Quantitative estimation of proteins by Bradford method

Standard solution:

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

- 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:

BSA standard curve

standard solutions
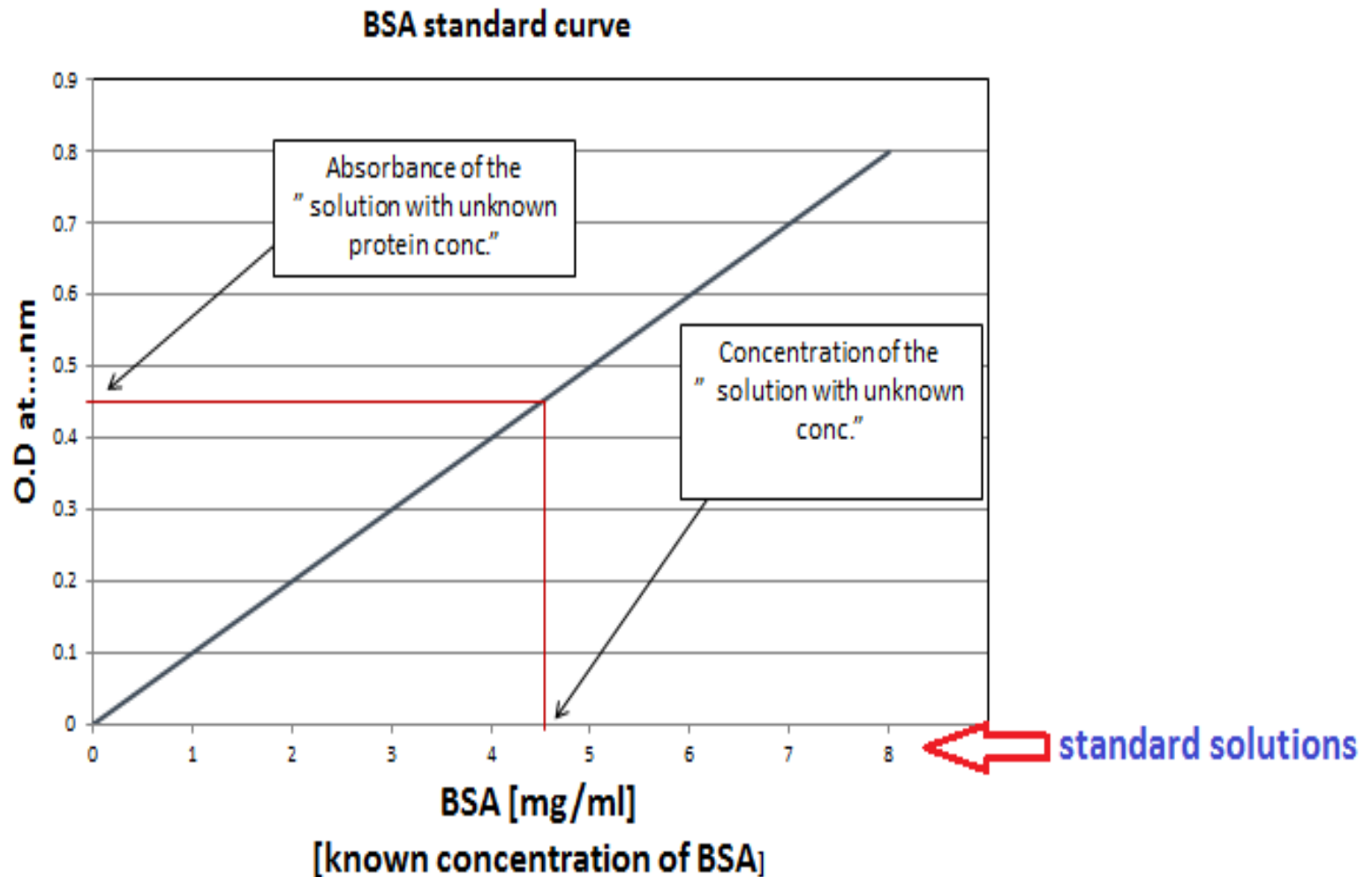
Practical part

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.*
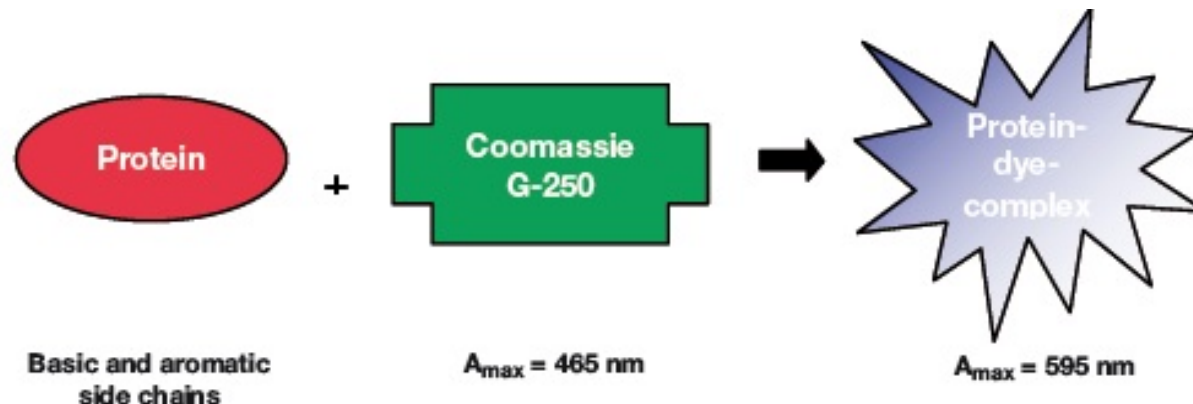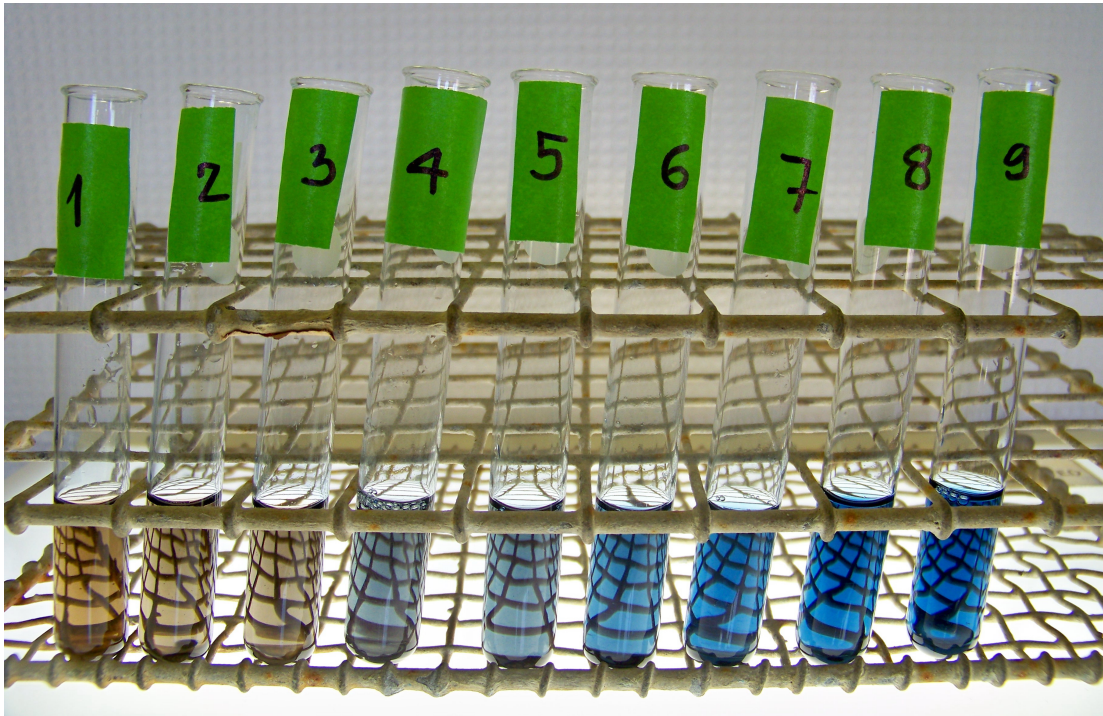- Stable color.

