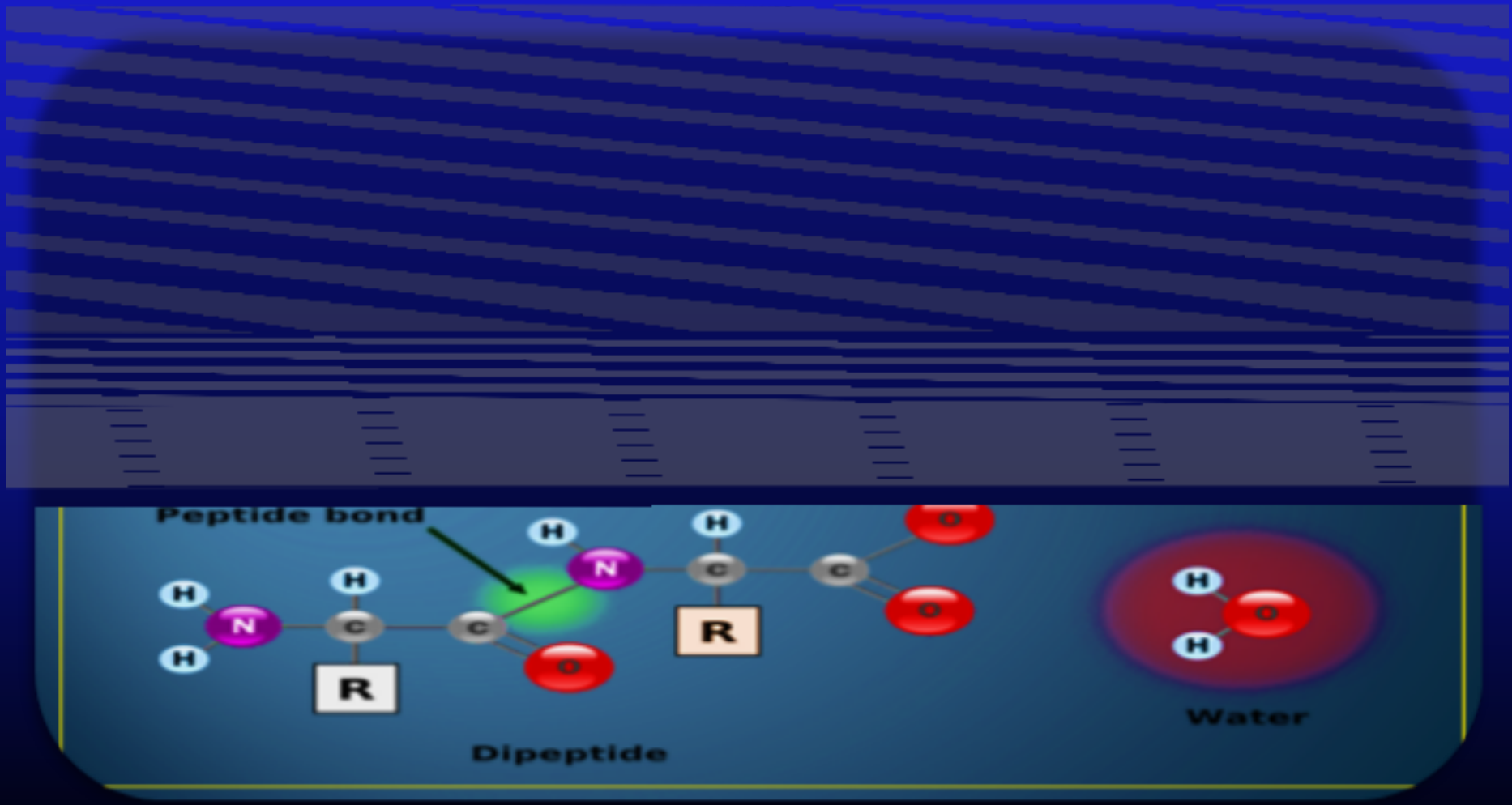
DETECTION &QUANTITATION OF PROTEINS

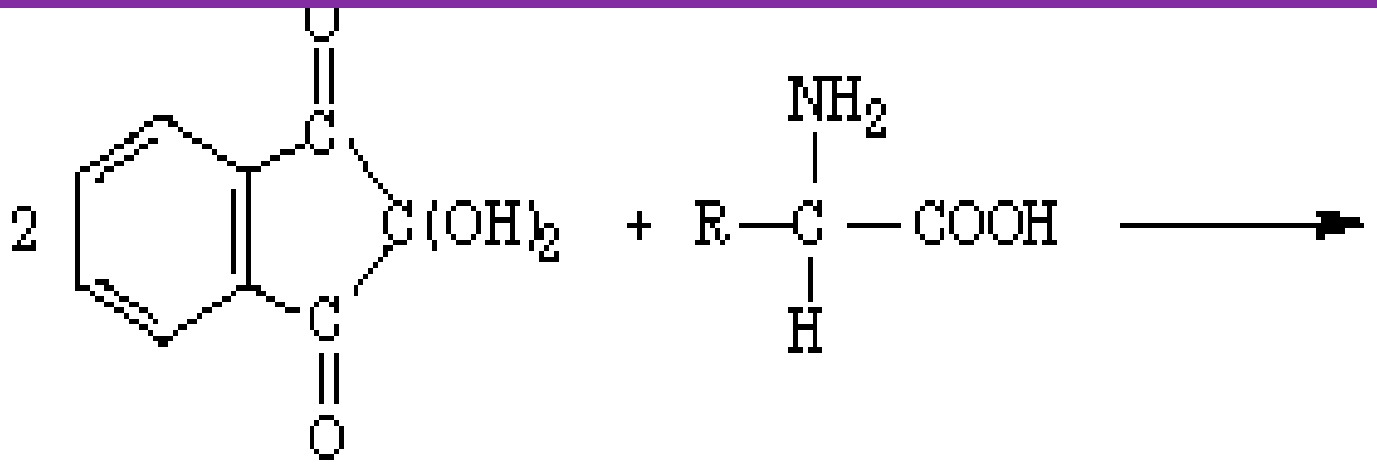
What is protein ?

Amino acid chains are linked by peptide bonds in condensation reactions

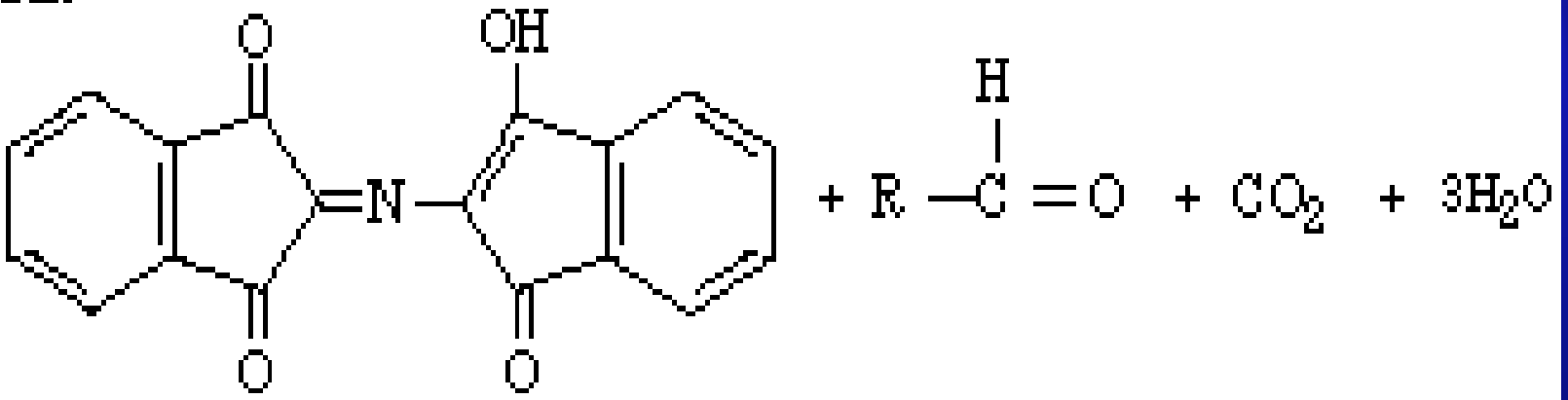


Ninhydrin Test

- Ninhydrin is a chemical used to detect free amino acid and proteins
- Amino acids(NH2) also react with ninhydrin at pH=4.
- The reduction product obtained from ninhydrin then reacts with NH3 and excess ninhydrin to yield a **dark blue or purple violet** colored substance.
- This reaction provides an extremely sensitive test for amino acids.

