Chapter 3C:

Proteins, Classification

Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

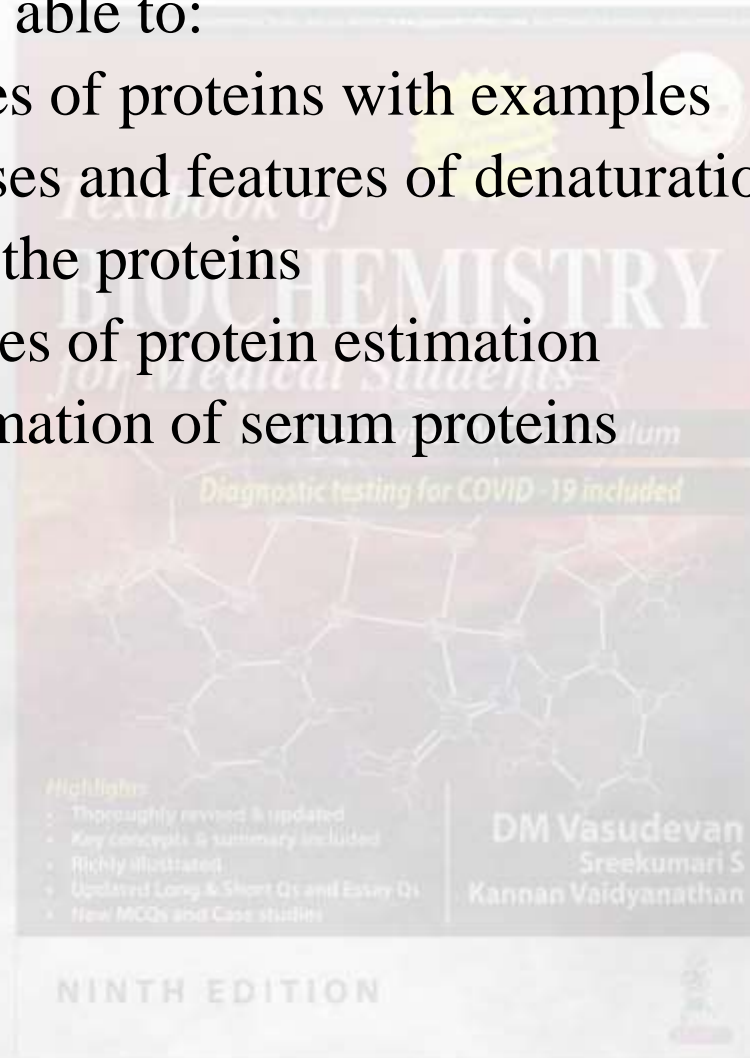TENTH EDITION

The learner will be able to:

- List the properties of proteins with examples
- Mention the causes and features of denaturation
- Broadly classify the proteins
- List the techniques of protein estimation
- Perform the estimation of serum proteins

Colloids

    Osmotic Pressure

Molecular Weight

    Insulin   **: 5,700**

    Hemoglobin   **: 68,000**

    Albumin   **: 69,000**

    Immunoglobulin G   **: 1,50,000**

Shape

    Globular   **: Insulin**

    Oval   **: Albumin**

    Elongated   **: Fibrinogen**

H+   OH--

+   +   --   --
+   +   --   --
+   +   --   --

Cation   pI   Anion
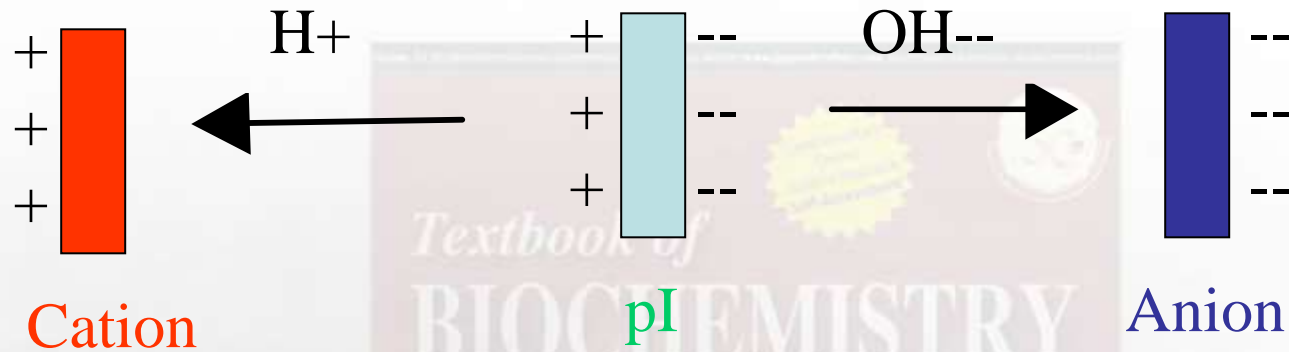
At iso-electric point
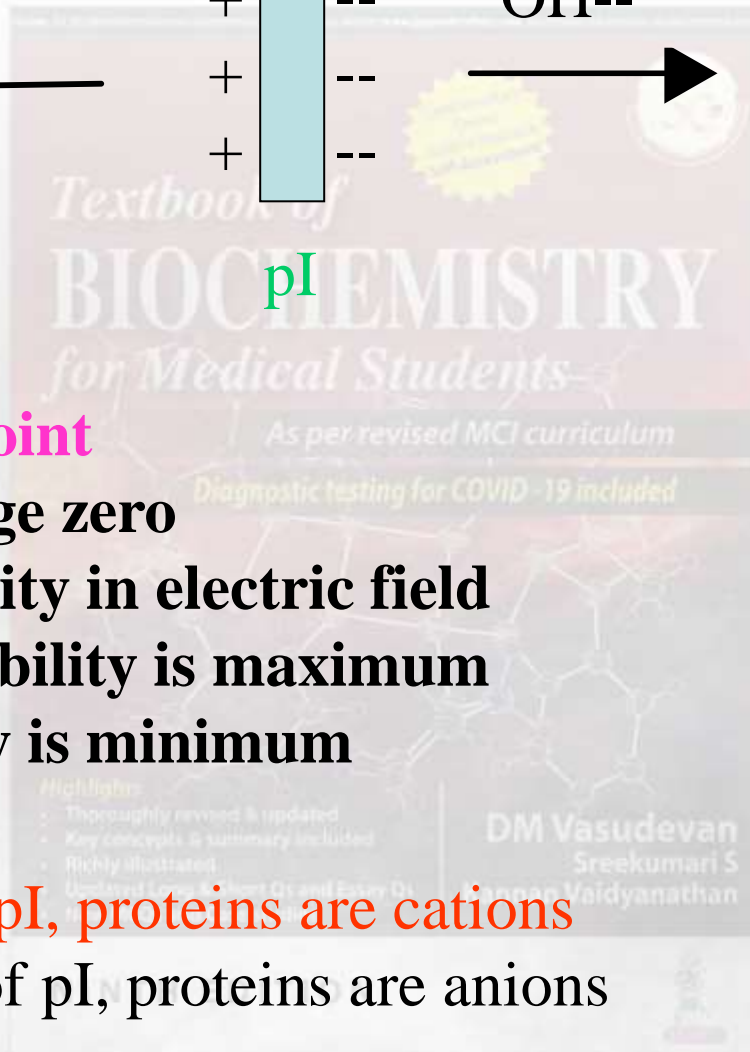> **Net charge zero**
> **No mobility in electric field**
> **Precipitability is maximum**
> **Solubility is minimum**

On acidic side of pI, proteins are cations
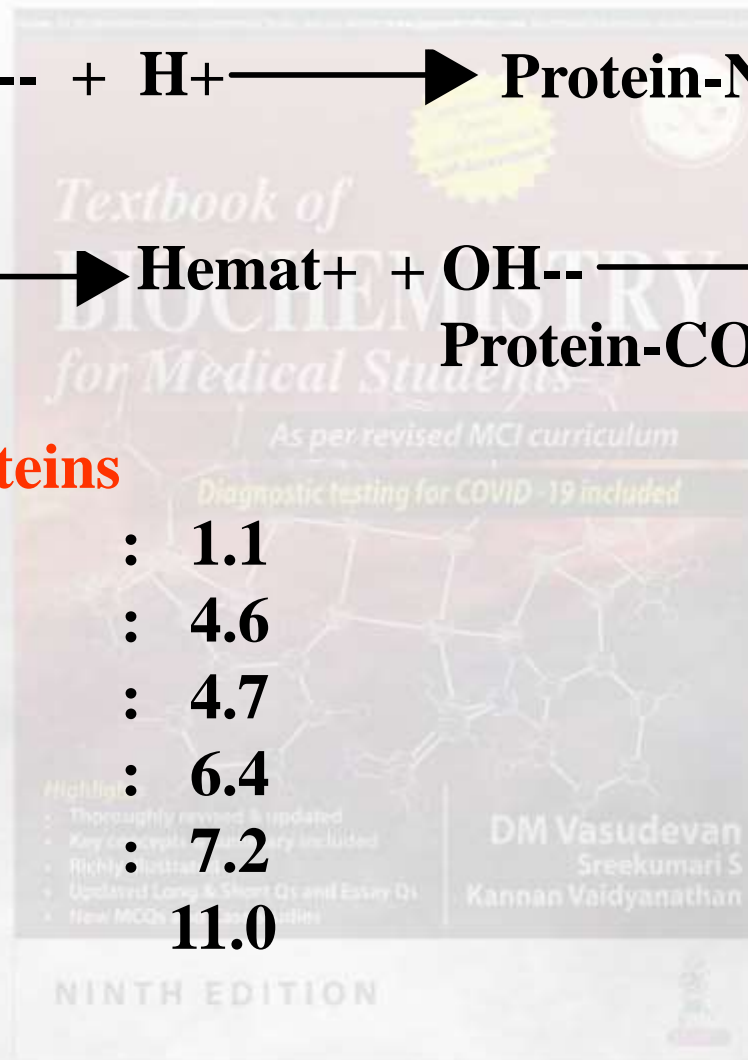On alkaline side of pI, proteins are anions

