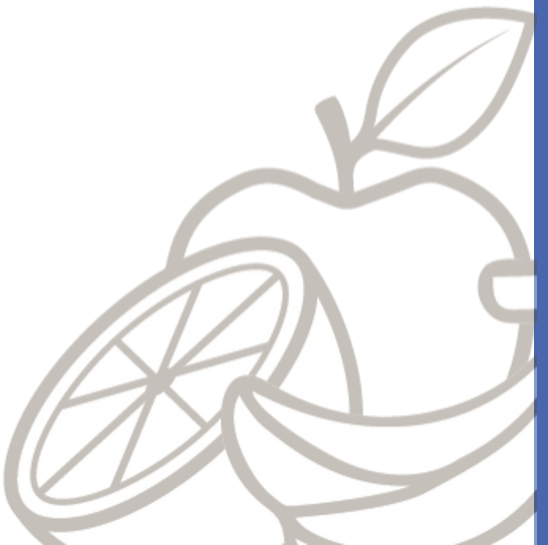
BCH 445 Biochemistry of Nutrition [Practical]

Estimation of total protein in milk and egg using turbidimetric method



Proteins in food

- **Proteins:** are nutrients that help build, repair and maintain body tissues and a source of energy.
- Proteins can only do their job if you consume carbohydrates and fats for your energy needs, otherwise your body uses protein for energy, rather than building and repairing.

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.

Proteins in food

- **Food analysts** are interested in knowing the total concentration, type, molecular structure and functional properties of the proteins in food.
- Proteins are also the major **structural components** of many natural foods, often determining their overall texture (*calpain and cathepsins* ?)
- Isolated proteins are often used in foods as **ingredients** because of their unique functional properties, i.e., their ability to provide desirable appearance, texture (*Gliadin and glutenin* ?)

Milk proteins

- Normal bovine milk contains **30–35 grams of protein per litre.**
- Milk proteins have **high** nutritional value compared to other proteins because of their relatively high content of essential amino acids and good digestibility.
- Primary group of milk proteins are the **caseins (aphosphoprotein and 80% of total protein).**
- **All other proteins** found in milk are grouped together under the name of **whey proteins.**
- **The major whey proteins** in cow milk are **alpha-lactalbumin** and **beta-lactoglobulin.**

- That fat content of human milk is similar to that of cow`s milk but there is more lactose and less protein, calcium and phosphorus.
- **Colostrum** is more yellowish and contains about twice as much protein including immunoglobulin, the protein content falls with time reaching average levels after about one month.

Eggs proteins

- Protein content of an egg accounts to about **12.6%** by weight of the edible portion.
- They supply **all essential amino acids** for humans (a source of 'complete protein') composition of amino acids in an egg **matches the requirement** of amino acids by the human body.
- **Egg white** consists primarily of about 90% water into which is dissolved 10% proteins contains approximately 40 different 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%.

Practical Part

Objective:

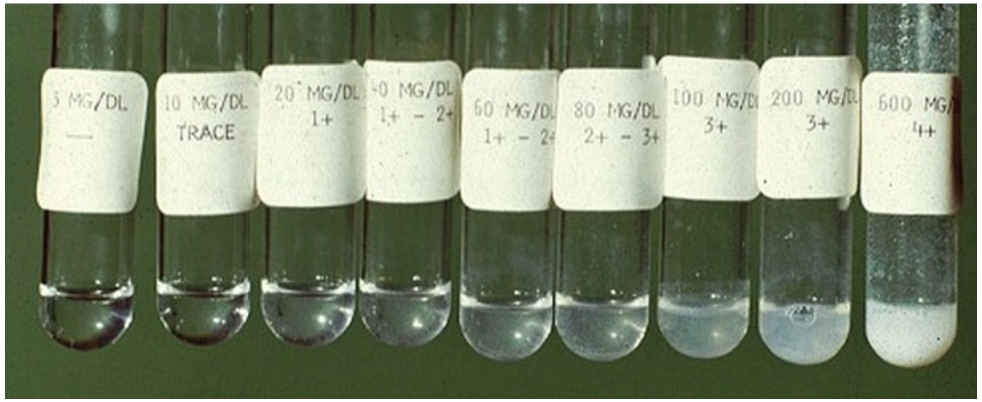
- Determine the total protein content in milk and egg using turbidimetric method (**by sulfosalicylic acid**).

When working with SSA, wear protective gloves, chemical goggles, suitable protective clothing and respiratory protection.

