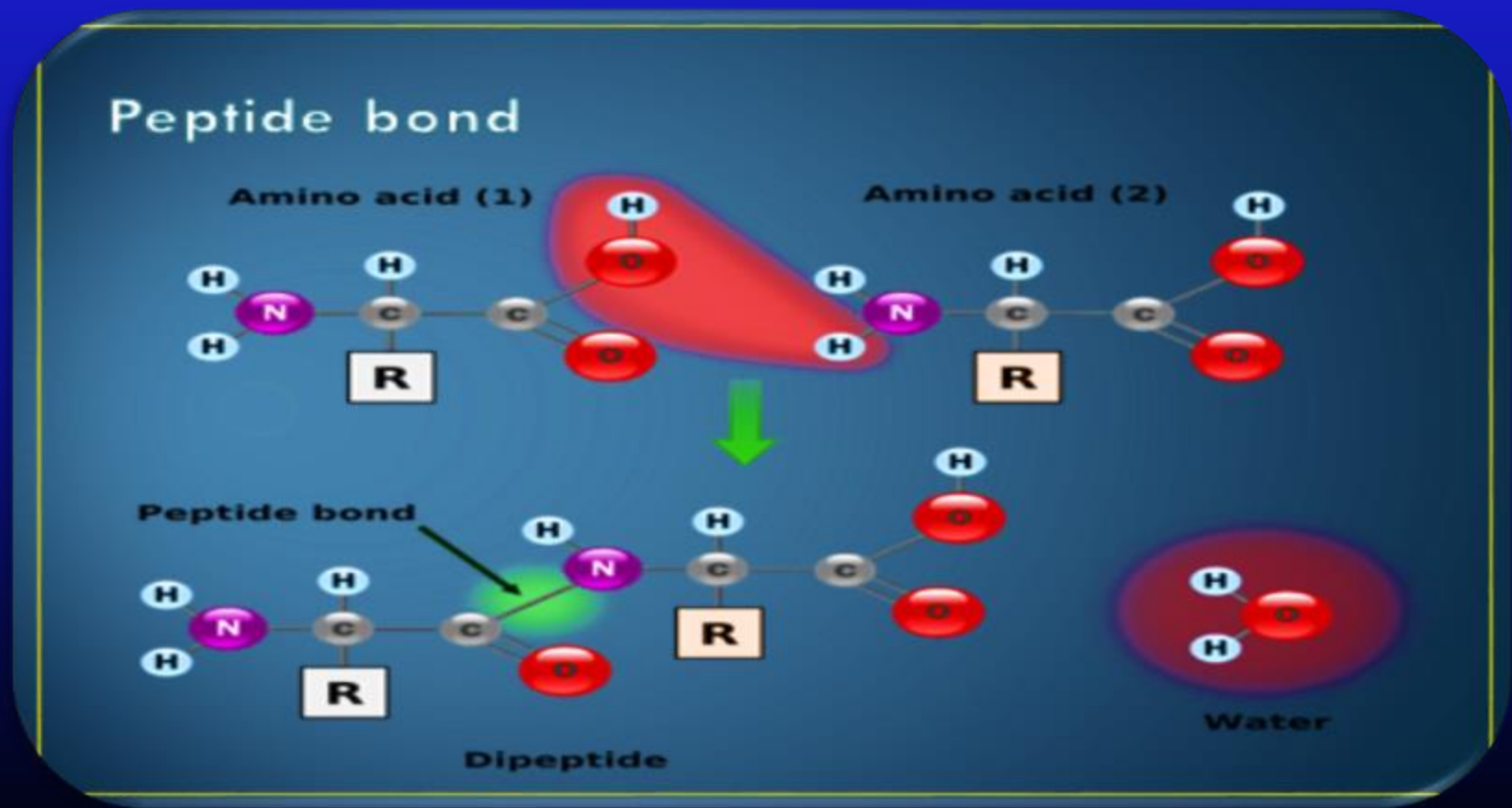


DETECTION & QUANTITATION OF PROTEINS

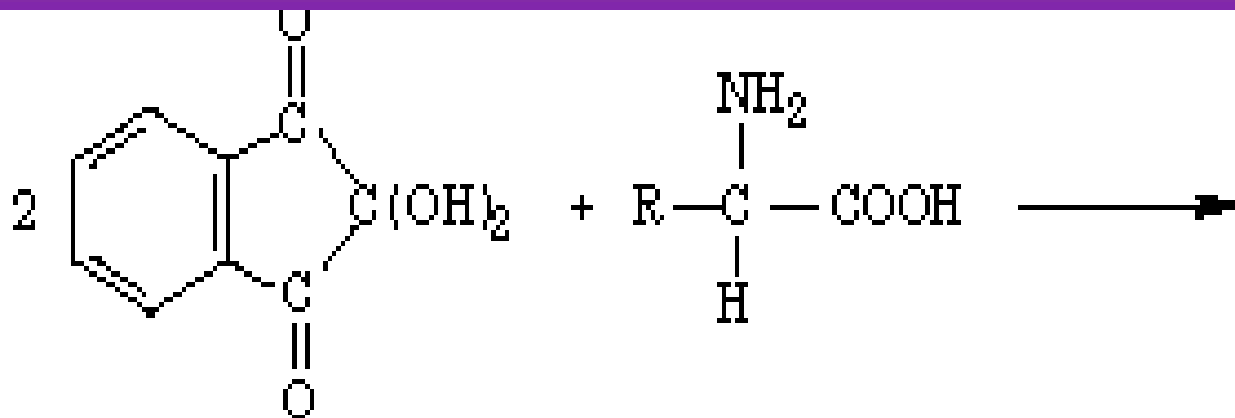
What is protein ?

Amino acid chains are linked by peptide bonds in condensation reactions

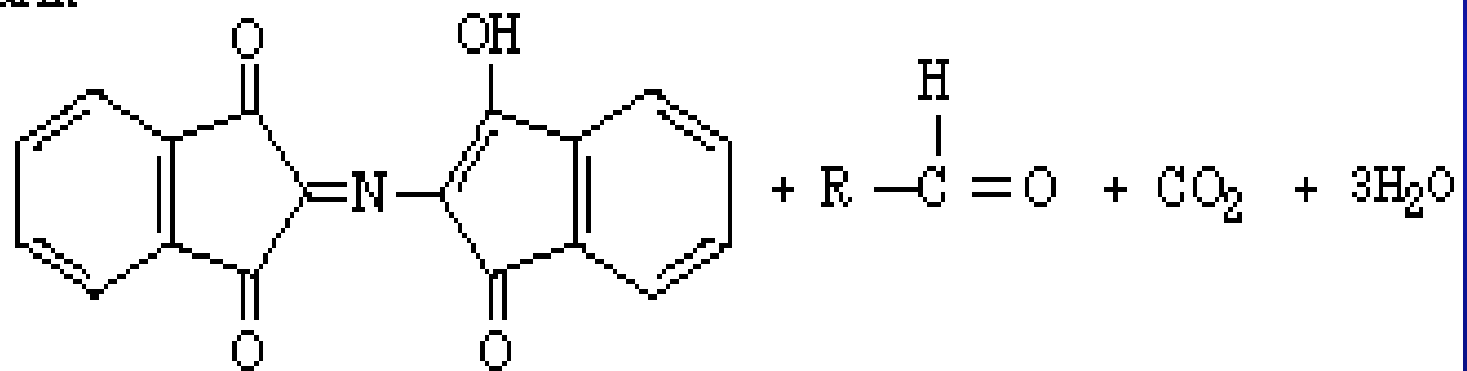


Ninhydrin Test

- ⦿ Ninhydrin is a chemical used to detect free amino acid and proteins
- ⦿ Amino acids(NH_2) also react with ninhydrin at $\text{pH}=4$.
- ⦿ The reduction product obtained from ninhydrin then reacts with NH_3 and excess ninhydrin to yield a dark blue or purple violet colored substance.
- ⦿ This reaction provides an extremely sensitive test for amino acids.