Acidic Dye

Eosin $\longrightarrow$ E-- + H+ $\longrightarrow$ Protein-NH3+ E--

Basic Dye

Hematoxylin $\longrightarrow$ Hemat+ + OH-- $\longrightarrow$

Protein-COO-- Hemat+

pI of certain proteins

Pepsin	:	1.1
Casein	:	4.6
Albumin	:	4.7
Globulins	:	6.4
Hemoglobin	:	7.2
Lysozyme		11.0

Charge Shell of hydration Polar groups
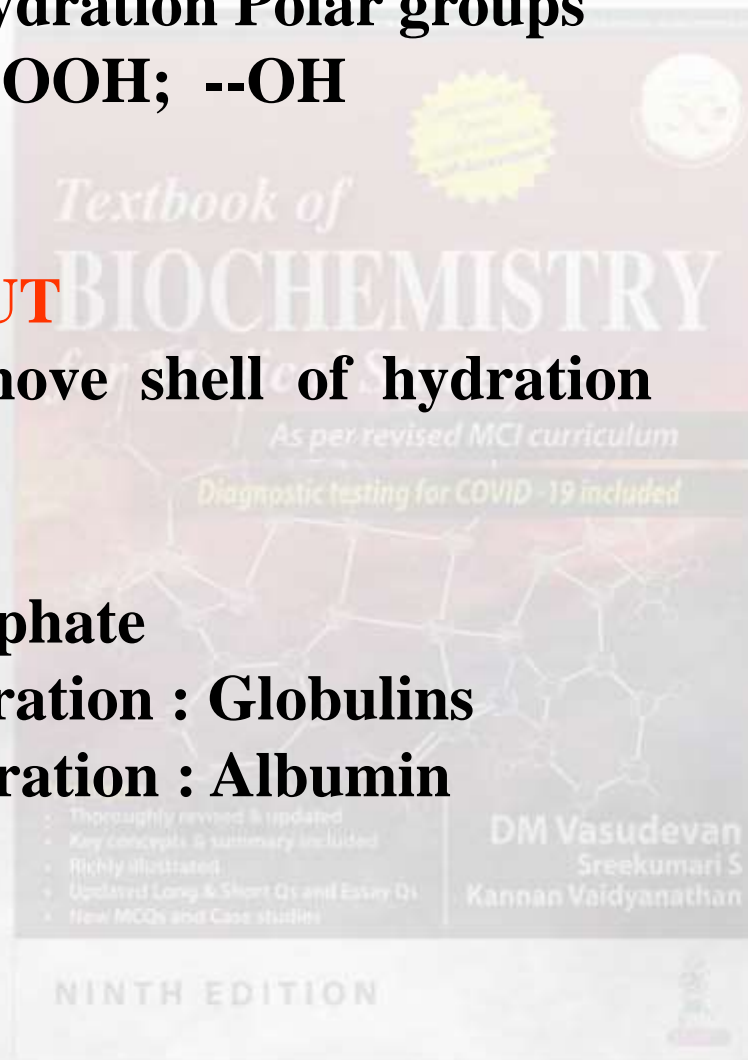**--NH2; --COOH; --OH**

1. SALTING OUT

Neutral salts remove shell of hydration

Ammonium Sulphate
Half saturation : Globulins
Full saturation : Albumin

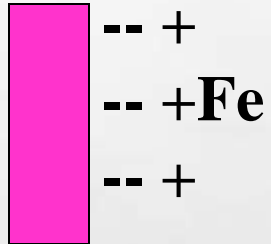## 2. ISO-ELECTRIC PRECIPITATION

Casein at pH 4.6
Curdling of milk

3. ORGANIC   SOLVENTS

Reduce dielectric constant
Ether, Alcohol
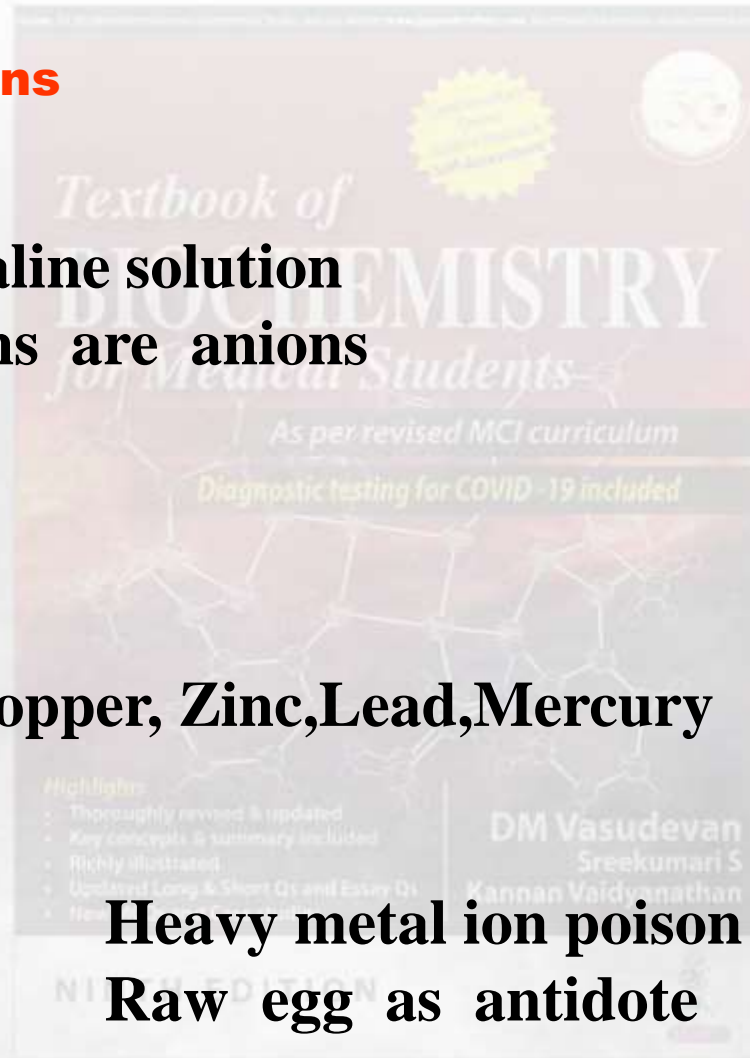**(Alcohol is a disinfectant)**

4. Heavy metal ions

-- +
-- +Fe
-- +

In alkaline solution proteins are anions

Iron,Copper, Zinc,Lead,Mercury

Heavy metal ion poison
Raw egg as antidote

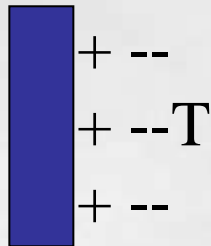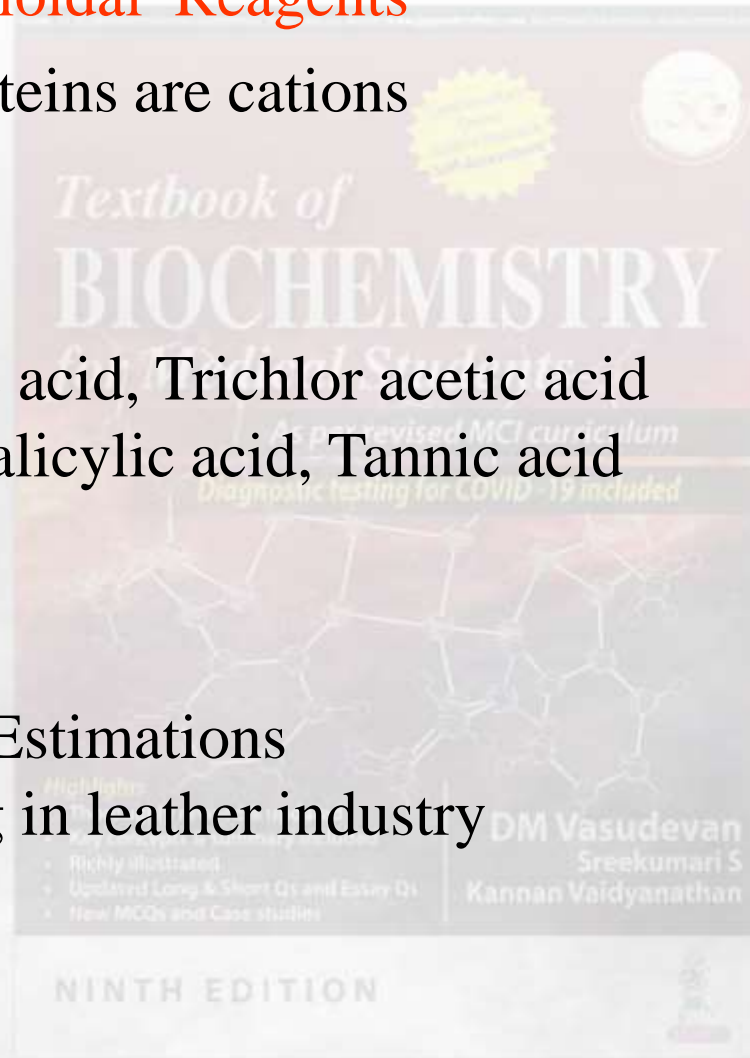## 5. Anionic or Alkaloidal Reagents

