Detection and quantitative estimation of proteins by different methods

Protein quantification:

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

- Depending on the **accuracy** required and the **amount and purity of the protein** available, different methods are appropriate for determining protein concentration.

Methods:

1. **Direct assay:** measure the absorbance at 280 nm (UV range).
2. **Colorimetric and fluorescent, 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 techniques

biuret test, Bradford test, bicinchoninic acid assay (BCA assay) and Lowry test.

Choosing the compatible method:

- Each method has its advantages and disadvantages.

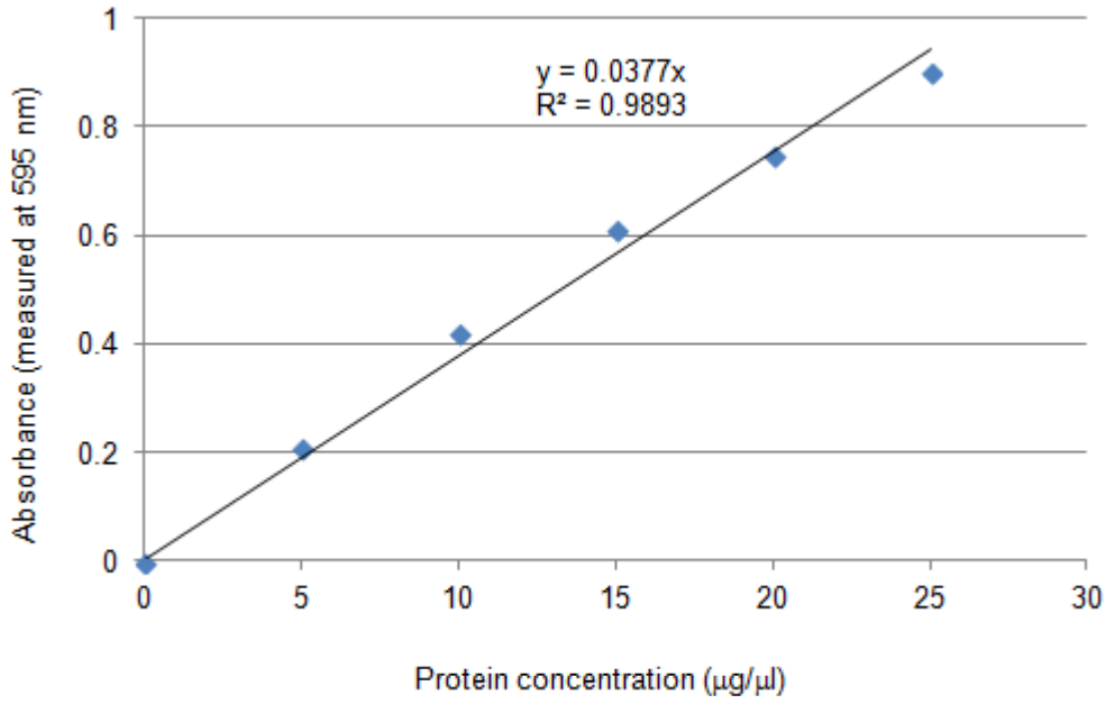
- Choosing an appropriate assay:
- Interfering substances
- Accuracy
- Incubation time
- Availability

Table 1. Comparison of various methods used for total protein concentration determination.