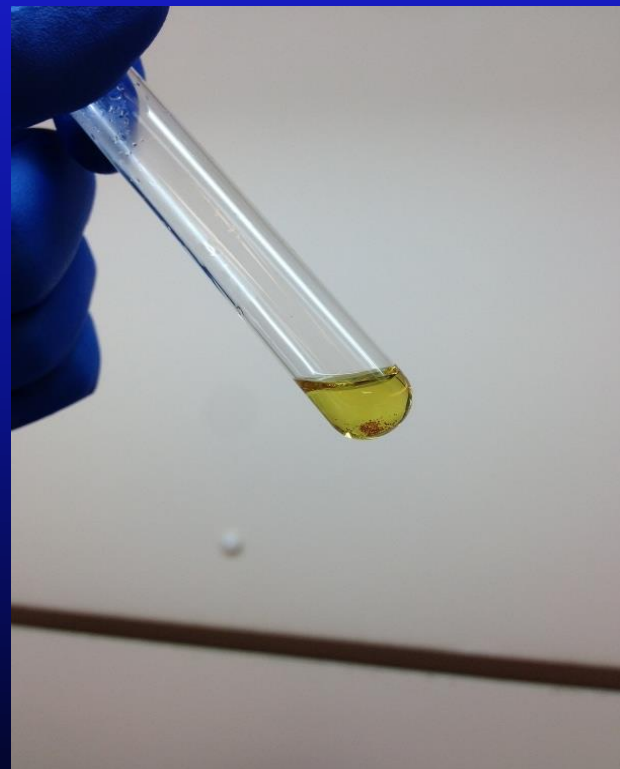


Ninhydrin

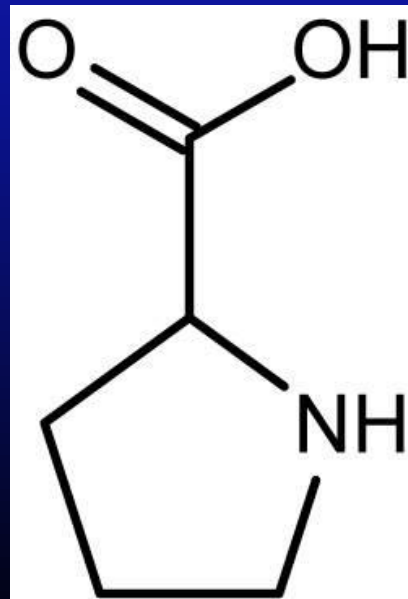


"Purple color"

- With all amino acids will give purple violet or deep blue with exception **Proline** gives **yellow** not violet (why)



- Proline reacts with ninhydrin, but in a different way. While most ninhydrin tests result in a purple violet color, the proline reaction is more yellow due to substitution of the alpha amino group that ninhydrin reacts with carbon rings



Procedure: ○

- To 1 mL solution add 5 drops of 2% ninhydrine solution
- Boil over a water bath for 2 min.
- Allow to cool and observe the purple color formed.

Bradford method

- use of coomassie brilliant blue dye in a colorimetric reagent for the detection and quantitation of total protein.
- In the acidic environment of the reagent protein binds to the coomassie dye
- This results in a special shift from the reddish/brown form of the dye absorbance maximum at 465 nm to the blue form of the dye absorbance maximum at 610 nm

-
- The differences between the two forms of the dye is greatest at 595nm, so that is the optimal wavelength to measure the blue color from the coomassie dye protein complex.
 - development of color in coomassie dye based bradford protein assays has been associated with the presence of certain basic amino acids primarily arginine, lysine ,histidine in the protein.