Turbidimetric method

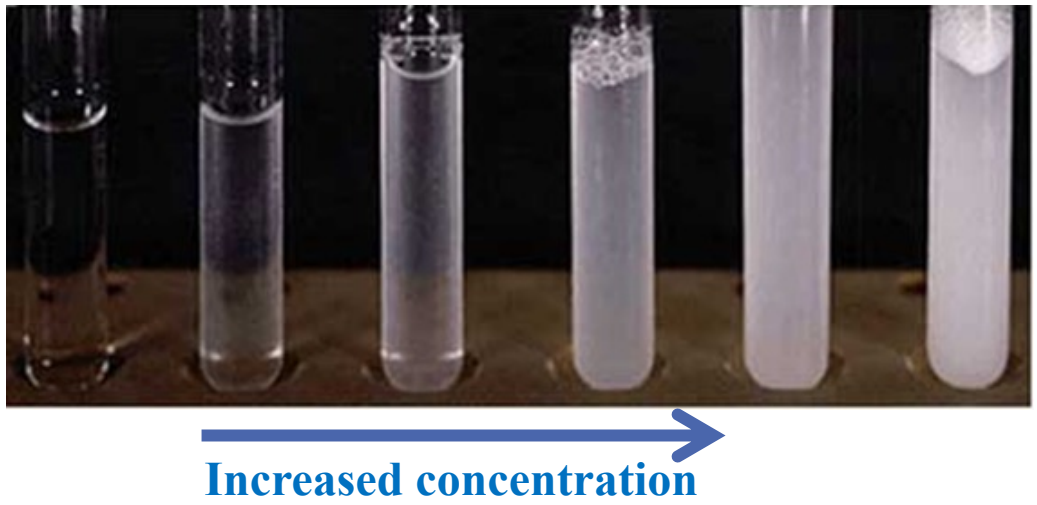
- Determination of total protein by measurement of **protein turbidity** produce by mixed with an anionic organic acid such as **sulfosalicylic acid** , TCA , or benzethonium chloride.
- These methods are sensitive, but the reagent **does not react equally** with each protein fraction.
- Proteins are **precipitated** as fine particles, turbidity is measured by spectrophotometry.

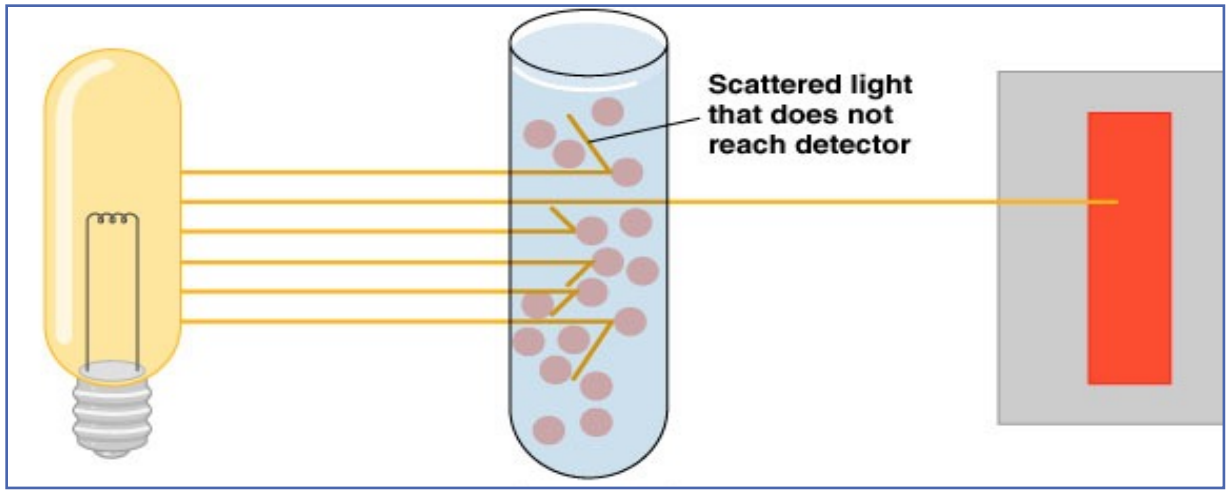
➔ The higher protein concentration, the higher turbidity.



Principle

- **Sulfosalicylic 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).