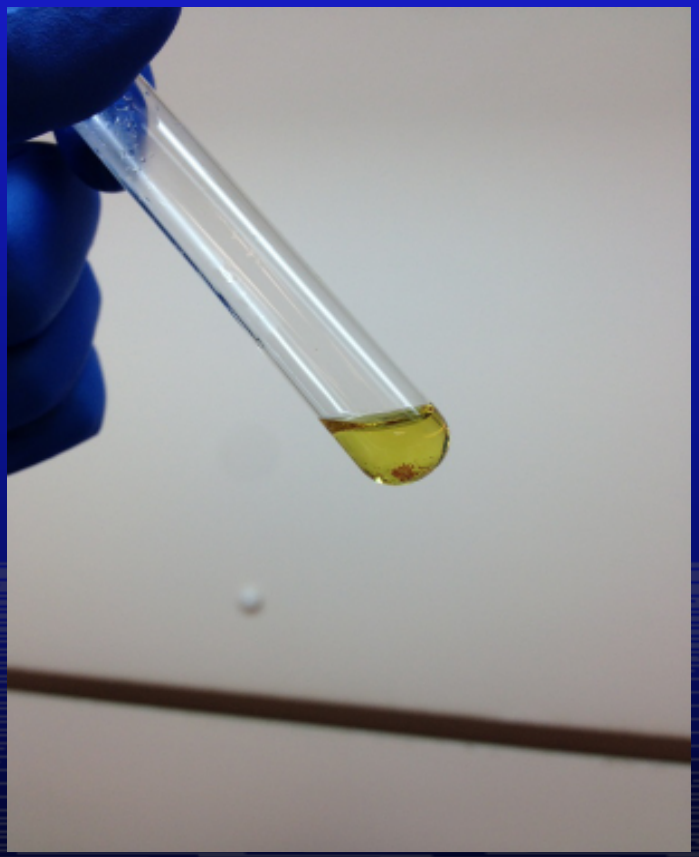


Ninhydrin

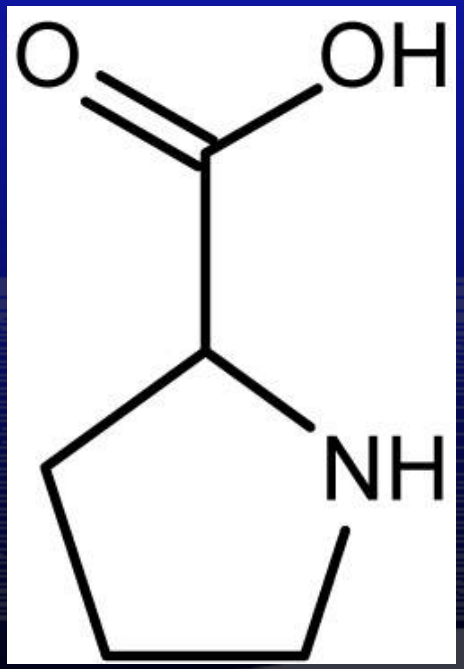


"Purple color"

- With all amino acid 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 465nm to the blue form of the dye absorbance maximunm at 610nm

- 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.

- **Advantages of the method include that:**

it is highly sensitive,

it is able to measure 1-20 µg of protein

it is very fast.