- **A dvantages of the method include that:**

it is highly sensitive,

it is able to measure 1-20 μg of protein

it is very fast.

Principle of Bradford Assay



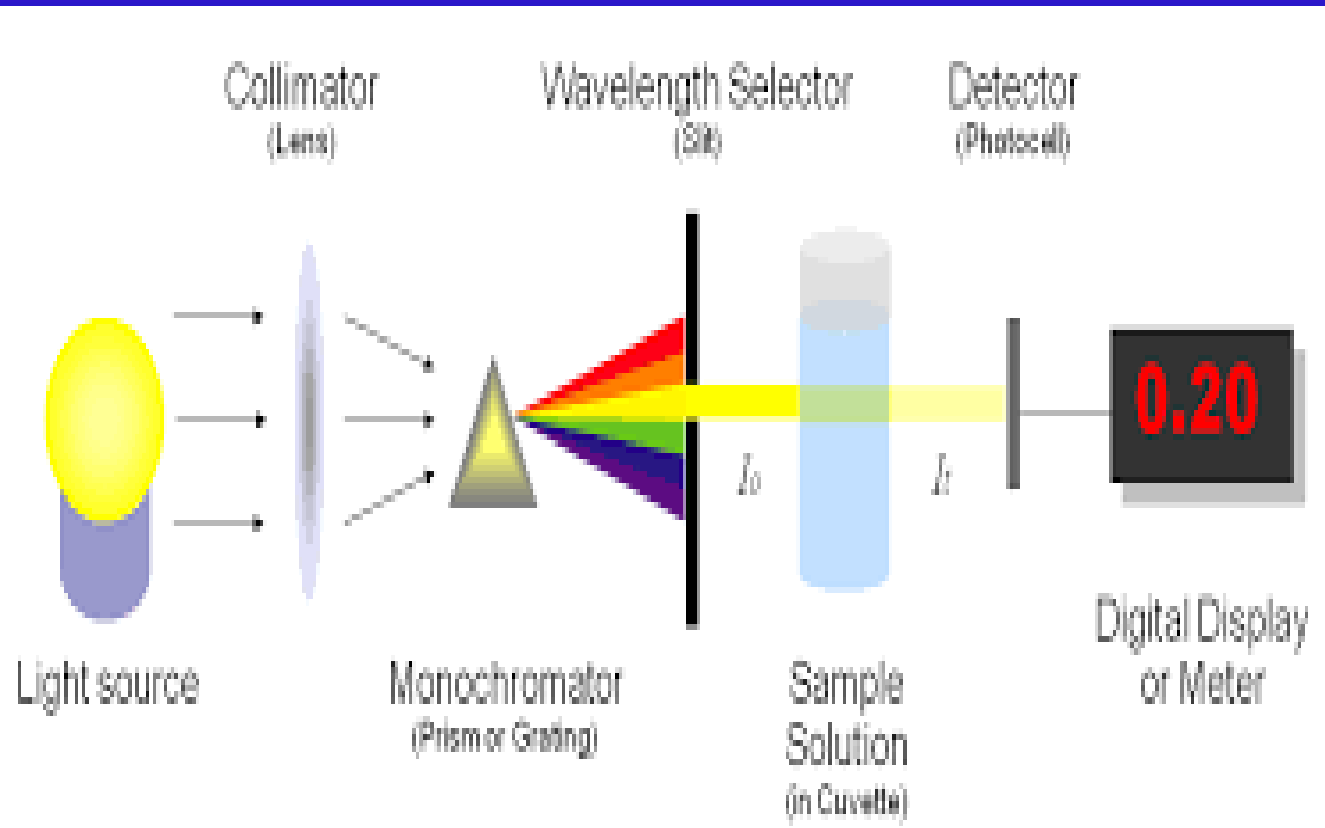
465 nm

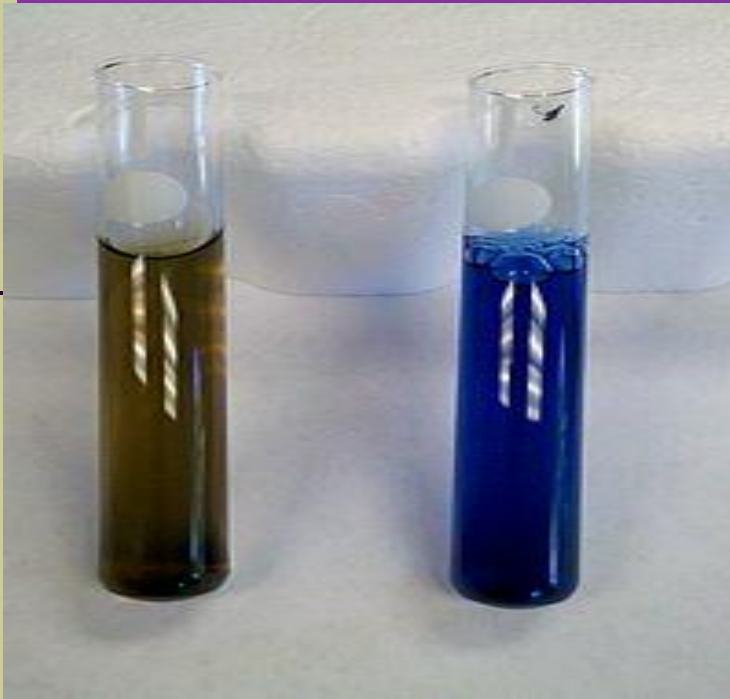


595 nm



Spectrophotometer