In acid solution proteins are cations

Tungstic acid, Trichlor acetic acid
Sulphosalicylic acid, Tannic acid

+ --
+ --T
+ --

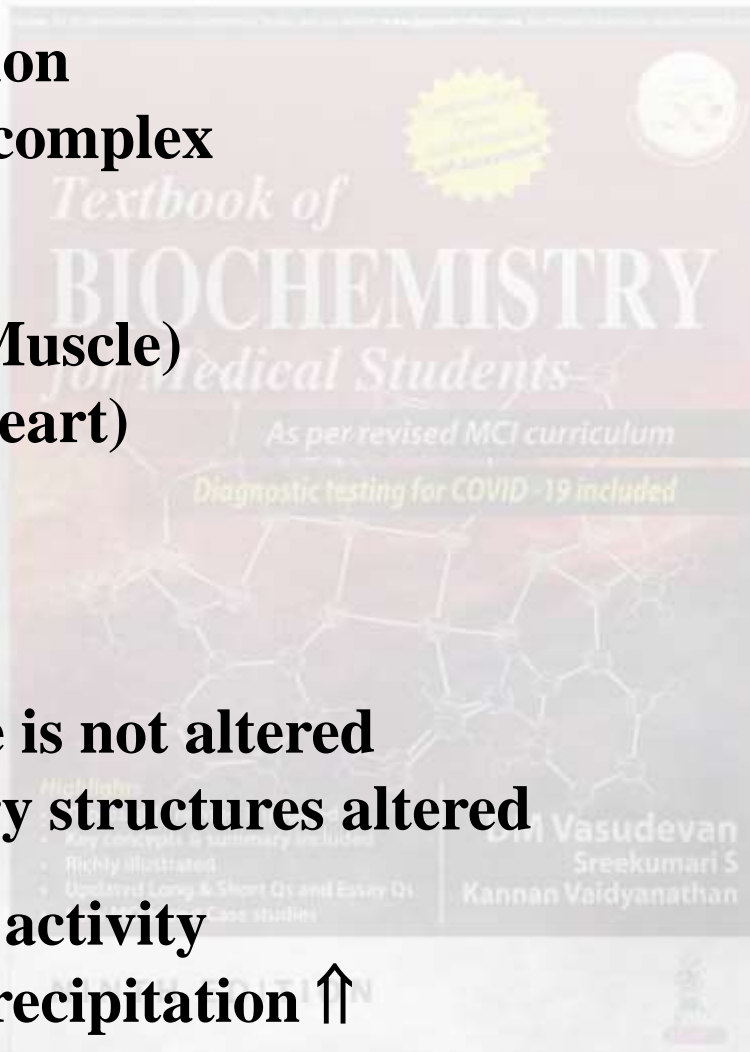Blood Estimations
Tanning in leather industry

6. Antibodies

Specific precipitation

Antigen-antibody complex

Creatine Kinase

CK-MM (Muscle)

CK-MB (Heart)

Denaturation

Primary structure is not altered

Secondary, tertiary structures altered

Loss of biological activity

Solubility $\Downarrow$ Precipitation $\Uparrow$

Urea, Salicylate
UV rays, High pressure
Vigorous shaking

Primary structure is not altered
Secondary, tertiary structures altered

Loss of biological activity
Solubility ⇓   Precipitation ⇑
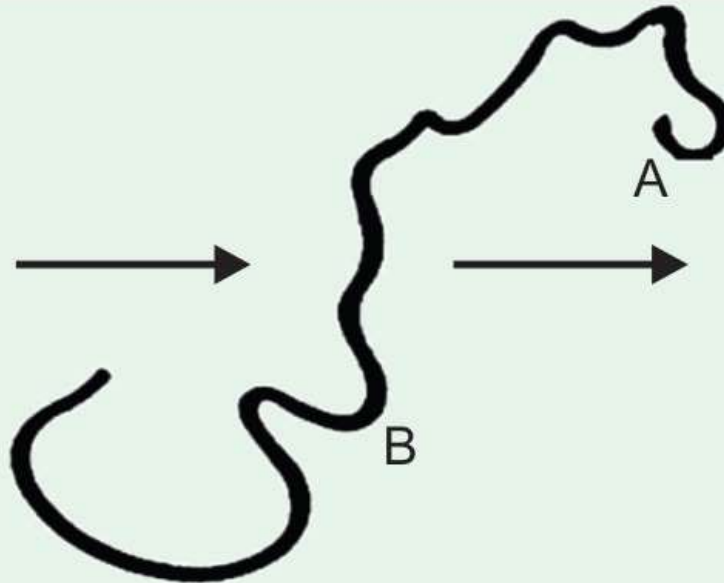
Cooking, denaturation
        Easily digested

Native protein with functional amino acids (A, B) are nearby; protein is functional

Denatured protein; Random coil structure; A and B are far apart; function is lost. Primary structure is intact.

Renaturation; native form is regenerated

Reversible denaturation

Ribonuclease / urea
Renaturation may or may not

Denature, proteins in solution

Alkaline phosphatase in serum

|	
20 •C	2 days
4 •C	7 days
--20 •C	20 days
--70 •C	90 days

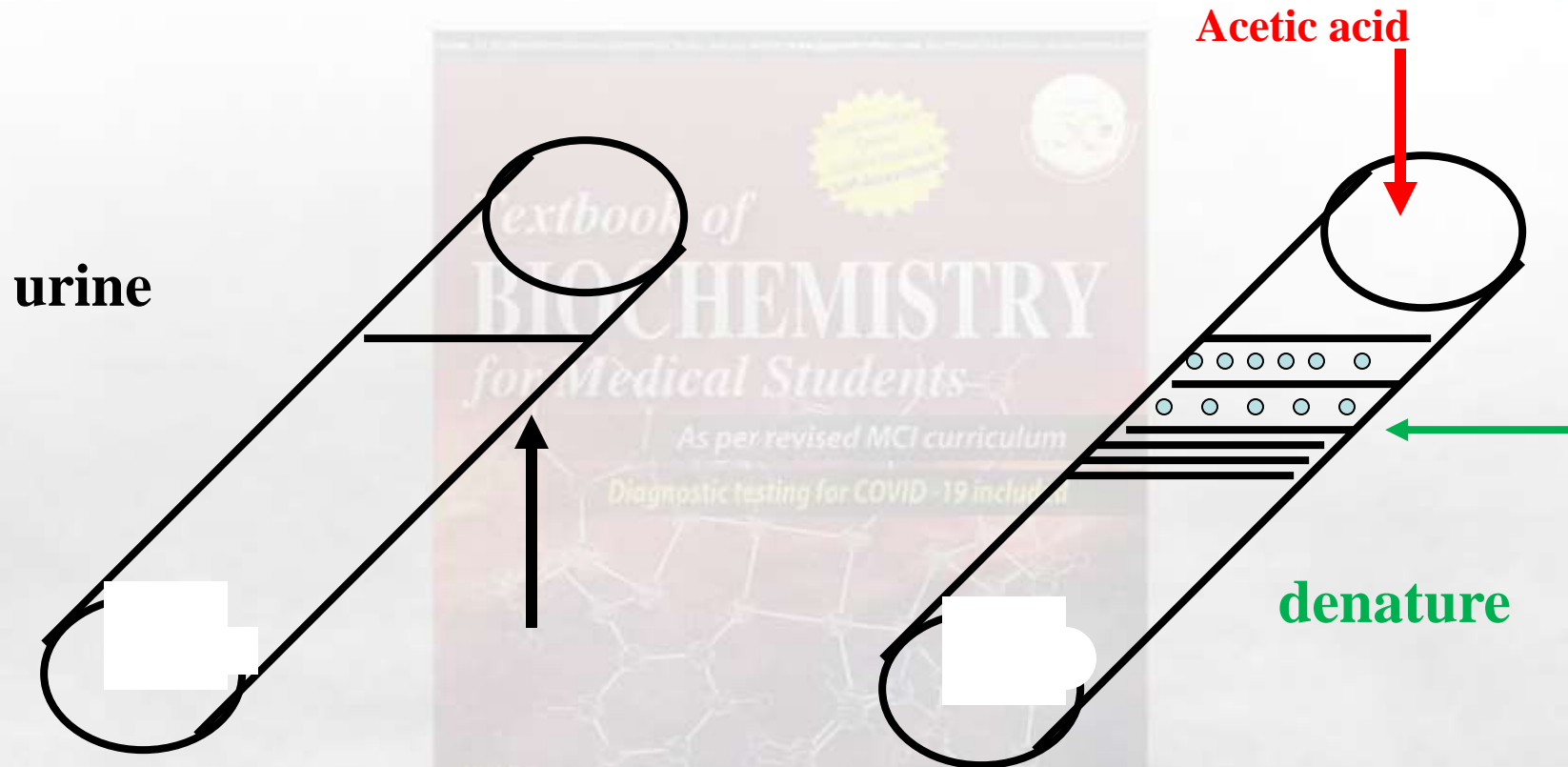**Lyophilisation** **Freeze drying**

Formaldehyde

Heat Coagulation

Heated at iso-electric point, proteins denature irreversibly thick floating conglomerates called coagulum.
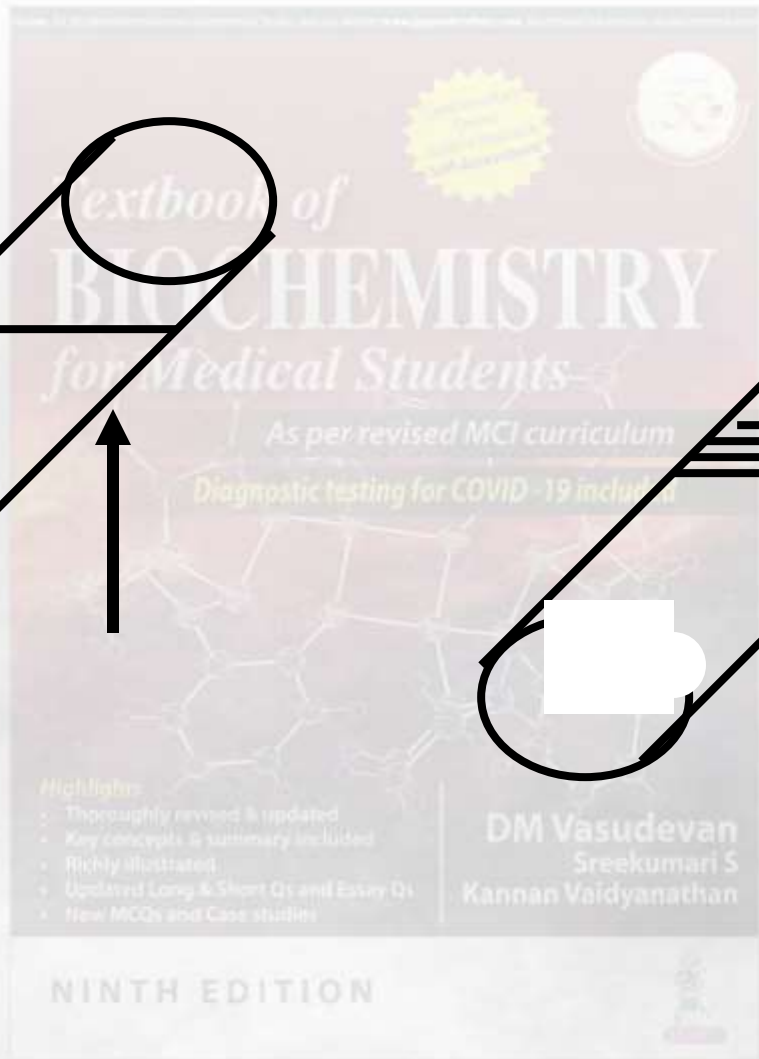
Albumin is easily coagulated Albumin when heated, denatured, but is still soluble. This is precipitated by bringing to iso-electric pH.

