Estimation of total protein in milk and egg using turbitmetric 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, 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.
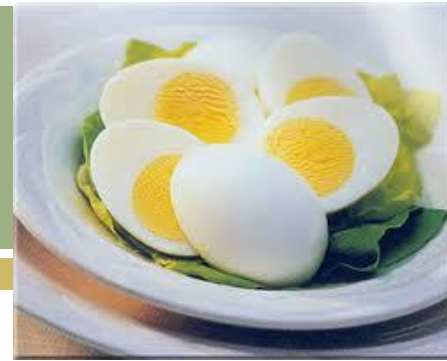
⁖ **Egg proteins:**

⁖ They supply all <u>essential amino acids</u> for humans (a source of '<u>complete protein</u>'),

⁖ Egg white consists primarily of about 90% water into which is dissolved
10% <u>proteins</u> (including <u>albumins</u>, <u>mucoproteins</u>, and <u>globulins</u>).

⁖ Unlike the yolk, which is high in <u>lipids</u> (fats), egg white contains almost no fat, and the <u>carbohydrate</u> content is less than 1%.

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

In this lab turbidimetric 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.
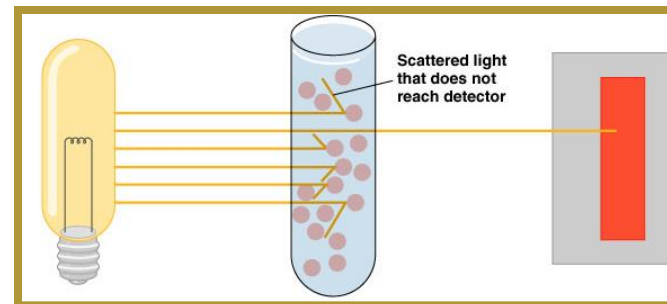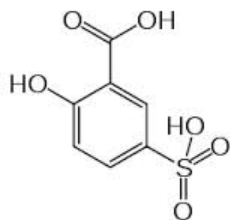
Continue- principle

- 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.
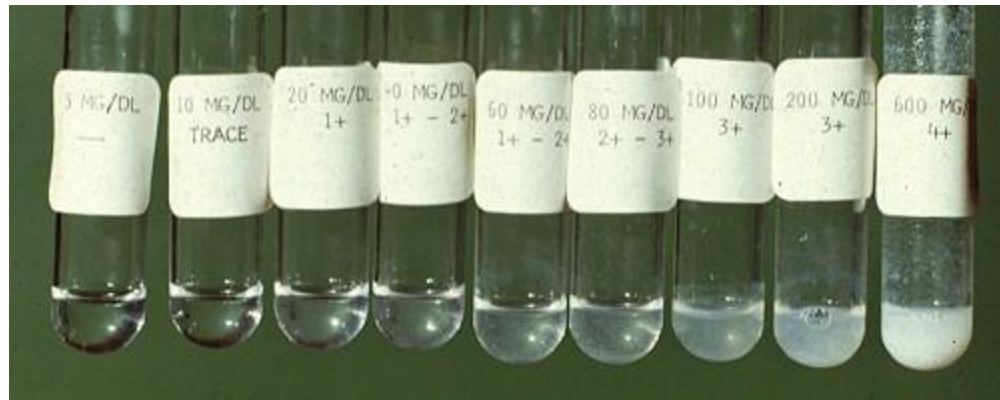
Method

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

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

2-Label a fresh set of test tubes 1 to 6 , blank, egg sample, protein sample:
-Add 8 ml of sulphosalisalic acid to each test tube
-Into tube 1 pipette 2 ml of protein solution 1
-Into tube 2 pipette 2 ml of protein solution 2,,,,,,etc
-in the blank add 2 ml water,
Add 0.5 ml of egg sample and the 1.5 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) in the cuvette of the spectrophotometer set the transmission at 500 nm
-Then record the transmissions

- Result:

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

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

-Read 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 =.............