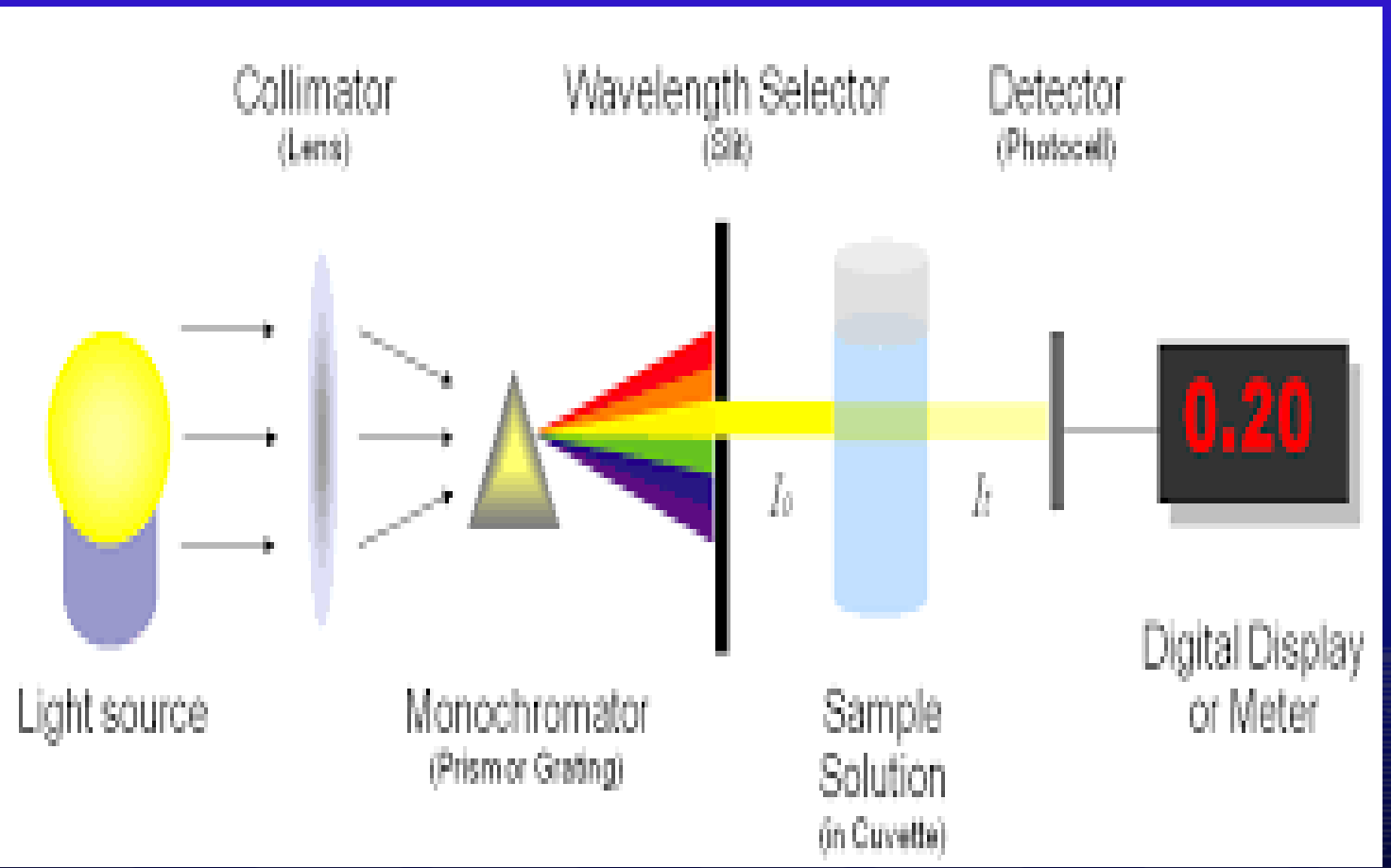
Principle of Bradford Assay

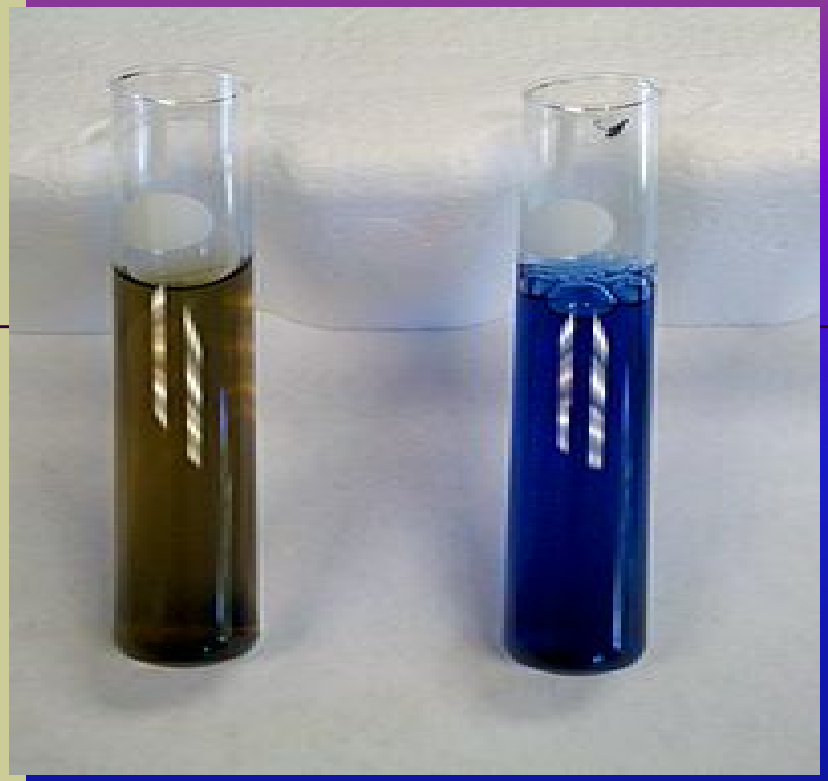
465 nm

595 nm

↑ Abs 595 → ↑ Blue → ↑ Protein concentration