'Heat and Acetic Acid Test'

urine

Acetic acid

denature
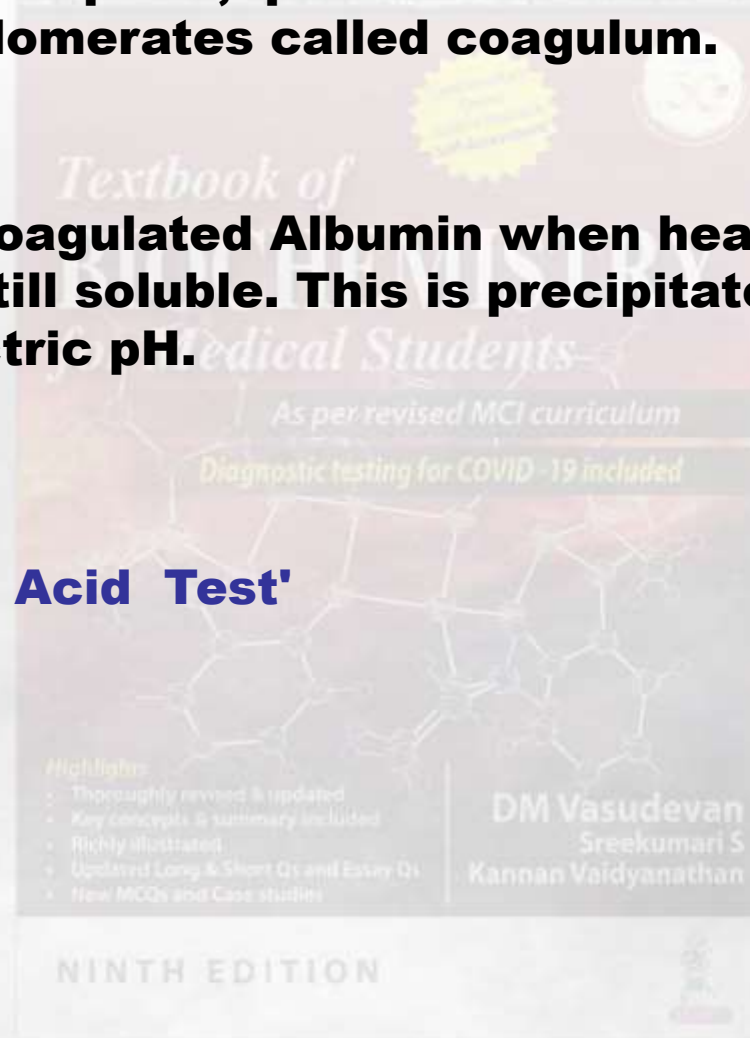
**Heat and
acetic acid
test
for
albuminuria**

Heat upper portion

Cloudy precipitation in upper part Coagulation

Take 5-10 ml urine and boil the upper portion

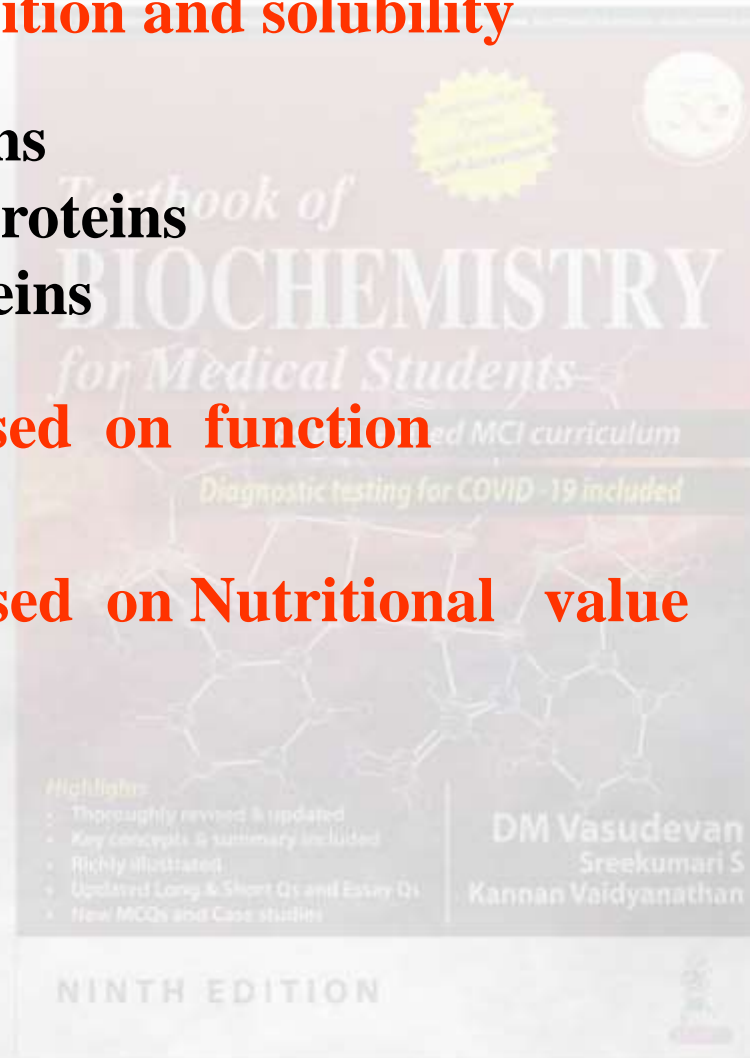Check for cloudiness and add 2-3 drops of glacial acetic acid

Based on Composition and solubility

I.   Simple proteins
II. Conjugated proteins
III. Derived proteins

Classification based on function
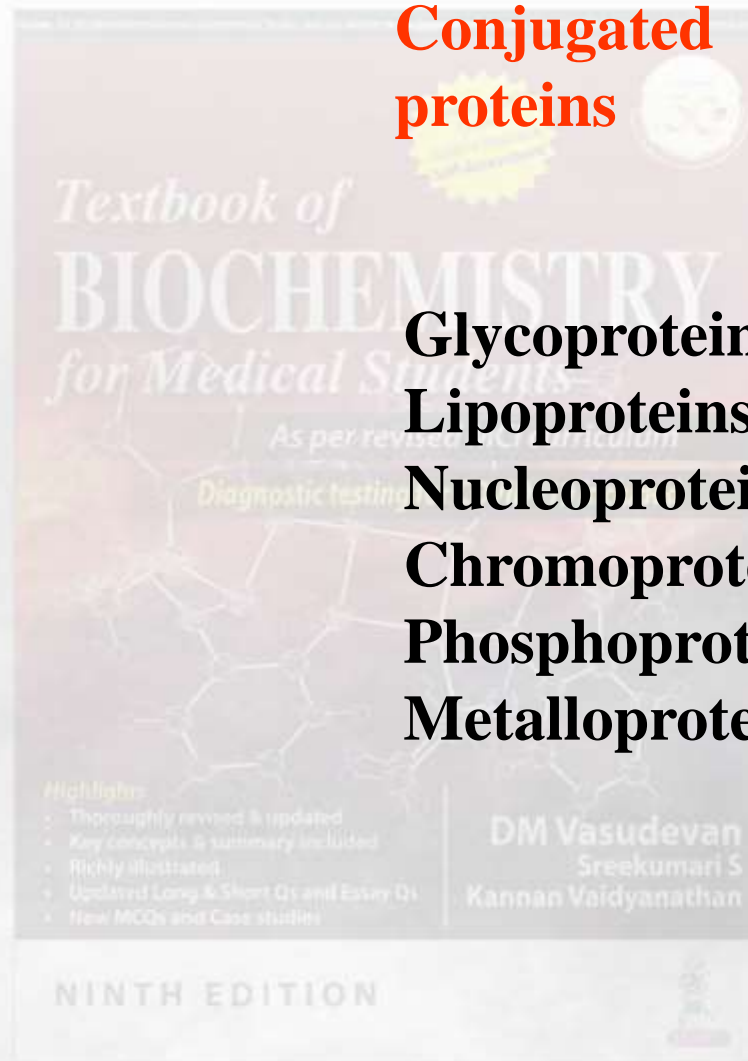
Classification based on Nutritional value