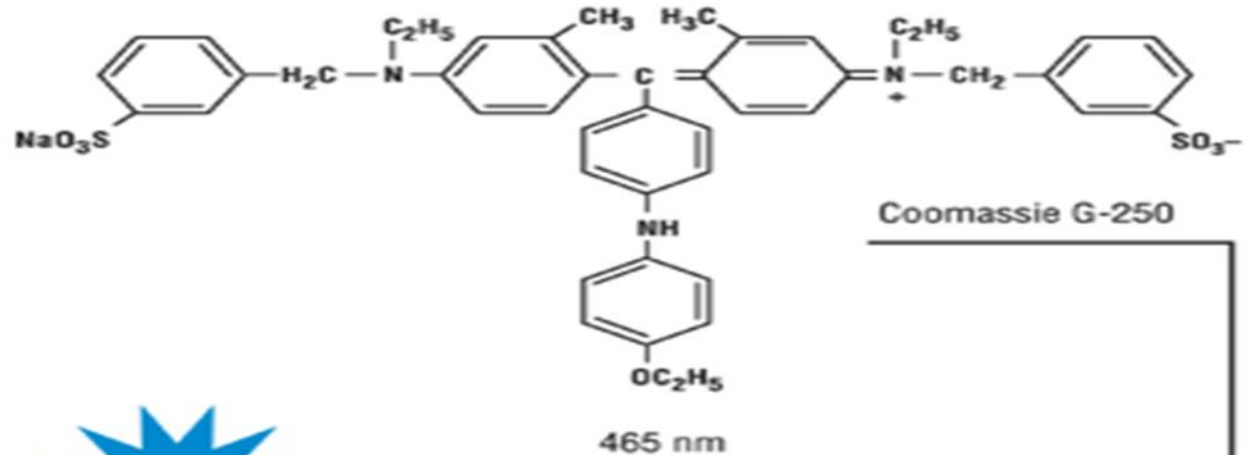




Samples treated with the Bradford assay. The brown sample (lower absorbance) contains no protein, while the blue sample (higher absorbance) contains protein. The amount of protein in the second sample can be determined by comparison to a standard curve

