Estimation of total protein in milk and egg using turbitmetric method

Diet protein sources:

- Proteins in human diet are derived from two main sources namely:
  1. Animal proteins (e.g. egg, milk, meat and fish).
  2. Plant proteins (e.g. pulses, cereals, nuts, beans and soy products).

- Animal proteins are more "**biologically complete**" than vegetable proteins.

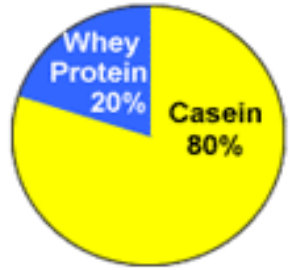
- **Food analysts** are interested in knowing the total concentration, type, molecular structure and functional properties of the proteins in foods.

- Proteins are also the major structural components of many natural foods, often determining their overall texture.

- Isolated proteins are often used in foods as ingredients because of their unique functional properties, i.e., **their ability to provide desirable appearance, texture.**

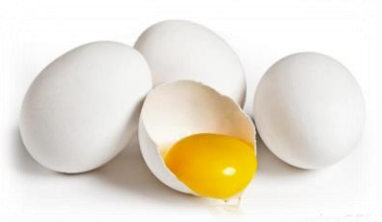
Milk proteins:

- Normal bovine milk contains **30–35 grams of protein per liter.**
- Primary group of milk proteins are the caseins 80%.



- All other proteins found in milk are grouped together under the name of whey proteins.
- The major whey proteins in cow milk are beta-lactoglobulin and alpha-lactalbumin.

Egg proteins:



- They supply all essential amino acids for humans (a source of 'complete protein').
- **Egg white** consists primarily of about 90% water into which is dissolved 10% proteins (including albumins, mucoproteins, and globulins).
- Unlike the **yolk**, which is high in lipids (fats), egg **white** contains almost no fat, and the carbohydrate content is less than 1%.

Fat & Protein	Yolk	White
Cholesterol	389 mg	0 mg
O3 FA's	71.8 mg	0.0 mg
Saturated Fat	3.0 g	0.0 g
Mono Fat	3.7 g	0.0 g
Protein	5.0 g	22.7 g

Practical Part

Objective:

- Determine the total protein content in milk and egg using turbidimetric method (by sulfosalsalyic acid).

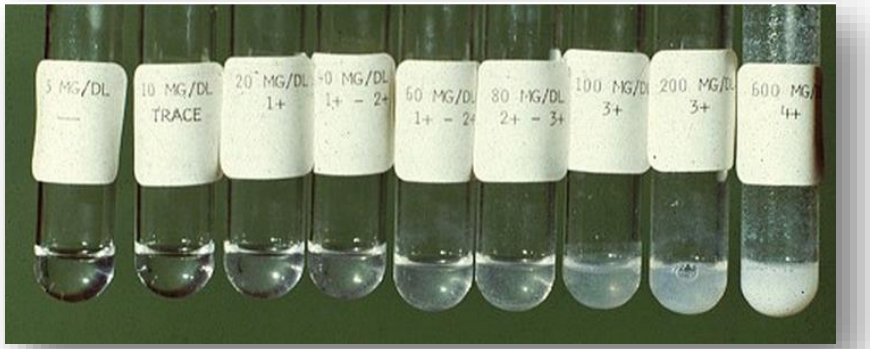
Turbidimetric method:

- Determination of total protein by measurement of **protein turbidity** produce by mixed with an anionic organic acid such as sulfosalicylic acid , TCA , or benzethonium chloride.

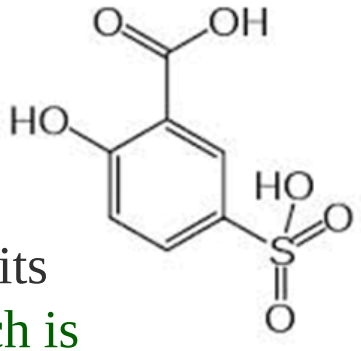
- These methods are sensitive, but the reagent does not react **equally** with each protein fraction.