Simple Proteins

Albumins
Globulins
Globins
Protamines
Prolamines
Lectins
Scleroproteins

Conjugated proteins

Glycoproteins
Lipoproteins
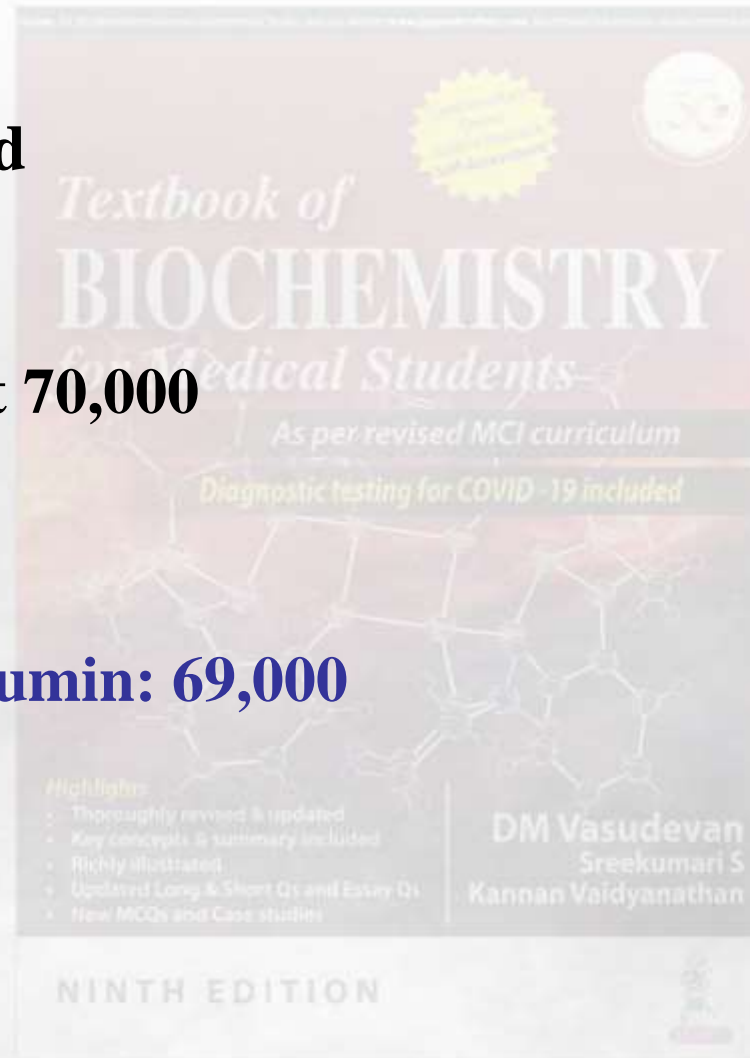Nucleoproteins
Chromoproteins
Phosphoproteins
Metalloproteins

Soluble in water
Easily Coagualated

Mol. Weight about 70,000
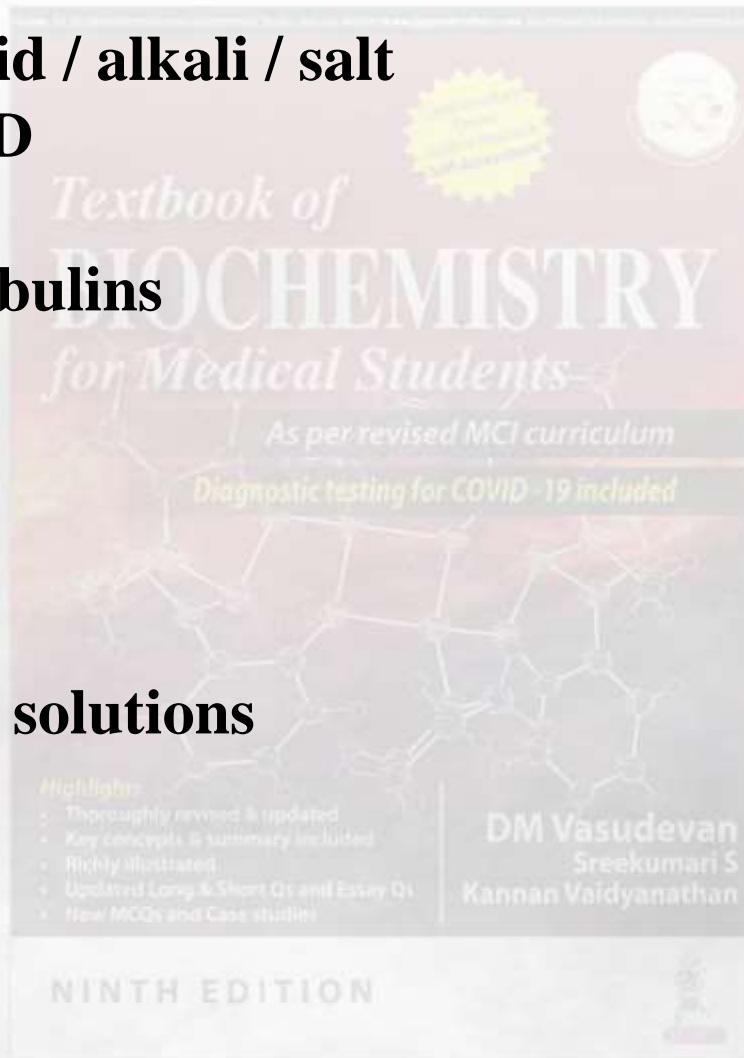
Human serum albumin: 69,000
Egg albumin
Lactalbumin

GLOBULINS

Soluble in mild acid / alkali / salt

Mol. Wt. 150,000 D

Coagulation

Human serum globulins

GLOBINS

Not soluble in salt solutions

Ex: Hemoglobin

PROTAMINES

Soluble in water

Not coagulated

Arg / Lys; strongly basic
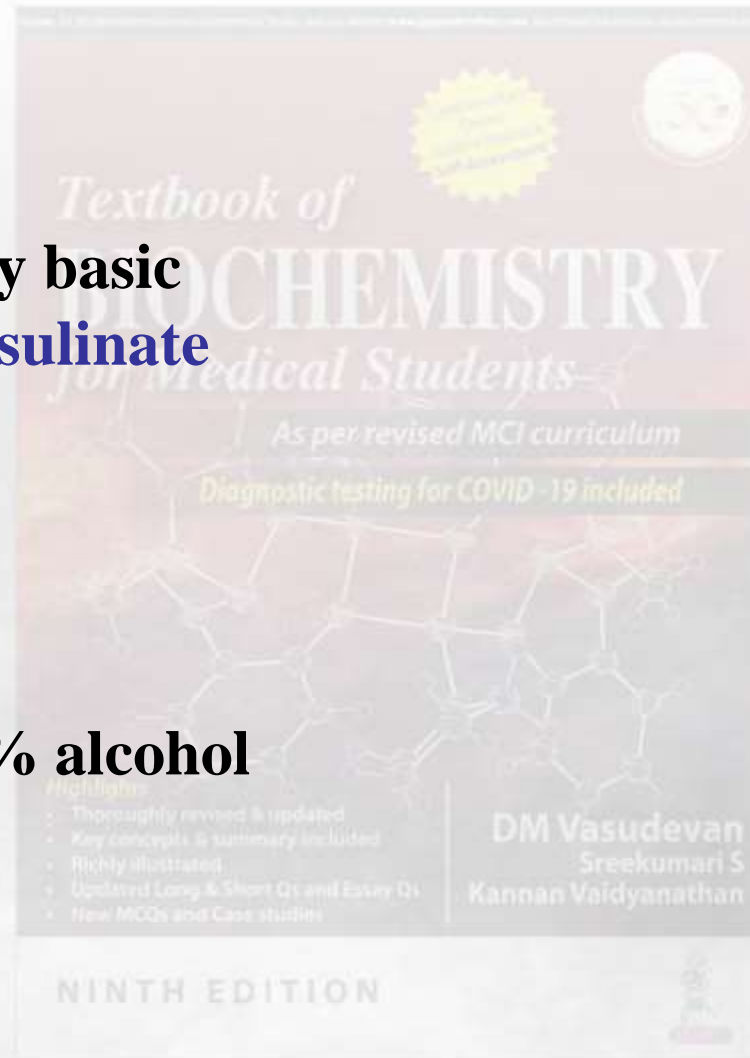
Protamine zinc insulinate

PROLAMINES

soluble in 70 - 80% alcohol

Rich in proline

Zein in corn

Gliadin of wheat

LECTINS

Precipitated by 30-60% saturated ammonium sulphate

Plant proteins with affinity to sugar groups

Dolichos :
RBC A group; Gal-Nac

Phytohemagglutinin (PHA) from Phaseolus vulgaris

SCLEROPROTEINS

Insoluble in water / mild acid / organic solvents
Soluble only in hot strong acid

Collagen
bone, cartilage

Keratin
Hair, nail

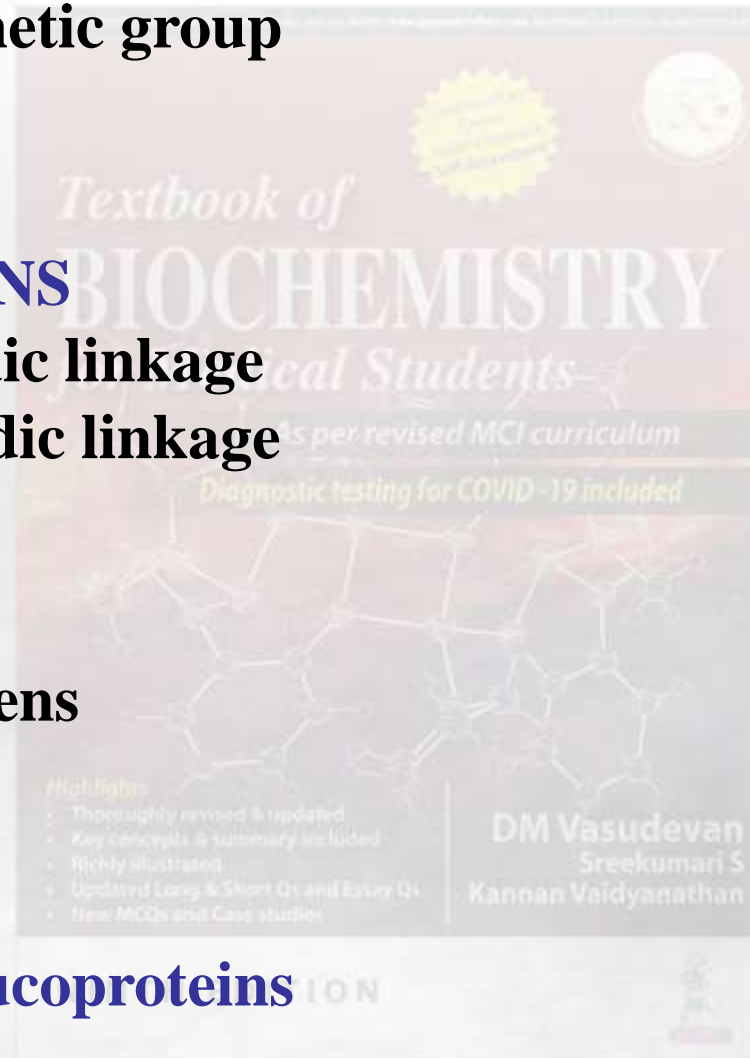**Protein + Prosthetic group**

GLYCOPROTEINS
S / T : O-glycosidic linkage
N / Q : N-glycosidic linkage

Cell surface antigens

More than 10% :