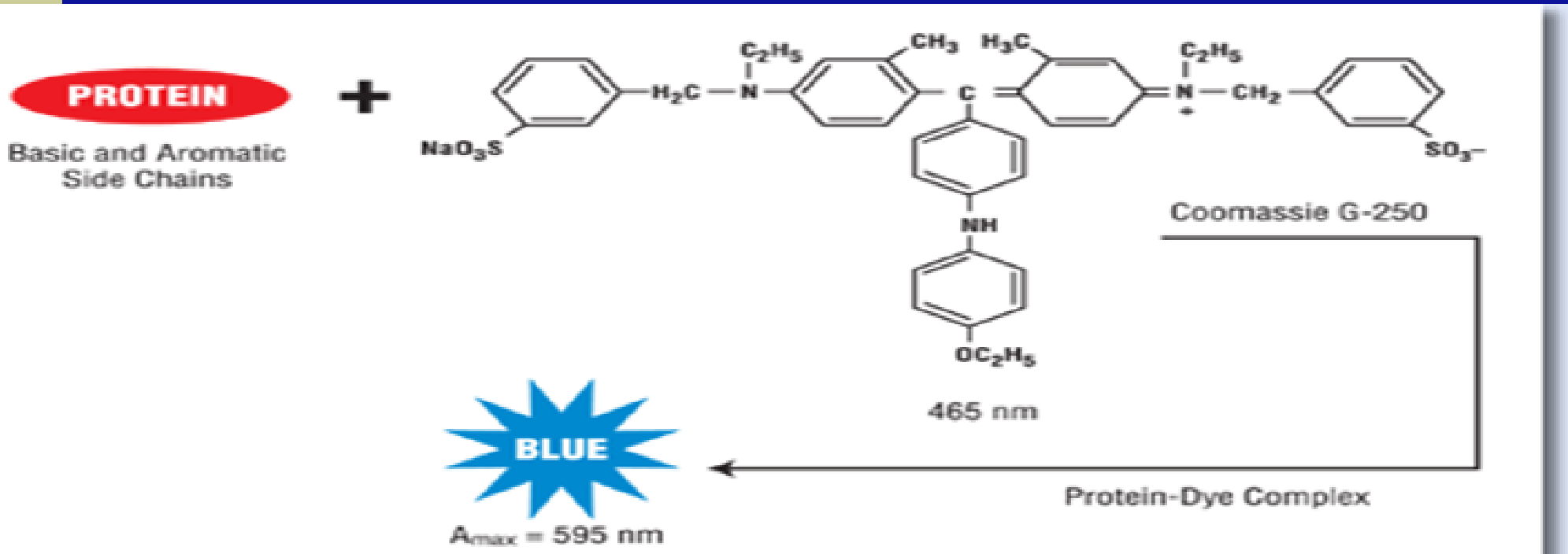
Spectrophotometer





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

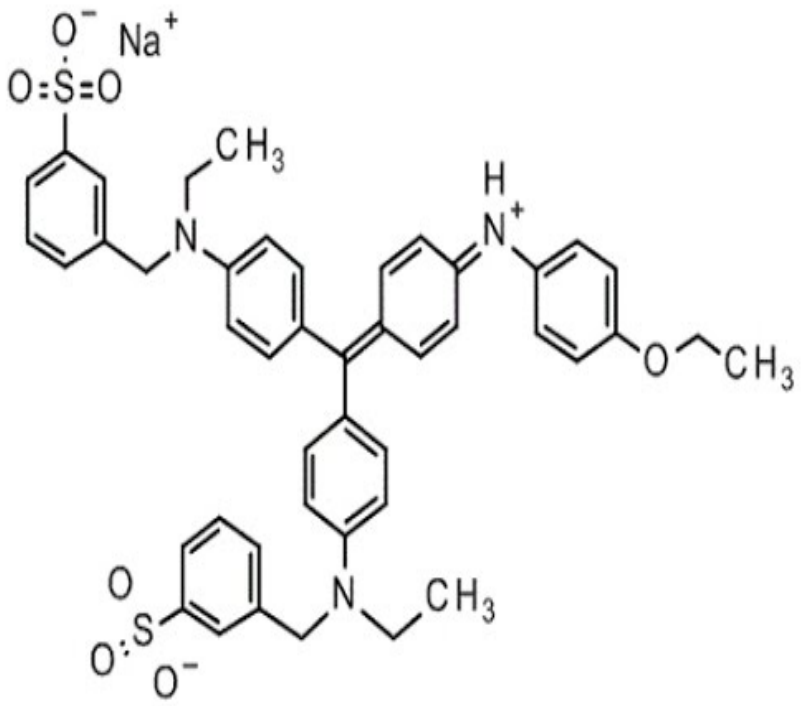