- Proteins are precipitated as fine particals, turbidity is measured spectrophotometry.

➔ The higher protein concentration , the higher turbidity.



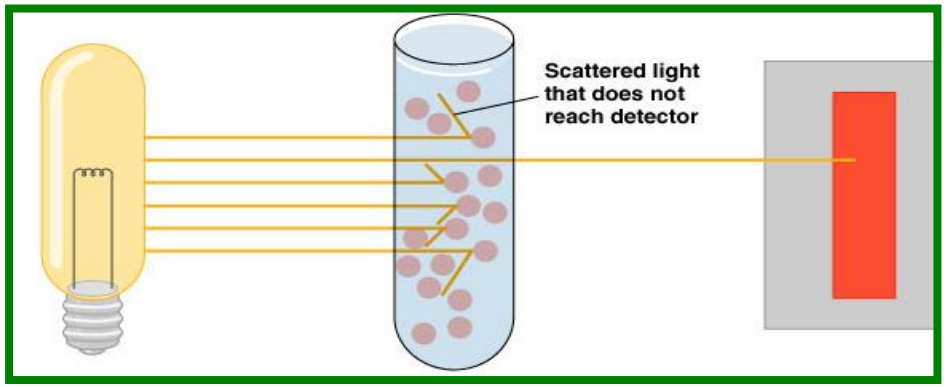
Principle:



- **Sulfosalsalyic acid** is an anion(-) which neutralizes the protein cations(+) leading to its precipitation (pH in highly acidic media, the protein will be positively charged, which is attracted to the acid anions that cause them to precipitate).

- Then the radiation of a wavelength which is **not absorbed** by the solution is made to pass through the suspension and the apparent absorption will be solely because of the scattering by the particles. ➔ (The higher protein concentration , the lower transmittance value).

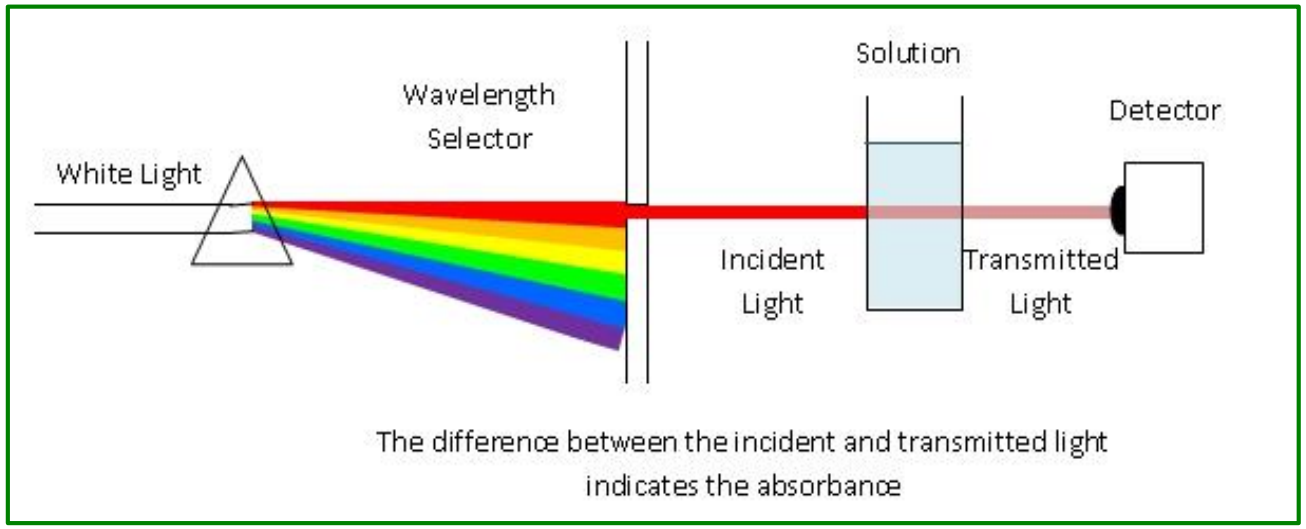
Increased concentration



Principle:

- So, the transmitted light will have lower intensity as compared to that of the incident light.

