

Quantitative estimation of proteins by Bradford method

BCH303 [Practical]

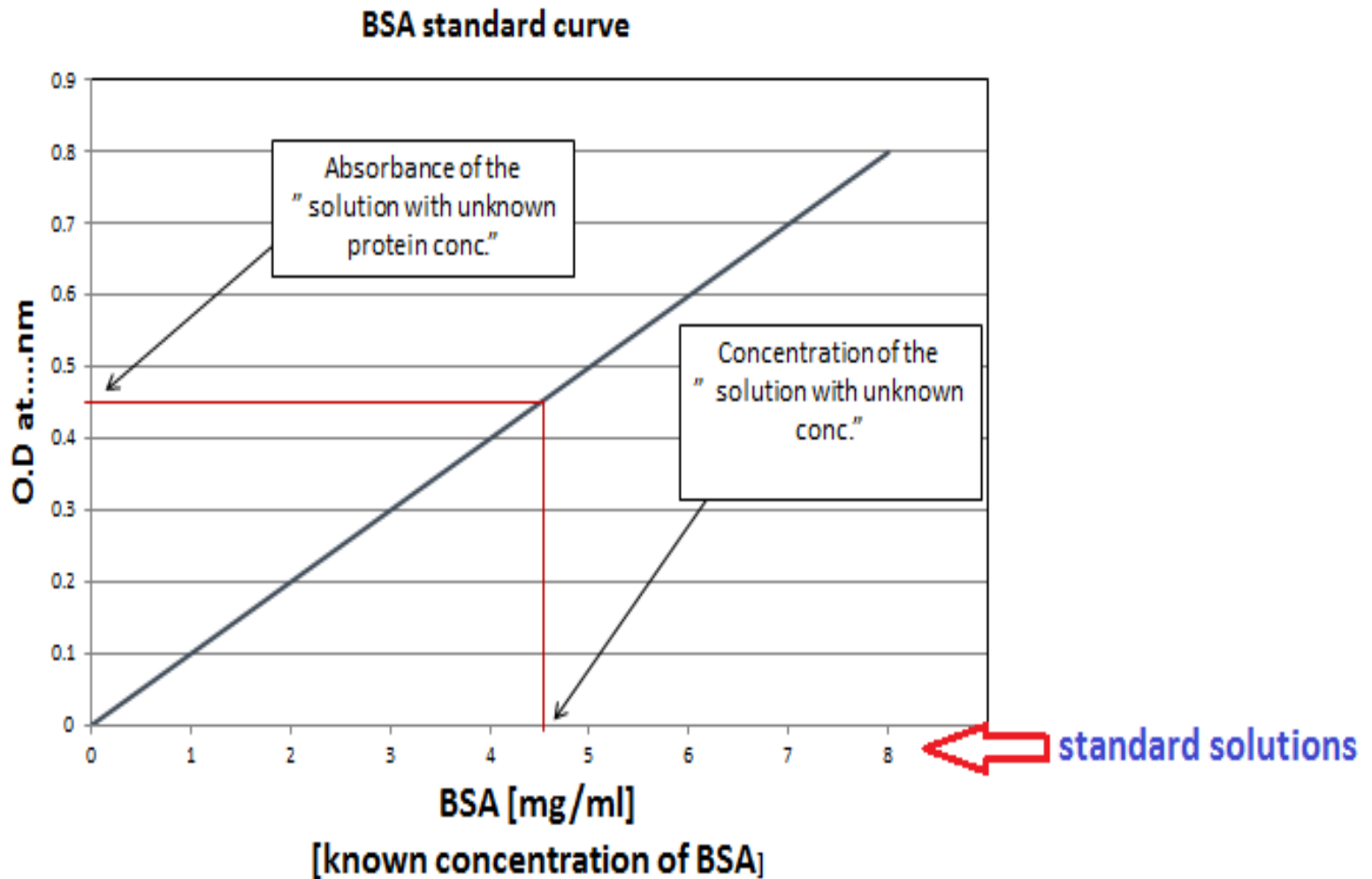
Standard solution:

- Protein concentration is determined by reference to a standard curve consisting of known concentrations of a **purified reference protein**.
- Because proteins **differ in their amino acid compositions**, each one responds somewhat **differently in each type of protein assay**.
- **How to chose a reference standard for your assay ?**
- Bovine serum albumin (BSA).

Constructing a standard curve:

- It is essential to include a standard curve each time the assay is performed.
- 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

Quantitative estimation of proteins by Bradford assay:

Objective:

- To determine the concentration of extracted protein by Bradford assay

Principle:

- Bradford reagent consists of ?
- Blue color.**
- The dye reagent reacts primarily with arginine residues and less so with histidine, lysine, tyrosine, tryptophan, and phenylalanine residues.
- Stable color.

