blank		
A		
B		
C		
D		
E		
F		
G		
Animal crude extract (D1)	_____	
Animal crude extract (D2)	_____	
Plant crude extract (D1)	_____	
Plant crude extract (D2)	_____	

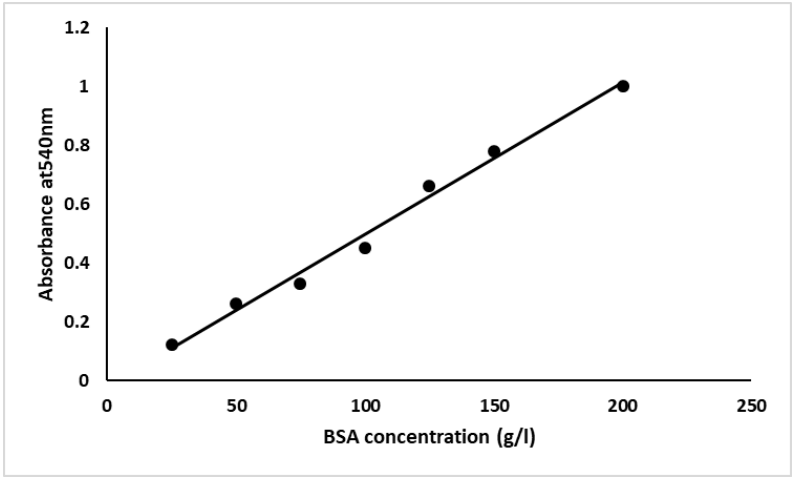


Figure 1. Standard curve of BSA using biuret method.