- As a result, if the intensity of the transmitted light is measured, it will give an idea of the number of particles in the suspension -proteins-. (inversely related)



Method:

- Set up a series of test tube as follows, label from 1- 6:

Tube	Protein solution	Water
1	4.5	**1.5**
2	3	**3**
3	2.4	**3.6**
4	1.5	**4.5**
5	0.9	**5.1**
6	0.3	**5.7**

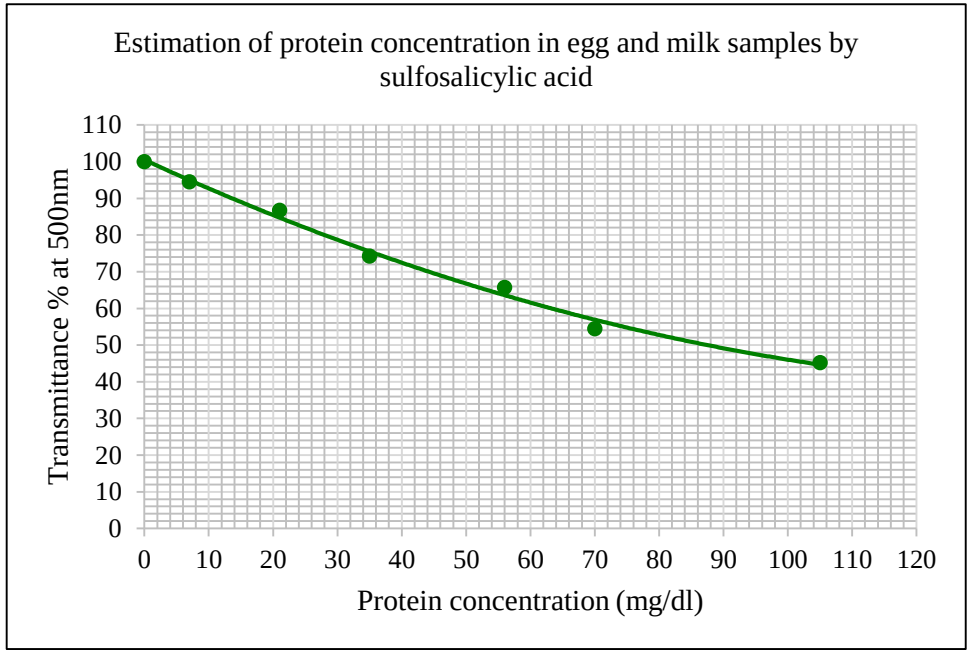
Method cont':

- Label a fresh set of test tubes 1 to 6. blank, egg sample and protein sample :
- Add 8 ml of sulphosalisalic acid to each test tube.
- Into tube 1 pipette 2 ml of protein solution 1(that you prepared before).
- Into tube 2 pipette 2 ml of protein solution 2 … etc
- In the blank add 2ml water.
- Add 0.5 ml of egg sample and 1.5 ml water.
- Add 2 ml of milk sample.
- Mix the content of each tube well and allow to stand for five minutes.
- Using solution 7 (Blank) to set transmittance 100% at 500nm.
- Then use solutions from 1-6, to recorded respective transmittance of each suspension.
- record your results.

Results :

- Plot transmittance against protein concentration on semi-logarithm paper (standard curve).
- Read the protein concentration of the “unknown samples” from the standard curve.
- Calculate the concentration of protein in the original sample (g/100 ml)

Tube	Transmittance at 500nm	Protein concentration (mg/dl)
B		
1		
2		
3		
4		
5		
6		
Milk sample		
Egg sample		



Calculations :

- The concentration from the standard curve (mg/dl) x dilution factor= ---------- mg/dl

- Dilution factor:
- Egg=……..
- Milk =…….