PROTEIN
Basic and Aromatic
Side Chains

+



Coomassie G-250

BLUE
 $A_{\text{max}} = 595 \text{ nm}$

465 nm

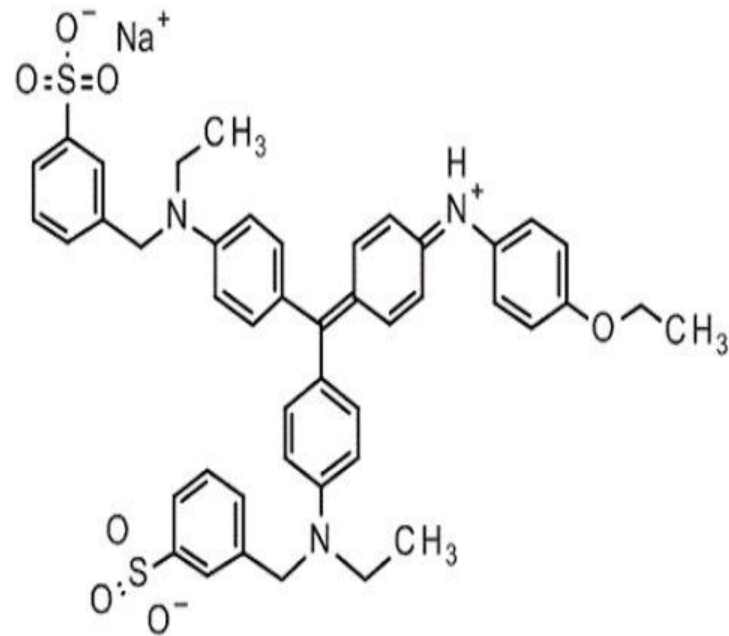
Protein-Dye Complex

Principle of Bradford Assay