Mucoproteins

Conjugated Proteins

LIPO PROTEINS

Cell membranes; serum

NUCLEO PROTEINS

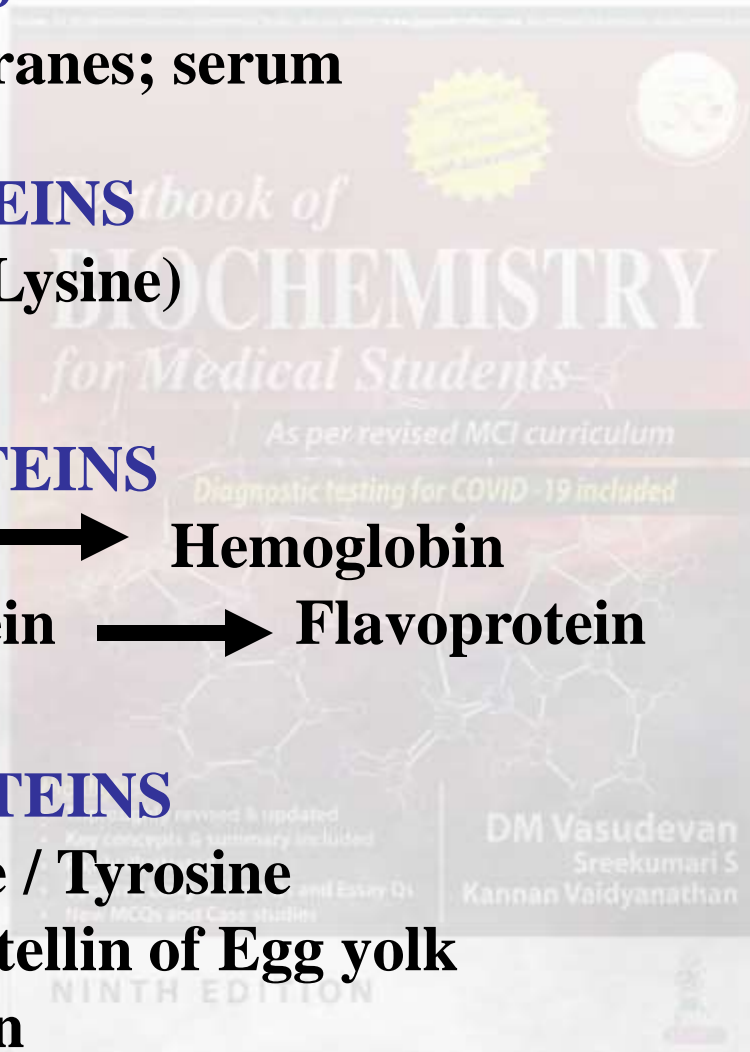Histones (Lysine)

CHROMO PROTEINS

Heme + Globin → Hemoglobin

Riboflavin + Protein → Flavoprotein

PHOSPHO PROTEINS

Serine / Threonine / Tyrosine

Casein of milk; Vitellin of Egg yolk

Enzyme activation

METALLO PROTEINS

Hemoglobin   : Iron
Myoglobin   : Iron
Cytochromes : Iron

Ceruloplasmin : Copper
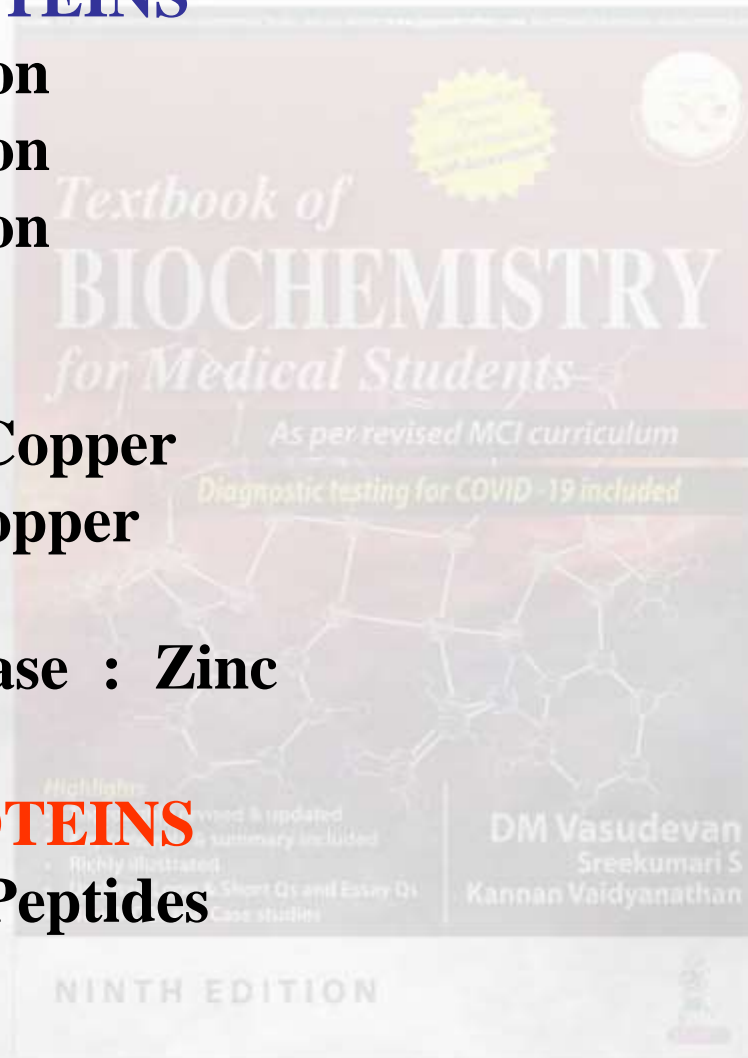**Tyrosinase   : Copper**

Carbonic anhydrase : Zinc

DERIVED   PROTEINS
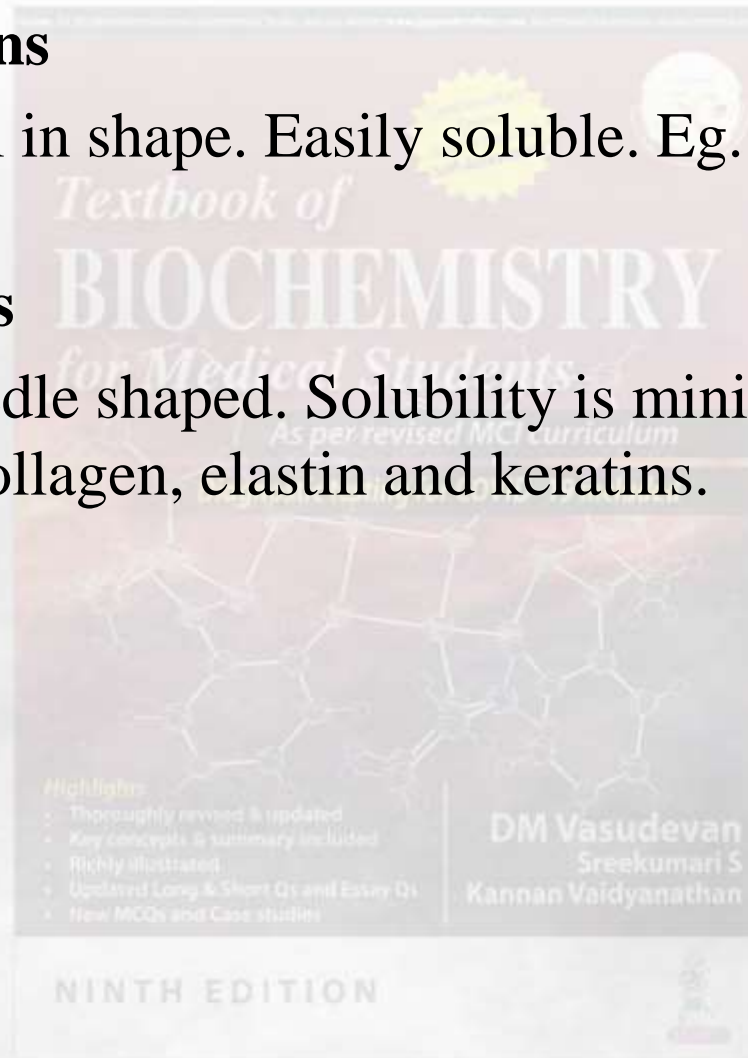
Peptones ⟶ Peptides
Amino acids

1. Globular Proteins

- Spherical or oval in shape. Easily soluble. Eg. albumins, globulins and protamines.

2. Fibrous Proteins

- Elongated or needle shaped. Solubility is minimum. Resist digestion. Eg. Collagen, elastin and keratins.