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.

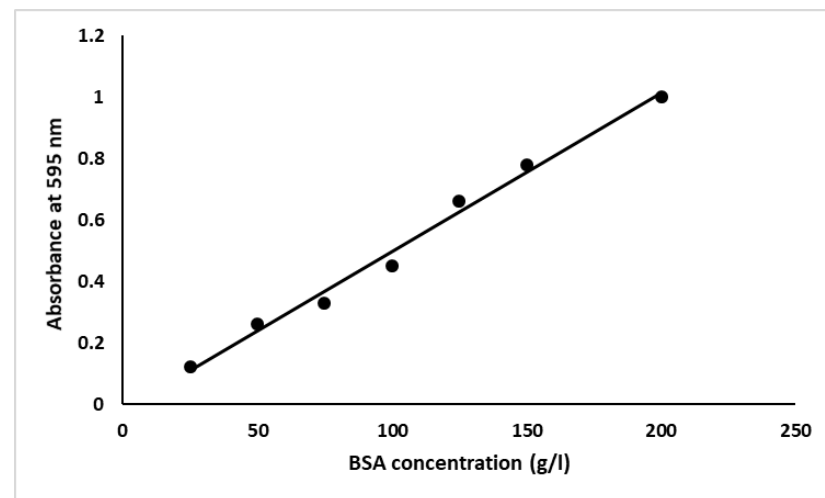| Test tube	Protein concentration (g/L) [X- axis]	Absorbance at 540 nm [Y- axis]
Blank		
A		
B		
C		
D		
E		
F		
G		
Animal crude extract (D1)		
Animal crude extract (D2)		
Plant crude extract (D1)		
Plant crude extract (D2)		

Figure 1. Standard curve of BSA using Bradford method.

Homework

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