Principle of Bradford Assay

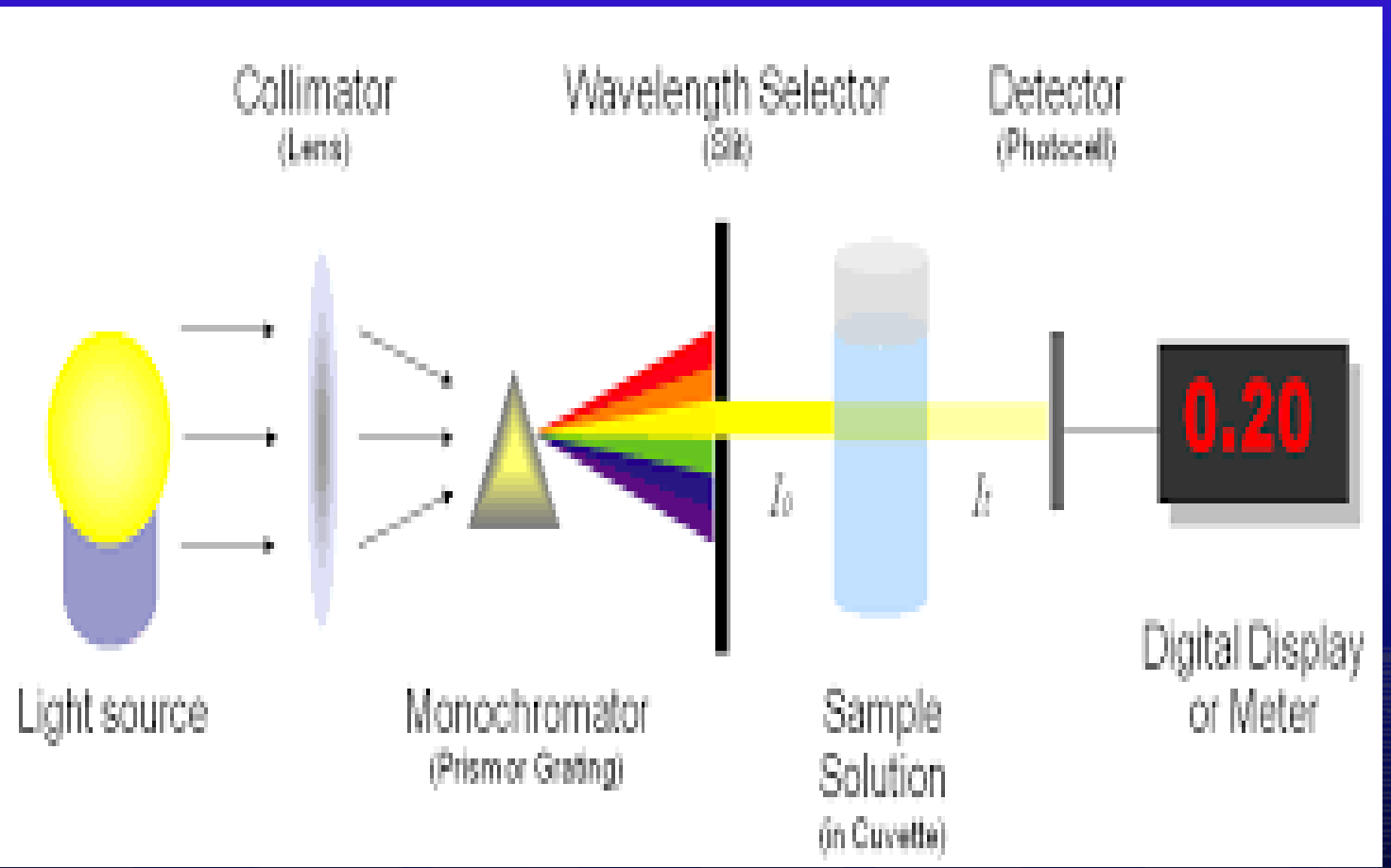
Unstable Cationic **CBB**

Protein

Stable Anionic **CBB**



Spectrophotometer