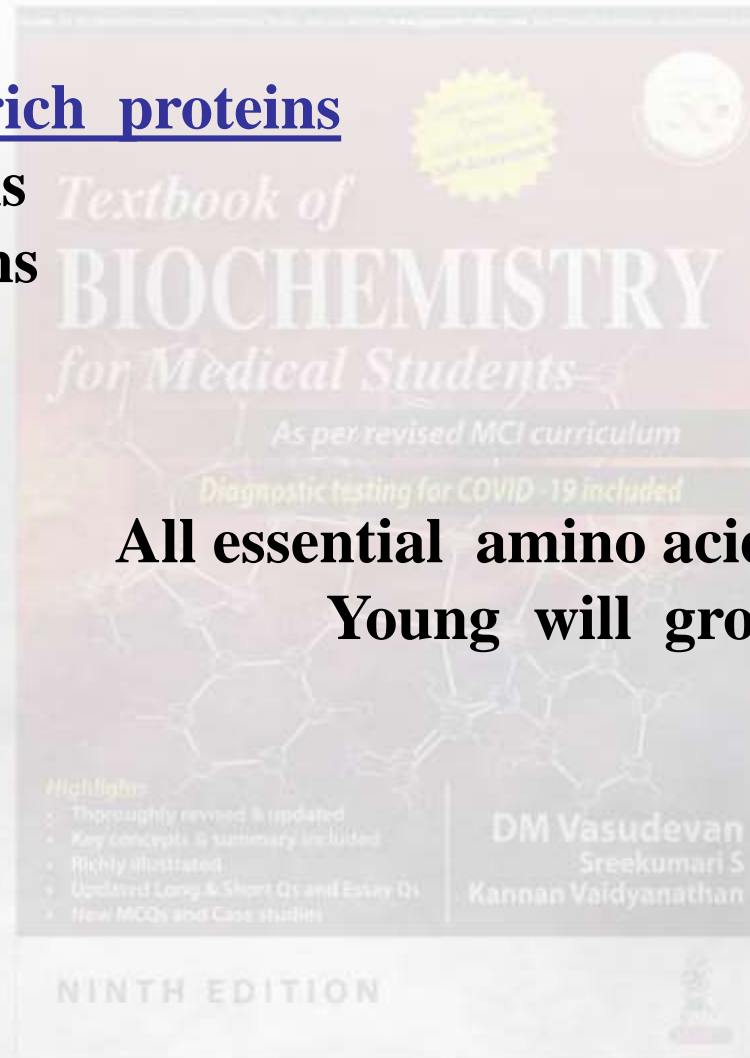**1. Nutritionally rich proteins**

Complete proteins

First class proteins

All essential amino acids

Young will grow

Casein of milk

2. Incomplete proteins
Second class proteins

Lack of one essential a.a.
Can sustain adult;

Pulses lack in Methionine
Cereals lack in Lysine
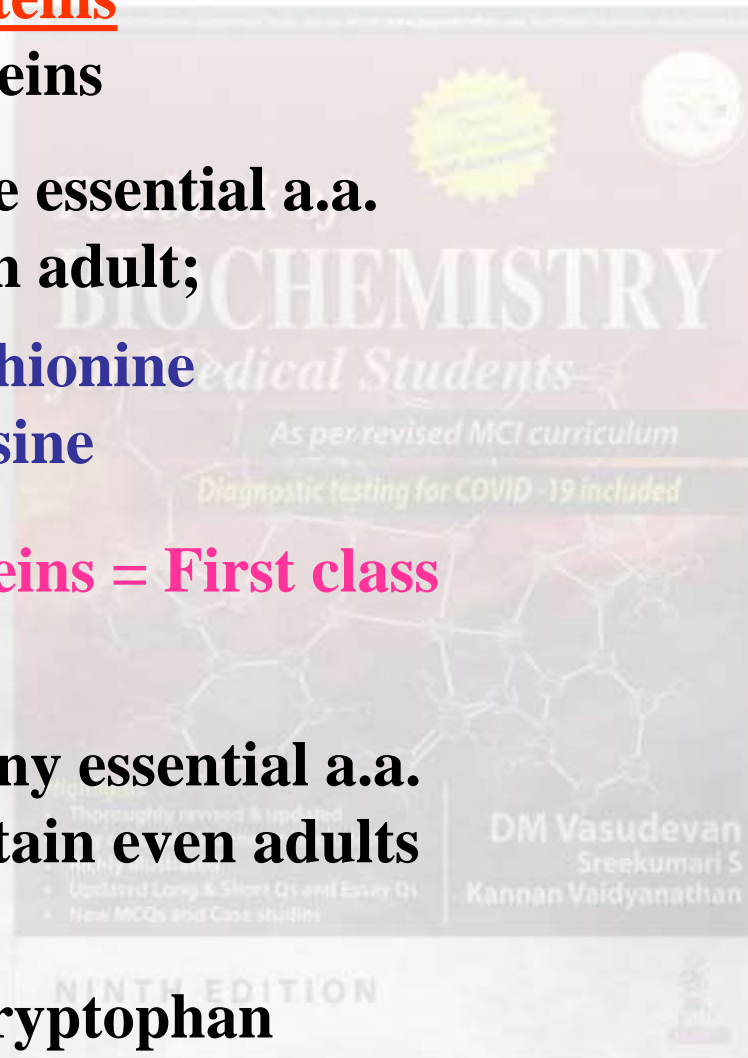
Two 2nd class proteins = First class

3. Poor proteins

Lack of many essential a.a.
Cannot sustain even adults

Zein from corn
lacks in Lys and Tryptophan

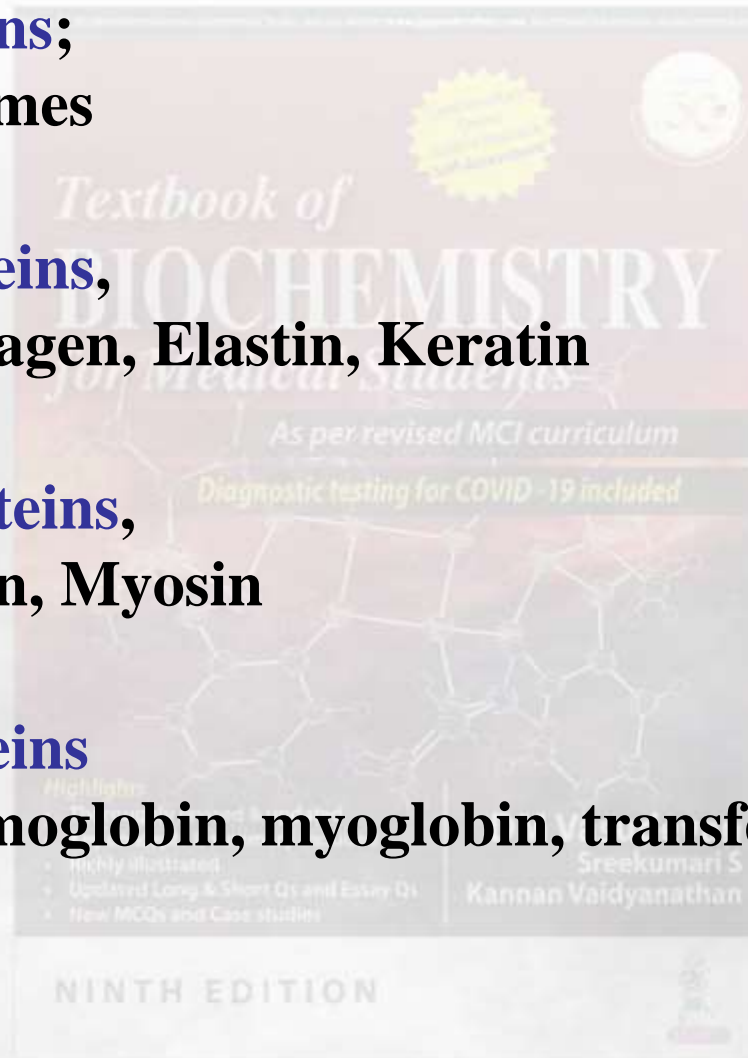# Classification Based on Functions of Proteins

1. Catalytic proteins;
            Enzymes

2. Structural proteins,
            Collagen, Elastin, Keratin

3. Contractile proteins,
            Actin, Myosin

4. Transport proteins
            Hemoglobin, myoglobin, transferrin

5. Regulatory proteins or hormones
        Insulin, Growth hormone

6. Genetic proteins
        Histones
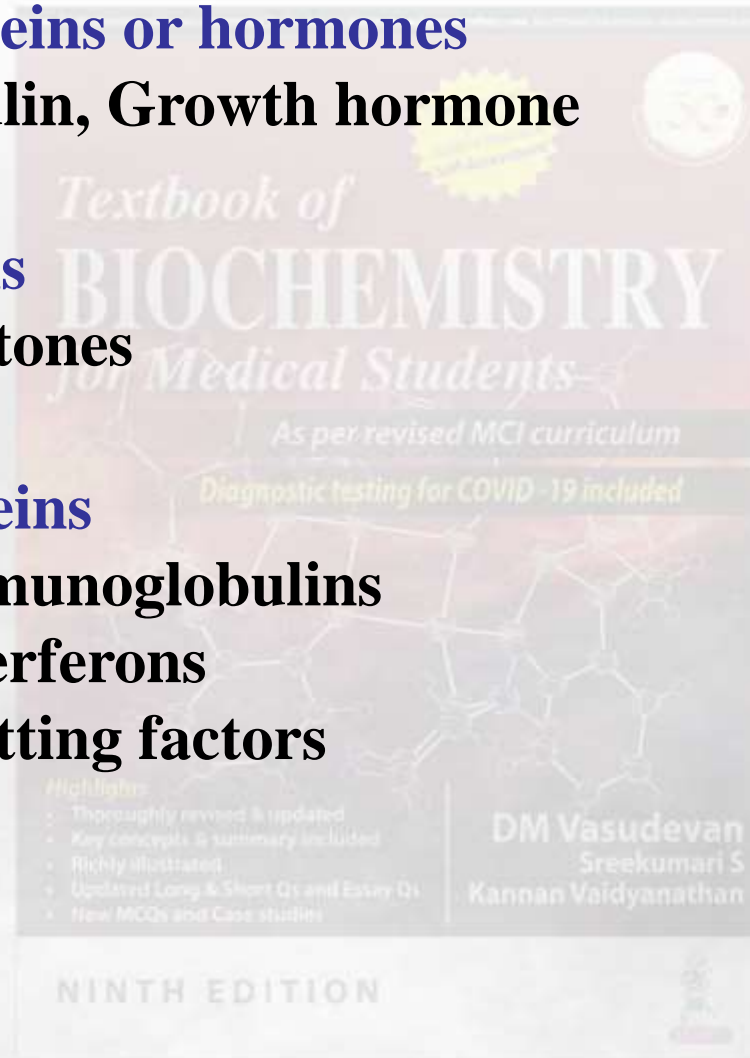
7. Protective proteins
        Immunoglobulins
        Interferons
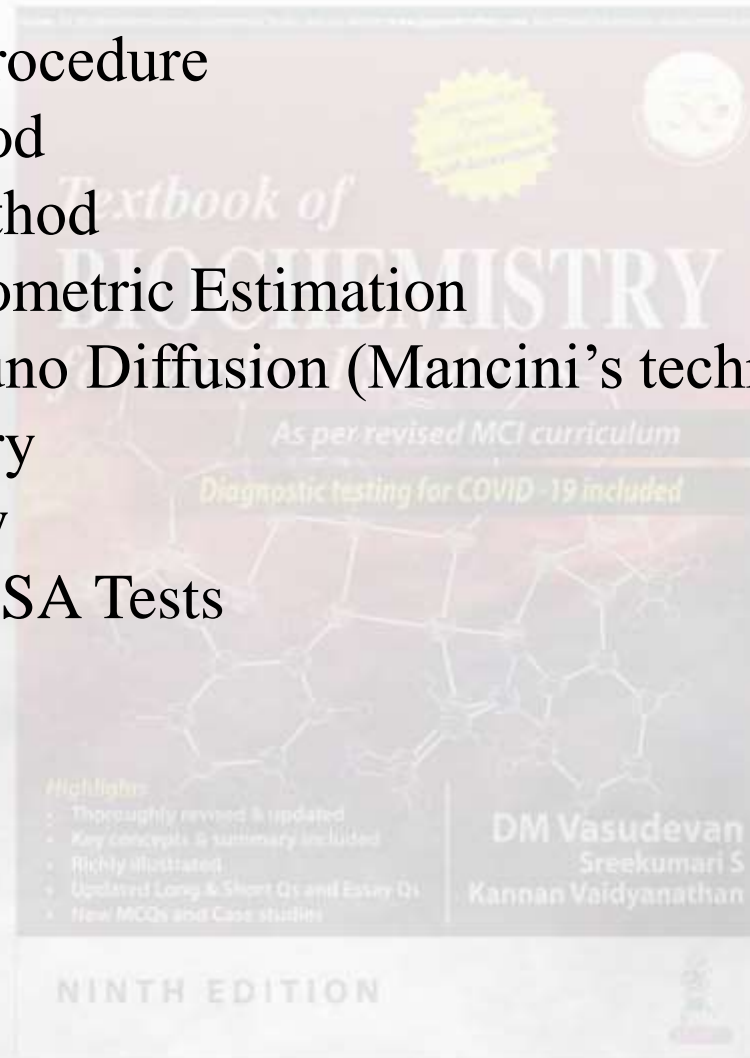   **Clotting factors**

