



Biochemistry of Proteins BCH 303 [Practical]

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**Lab (7) Quantitative estimation of proteins by  
Bradford's method**

Emtenan Mohammed Alkhudair

Office: Building 5, 3<sup>rd</sup> floor, Office No. 269

E.mail: [ealkhudair@ksu.edu.sa](mailto:ealkhudair@ksu.edu.sa)

Website: <http://fac.ksu.edu.sa/ealkhudair>

# Standard solution

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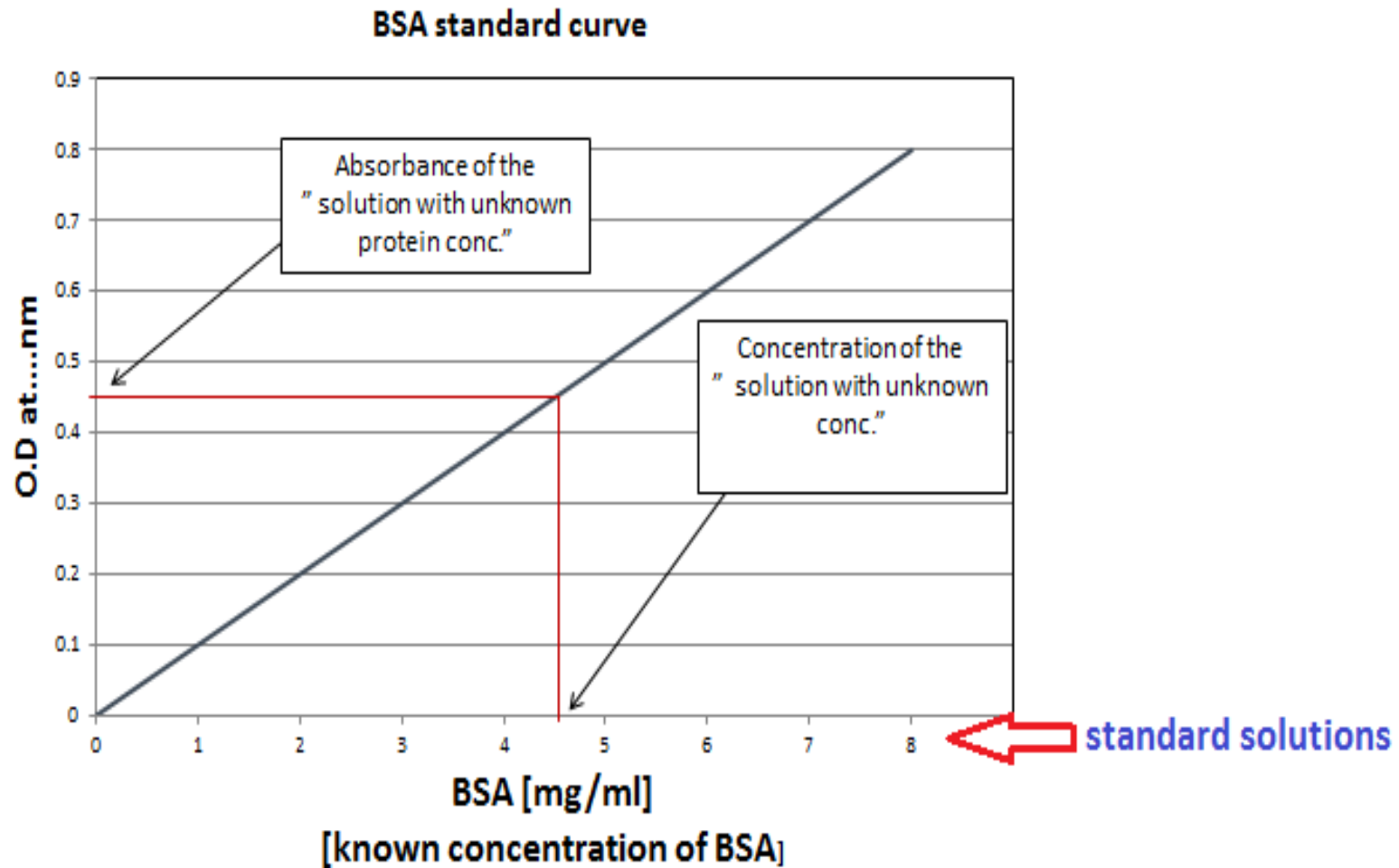
- Protein concentration is determined by reference to a standard curve consisting of known concentrations of a **purified reference protein**.
- Because proteins **differ in their amino acid compositions**, each one responds somewhat **differently in each type of protein assay**.
- **How to chose a reference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

# Constructing a standard curve

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- 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:

- The Bradford reagent consists of the dye **Brilliant Blue G** in phosphoric acid and methanol or ethanol.
- This method relies on forming a complex by the binding of the dye **Coomassie Brilliant Blue G-250** to the proteins resulting in a shift in the absorption maximum of the dye from 465 to 595 nm.
- The absorption at 595 nm is proportional to the amount of protein present in the sample.
- The dye reagent reacts primarily with arginine residues and less so with histidine, lysine, tyrosine, tryptophan, and phenylalanine residues.
- **Advantages:** 1. Simple to prepare 2. The colour develops rapidly 3. Resulting colour is stable

