# Estimation of total protein in milk and egg using Turbidmetric method

Proteins in human diet are derived from **two main sources**, namely **animal proteins** (e.g. egg, milk, meat and fish.) and **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**.

Proteins are often used in foods as ingredients because of their unique functional properties, *i.e.*, their ability to provide desirable **appearance** and **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%.

The protein content of foods can be determined by numerous methods.

In this lab **turbidmetric method** (by sulfosalsalyic acid) will be used to determine the total protein content **in milk and egg**.

# 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.

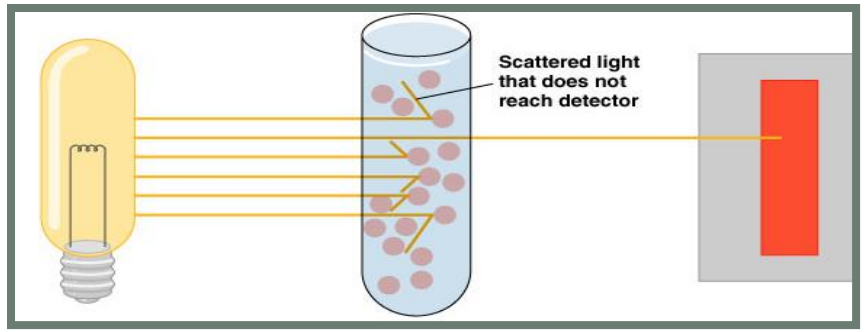
These methods are sensitive , but the reagent does not react equally with each protein fraction.

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

# Principle

Sulfosalsalyic acid is an **anionic** precipitant which neutralizes the protein cations leading to its precipitation (in highly acidic media, the protein will be positively changed, 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.



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.

- The transmission **decrease** with **increasing** protein concentration.

# Method

**1**-Set up a series of test tubes as follow:

| Tube | Protein Stock Solution ( 140 mg/dl) | water | Protein concentration mg/dl |
|---|---|---|---|
| **S1** | 4.5 | 1.5 | |
| **S2** | 3 | 3 | |
| **S3** | 2.4 | 3.6 | |
| **S4** | 1.5 | 4.5 | |
| **S5** | 0.9 | 5.1 | |
| **S6** | 0.3 | 5.7 | |
| **S7( Blank)** | 0 | 6 | |

2-Set another 9 test tube labeled and add in each one 8 ml of sulfosalicylic acid

| Tube | sulfosalicylic acid |
|---|---|
| **1** | 8 ml |
| **2** | 8 ml |
| **3** | 8 ml |
| **4** | 8 ml |
| **5** | 8 ml |
| **6** | 8 ml |
| **7( Blank)** | 8 ml |
| **Egg Sample** | 8 ml |
| **Milk sample** | 8 ml |

# Method

- 3-Into tube 1 pipette 2 ml of protein solution S1, into tube 2 pipette 2 ml of protein solution S2 etc. For the egg Sample pipette 0.5 ml of the Sample and 1.5 ml water, for the milk sample pipette 2ml of the sample.
- 4-Mix contents of each tube well and allow standing for 5 minutes.
- 5-Using solution 7 (Blank) to set transmittance at 100 at **500 nm**.
- 6-Then use solutions from 1-6, to recorded respective **transmittance** of each suspension.

# Result:

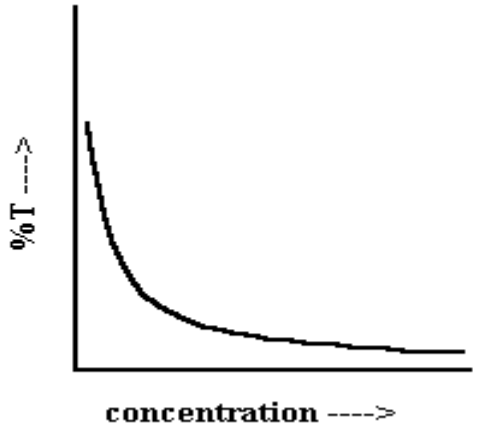
| Tube | Transmittance at 500 nm | Protein concentration mg/dl |
|---|---|---|
| 7( Blank) | 100 % | |
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |
| egg Sample | | |
| Milk sample | | |

# Method

- Plot transmittance against protein concentration on semi-logarithm paper (standard curve).

-Determine the protein concentration of the “unknown sample” from the standard curve.

- Calculate the concentration of protein in the original sample (g/100 ml)



# Calculation

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

Dilution factor:

Egg=……..

Milk =…….

**Note:**

*Milk was diluted in the sample preparation (1:100)

*1g of egg white was dissolved in 100ml of water