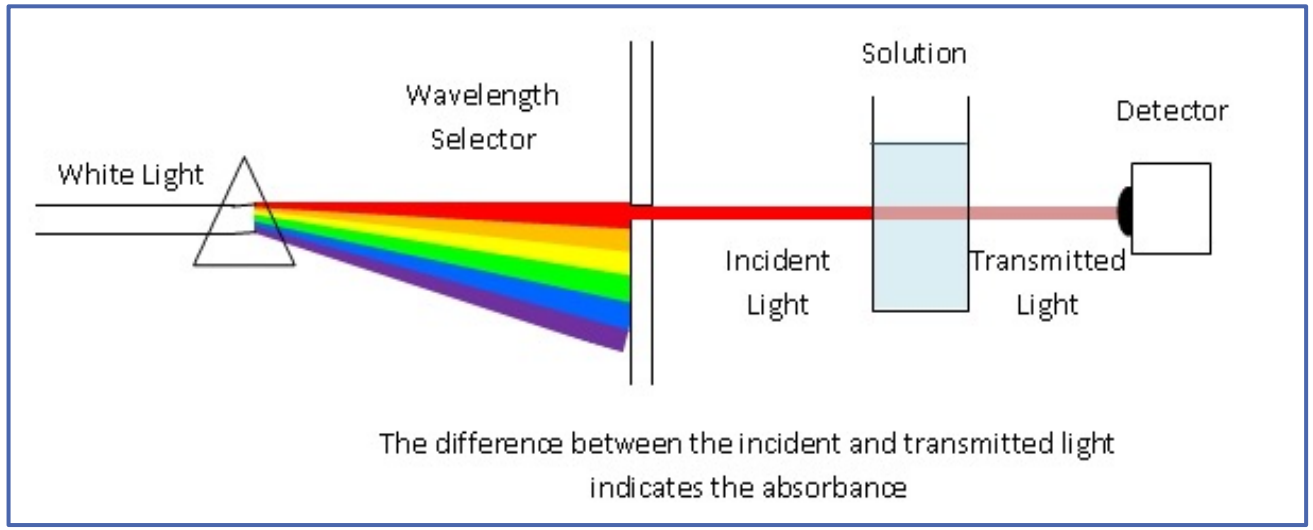




Principle

- 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)

↑ Protein concentration ↑ Turbidity ↓ Transmittance



Method

1- 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**

2- Set another 8 test tube labeled 1-7 and pipette in each one Add 8 ml of sulfosalicylic acid

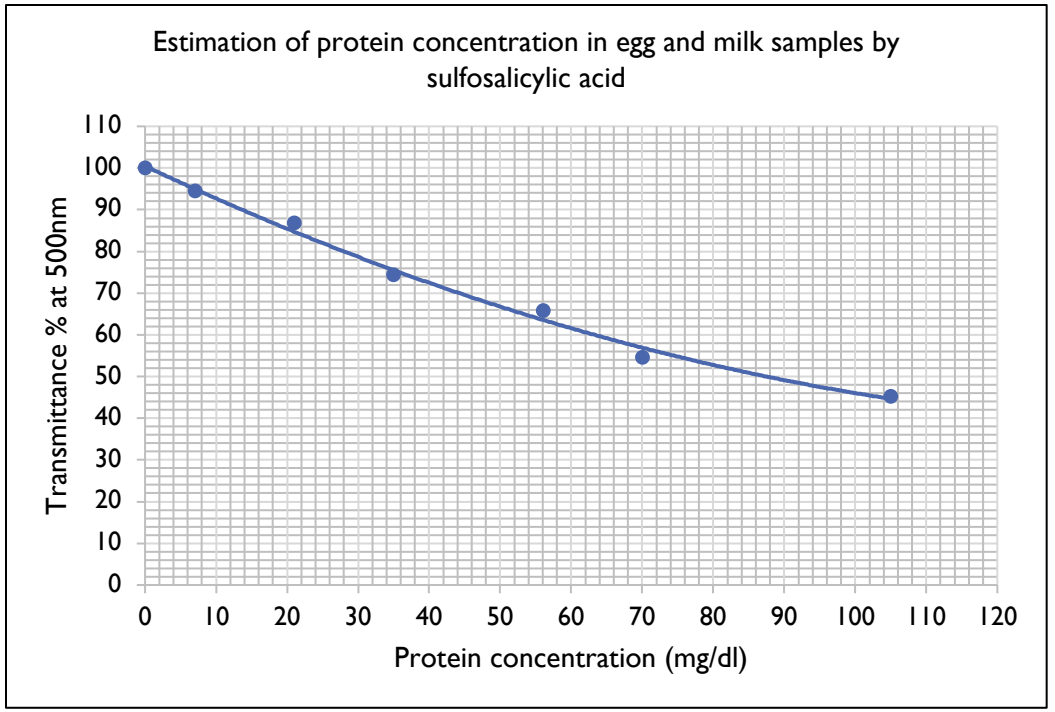
Method

- Label a fresh set of test tubes 1 to 6, blank **Egg sample** and **Milk sample**
- Add 8 ml of sulfosalicylic acid to each test tube
- In the blank add 2 ml 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 5 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=** final volume / aliquot volume

➔ Egg= ……..

➔ Milk =……

Homework

- Why animal proteins are referred to as first class protein?
- What causes the white color of milk?
- Several methods have been proposed for the determination of total protein, name 3 of them other than sulfosalicylic acid method.