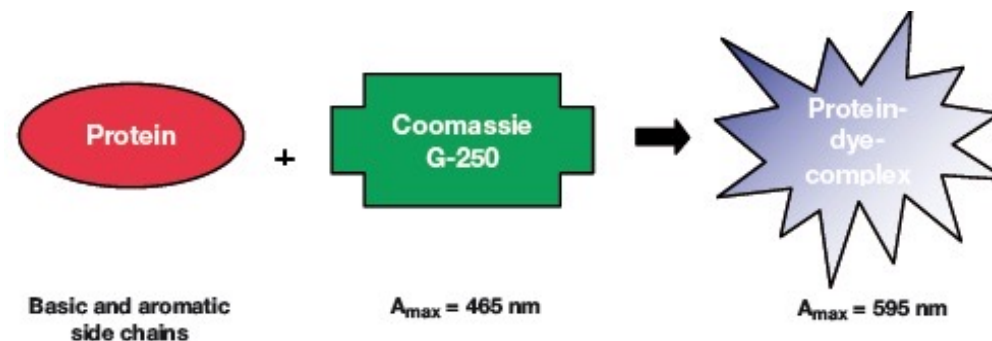
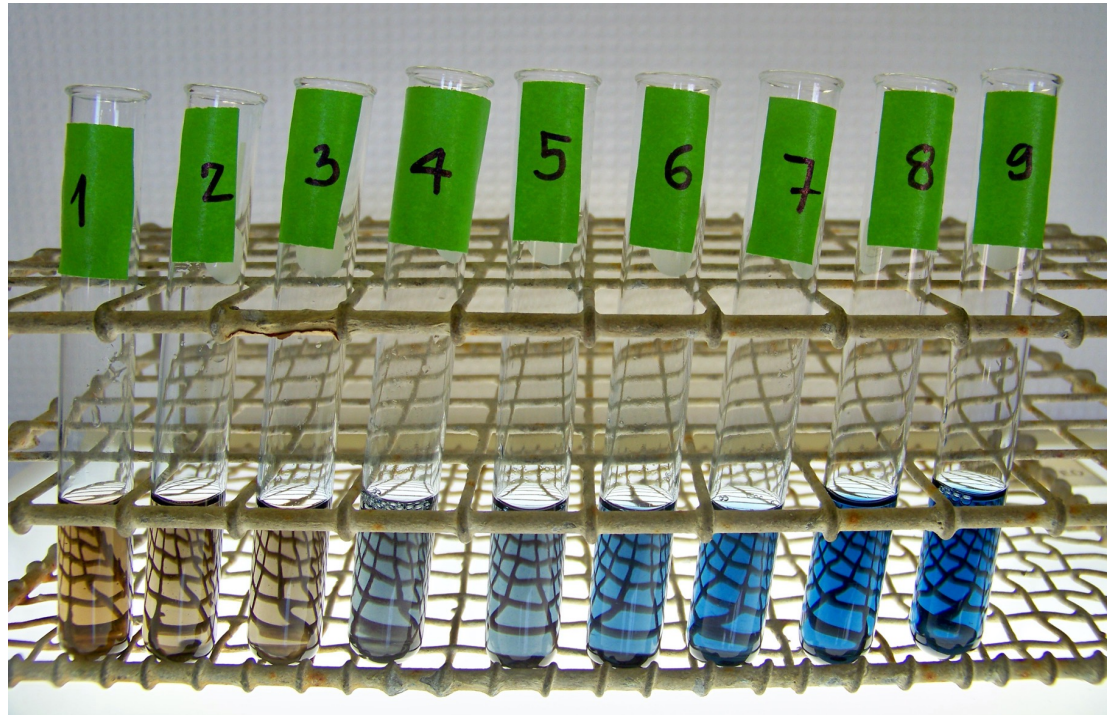


Figure 1. Protein estimation principle using the Bradford method

From lower to higher concentration



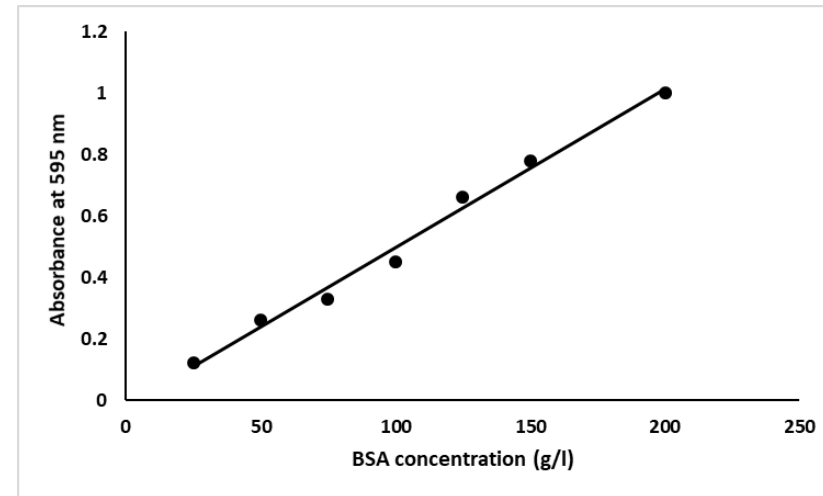
There is a **linear relationship** between blue color developed and protein concentration.

# 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.**