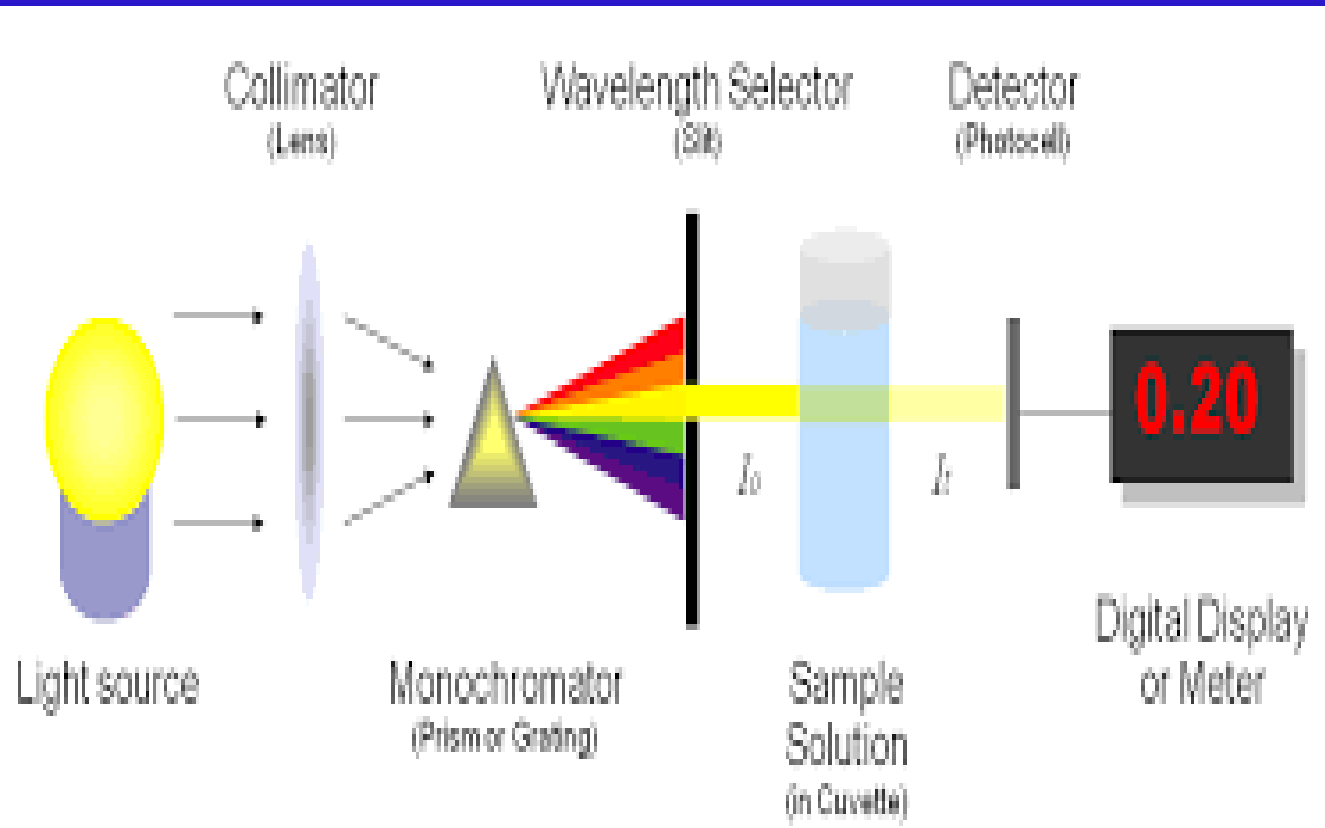
Unstable
Cationic
CBB

Protein

Stable
Anionic
CBB

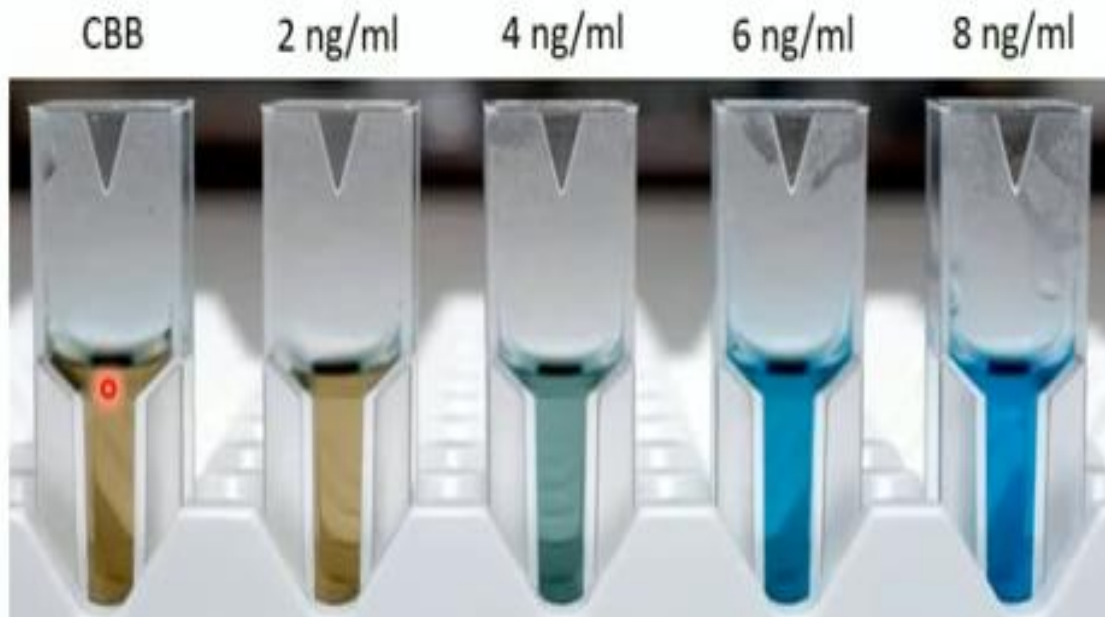


Spectrophotometer



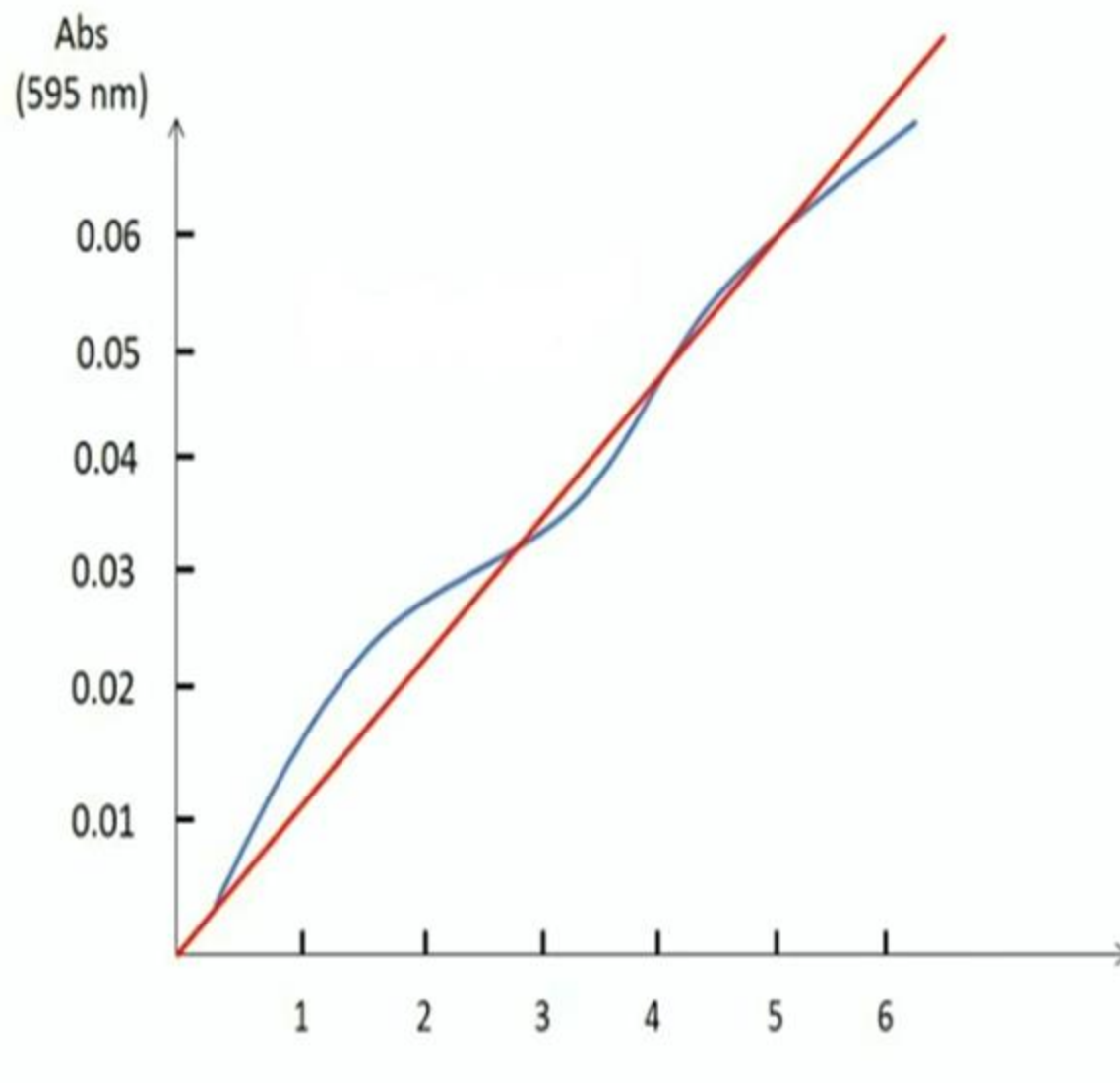
Measurement of the Protein Concentration

Calibration curve



Absorbance
(595 nm)

Measurement of the Protein Concentration



Calibration curve



Protein sample
Unknown concentration