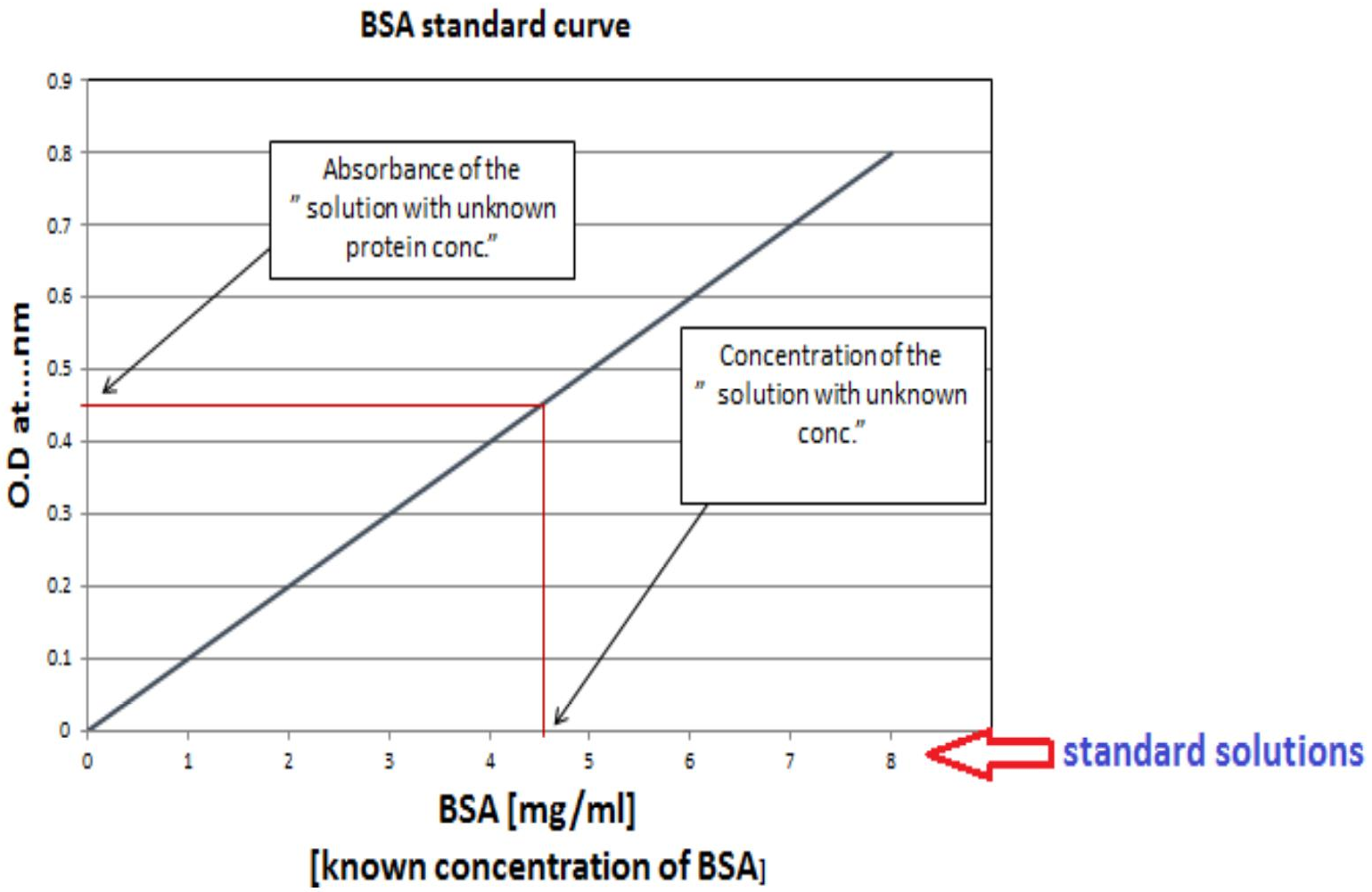
Method	Sensitivity	Time	Reagent	Interferences	Disadvantages and comments
Biuret	Low 1-20 mg	Moderate 20-30min	Alkaline copper sulphate	Zwitterionic buffers, Some amino acids	Similar color with all proteins. Destructive to protein samples.
Lowry	High ~ 5 µg	Slow 40-60min	Cu^{+2} Folin–Ciocalteau	Ammonium 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 Triton X-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.

Determination of protein concentration:

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



Standard curve:



Practical Part

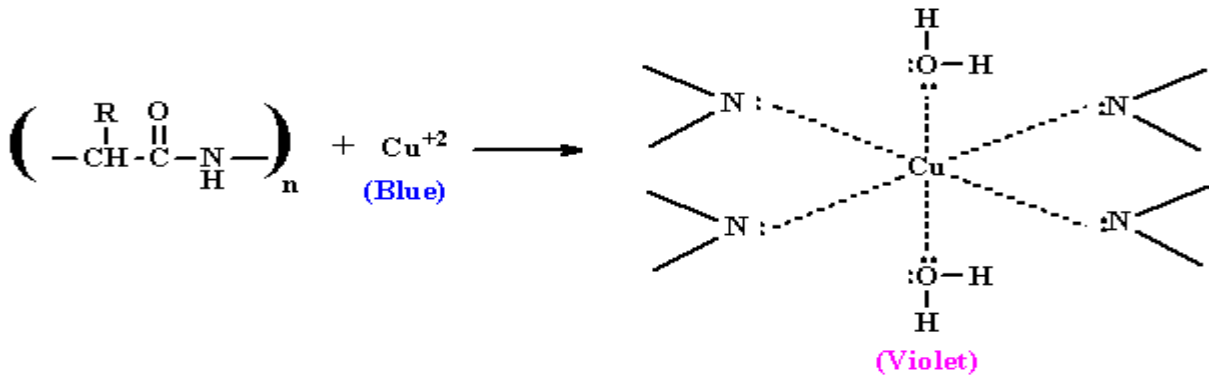
Experiment (1). Qualitative detection of proteins by biuret test:

Objective:

To detect the presence of a protein and peptides using Biuret test.