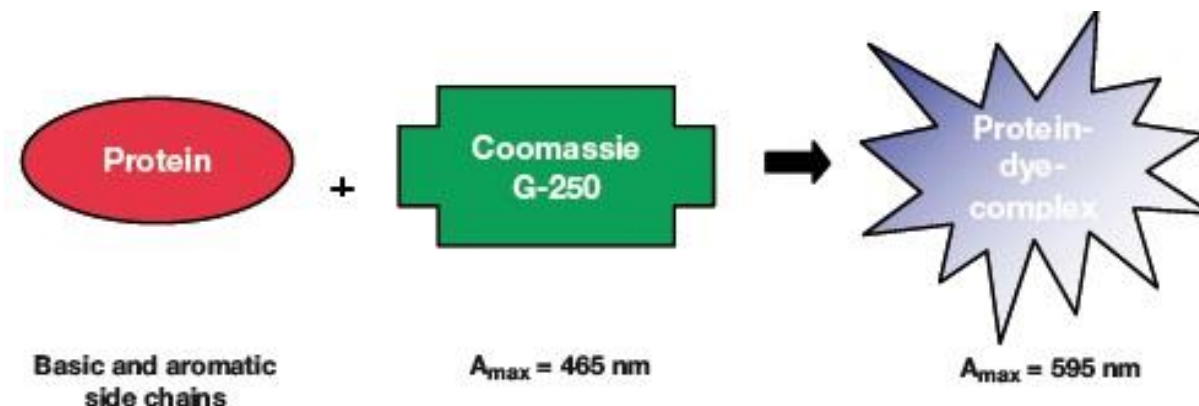
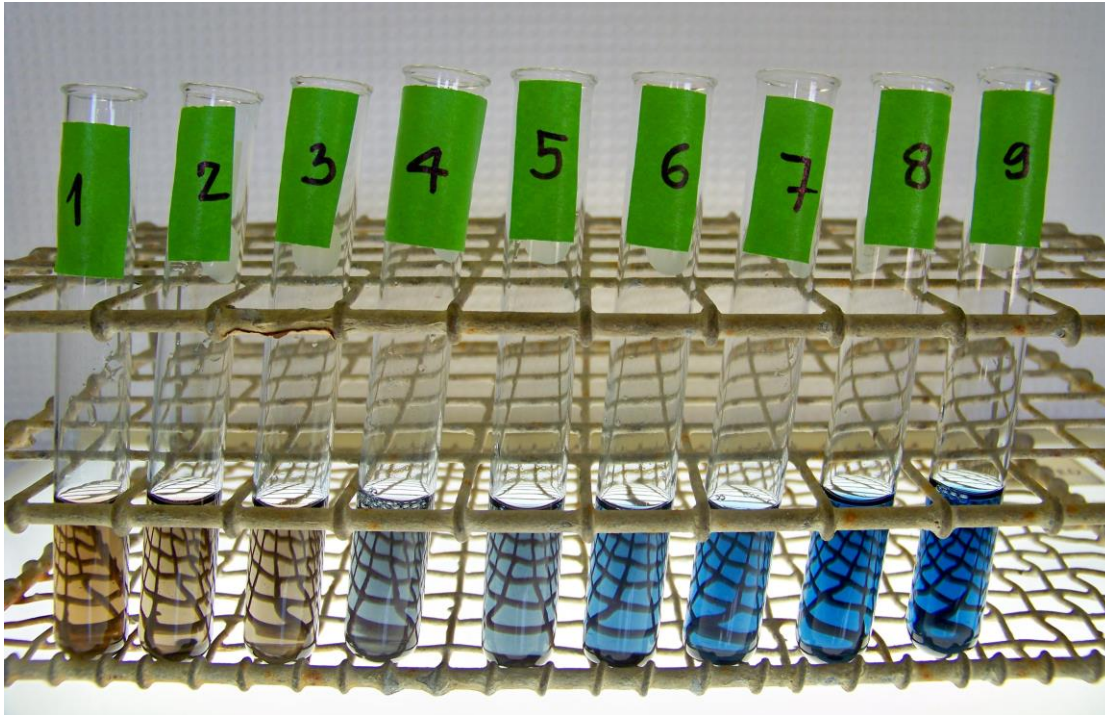


Figure 1. Protein estimation principle using the Bradford method

From lower to higher concentration



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

Quantitative estimation of proteins by Bradford assay:

Results:

Table 1. Concentration of standard BSA solution and their absorbance at 595 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)	_____	

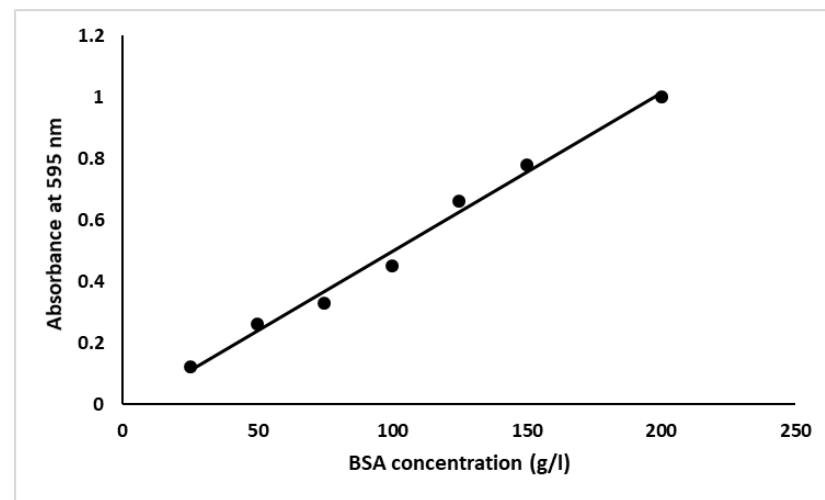


Figure 1. Standard curve of BSA using Bradford method.