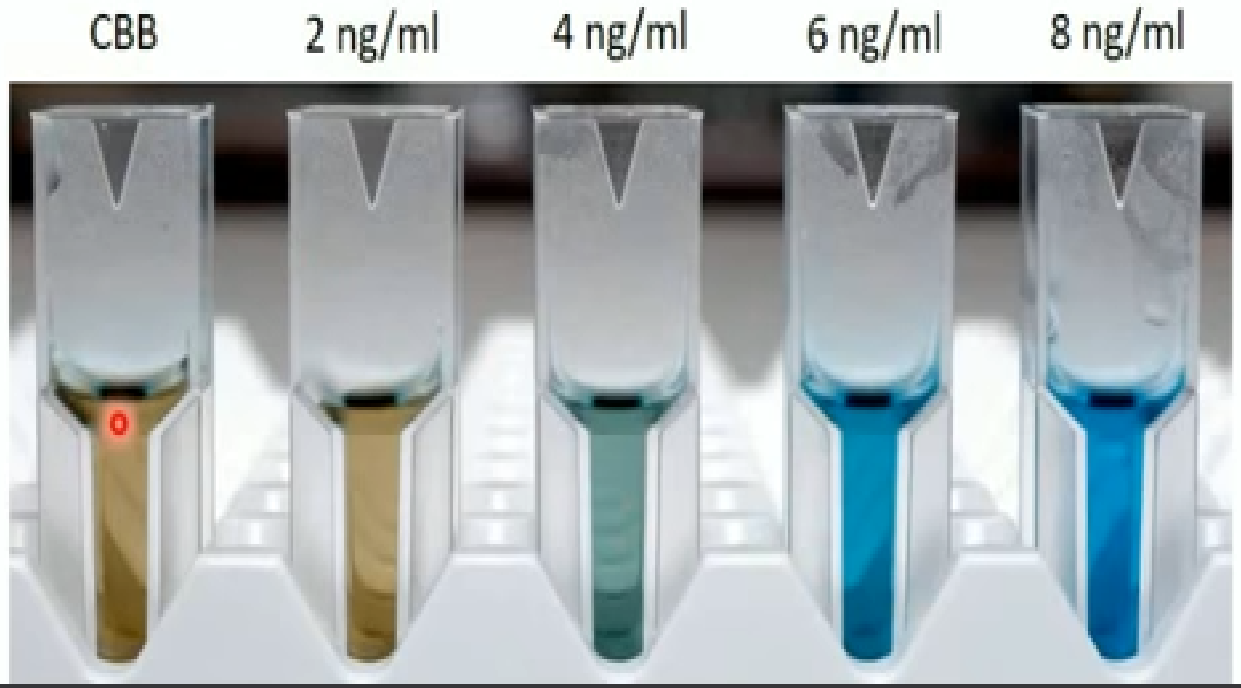


Measurement of the Protein Concentration

Calibration curve

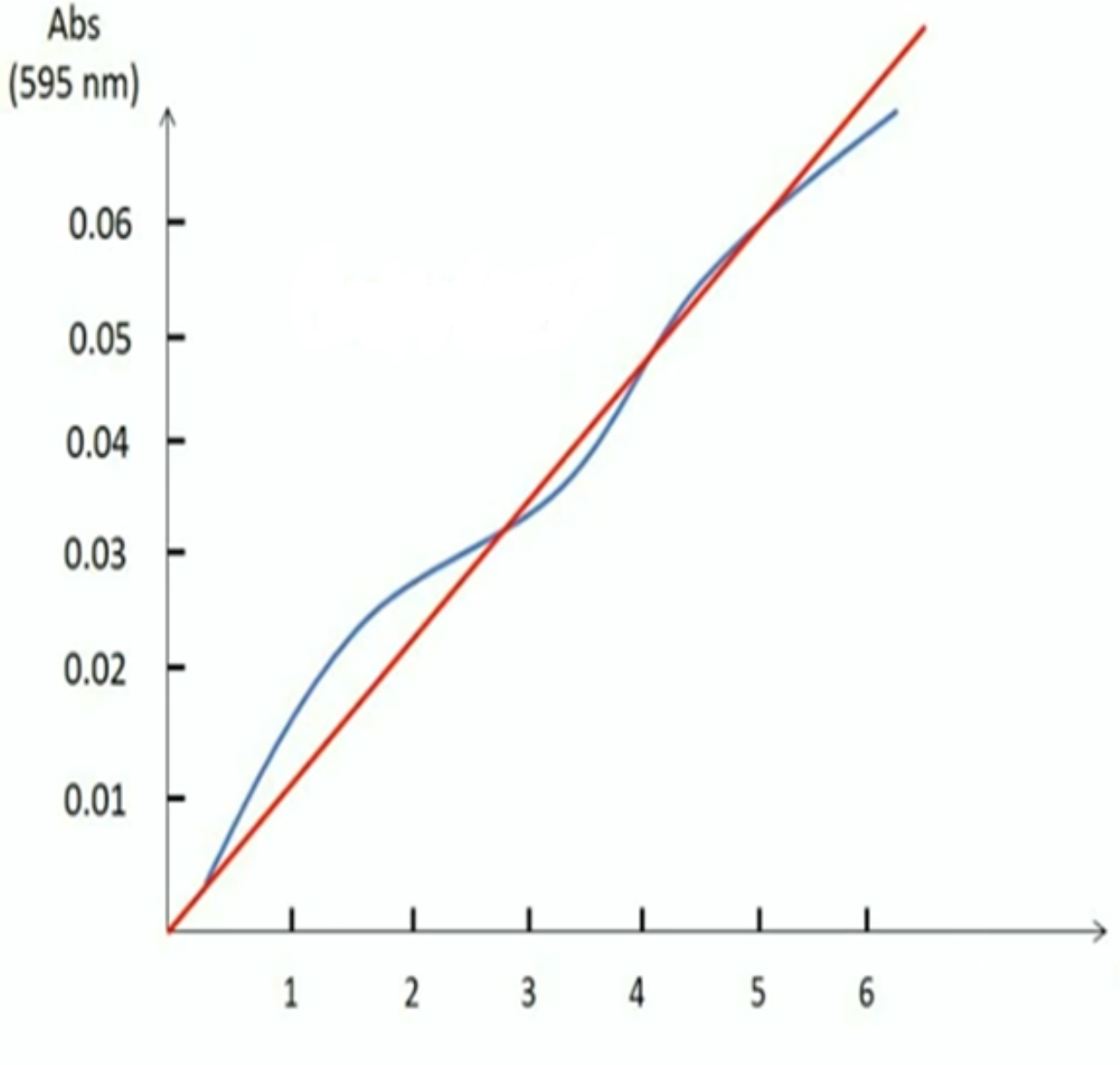
2 ng/µl

BSA (Bovine Serum Albumin)

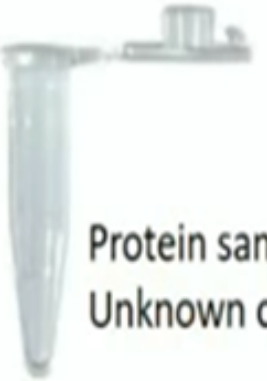


Absorbance (595 nm)

Measurement of the Protein Concentration



Calibration curve



Protein sample
Unknown concentration