Principle:

- peptide bonds in the proteins and peptides treated with an alkaline solution of dilute copper sulphate $CuSO_4$ (biuret reagent) forming a **purple colored complex**.
- The color density is **proportional** to the amount of proteins present.
- This test is specific for the peptide bond, positive result will be given if has two or more peptide bonds.



From lower to higher concentration

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.

Result

Tube	Observation
Animal crude extract	
water	

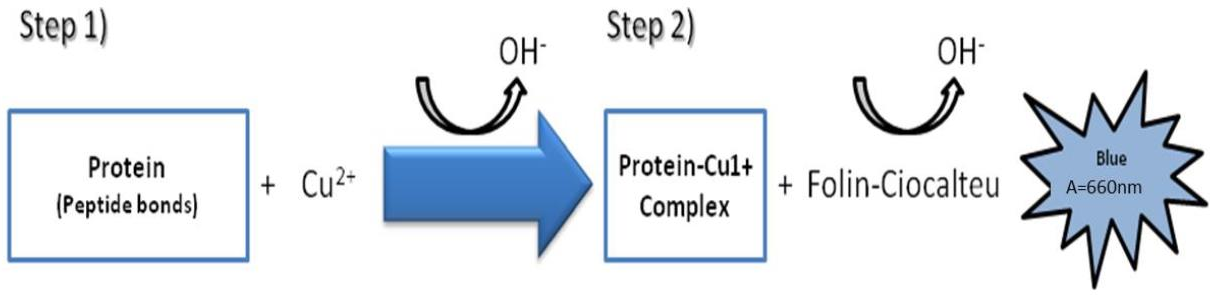
Experiment (2). Quantitative estimation of proteins by Lowry assay:

Objective:

To determine the concentration of extracted protein by Lowry assay.

Principle:

- When the Folin reagent (a mixture of sodium tungstate, molybdate and phosphate), together with a copper sulphate solution, is mixed with a protein solution, a **blue-purple color** is produced.
- The method is based on two chemical reactions.



Experiment (3). Quantitative estimation of proteins by biuret assay:

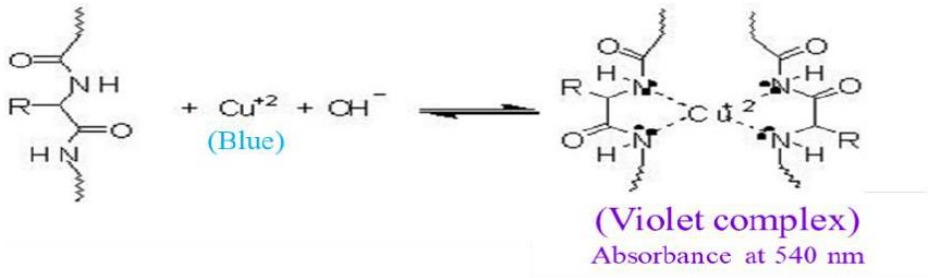
Objective:

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 color 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



Result

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)	_____	
40% pellet	_____	
Dialyzed sample	_____	

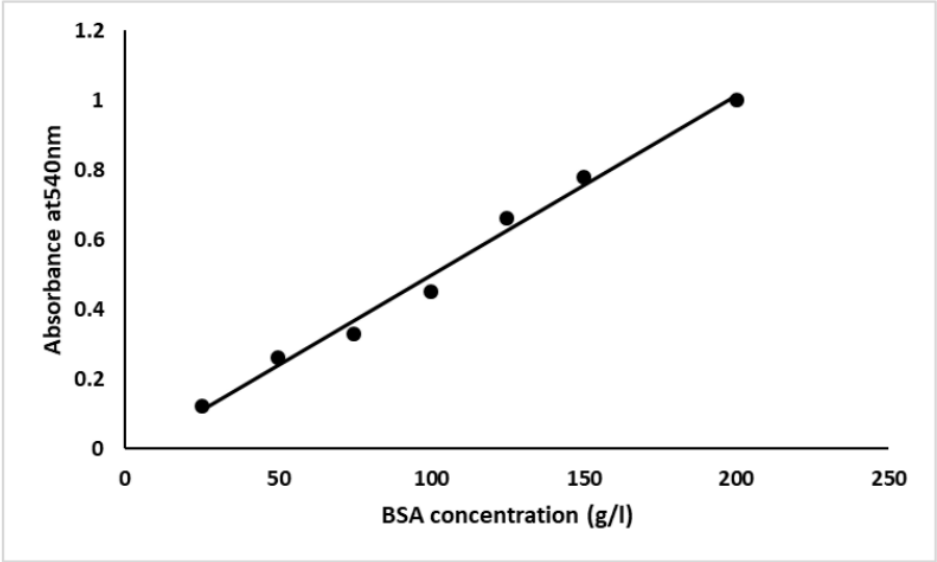


Figure 1. Standard curve of BSA using biuret method.