Bioactive Peptides

Bioactive peptides (BPs) are peptides mainly derived from natural sources by bacterial action during food processing. So they are called tertiary metabolites. Fermented milk (lactobacilli action) is a rich source of BP. Fish, egg and meat are also sources for several BP. Plant sources include cereals like rice, wheat, maize, soya and mushroom. They are used as nutraceuticals with a role in maintaining normal health and preventing several life style diseases. They are also used as food additives in food processing to prevent bacterial degradation of food. Several of these BPs have antihypertensive, antioxidant, antimicrobial, antithrombotic, anti-inflammatory and immunomodulatory effects which are beneficial to maintaining health.

Quantitative Estimation

1. Kjeldahl's Procedure
2. Biuret Method
3. Lowry's Method
4. Spectrophotometric Estimation
5. Radial Immuno Diffusion (Mancini's technique)
6. Nephelometry
7. Turbidimetry
8. RIA and ELISA Tests

KJELDAHL'S PROCEDURE

360 c; H2SO4; CuSO4

N $\longrightarrow$ NH3

Proteins have 16% nitrogen

N x 100 / 16

or N x 6.25

Advantage: Accurate Used to standardise

Disadvantage: Many days Unsuitable for clinical work

Biuret Method

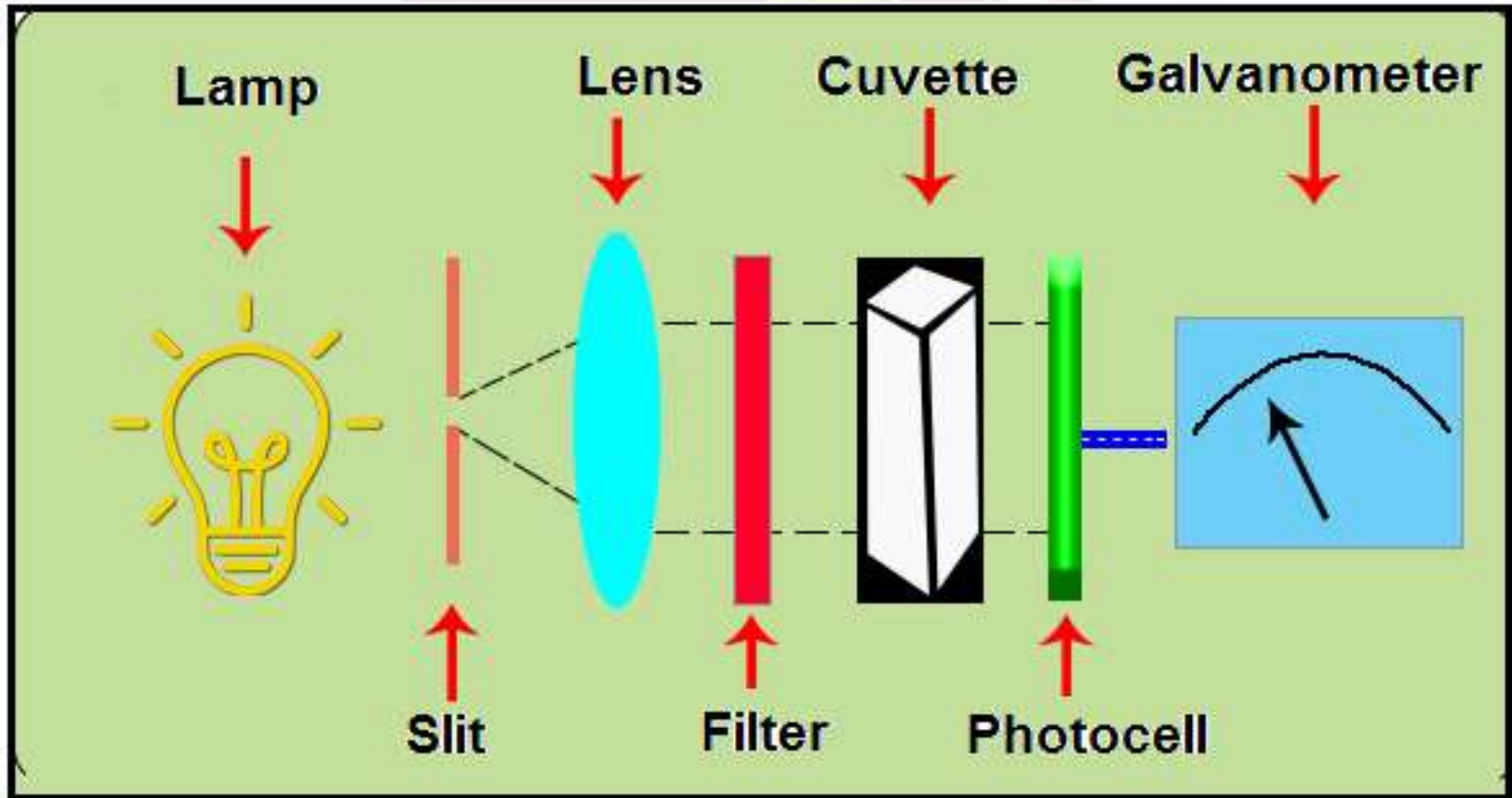Protein + Cu^{++} in alkaline

Peptide bonds ⟶ Violet color

Color in test is compared
with a standard

Biuret Method
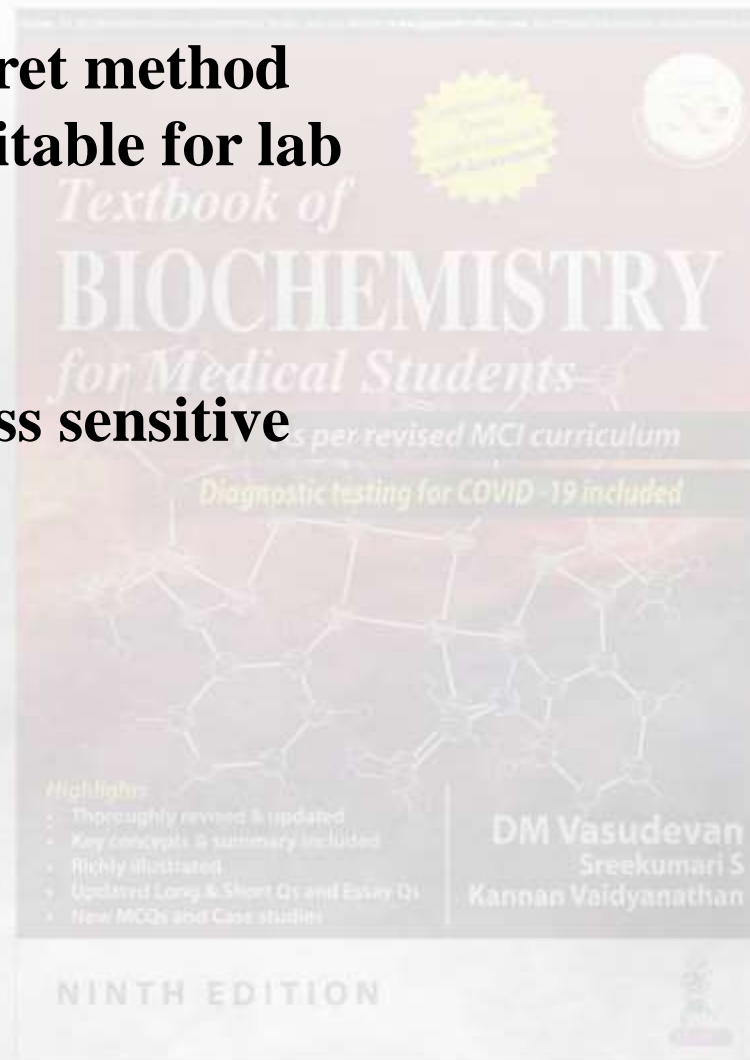
Colourimetry : Beer's law:
Intensity of colour is proportional to coloured particles

Advantage of Biuret method
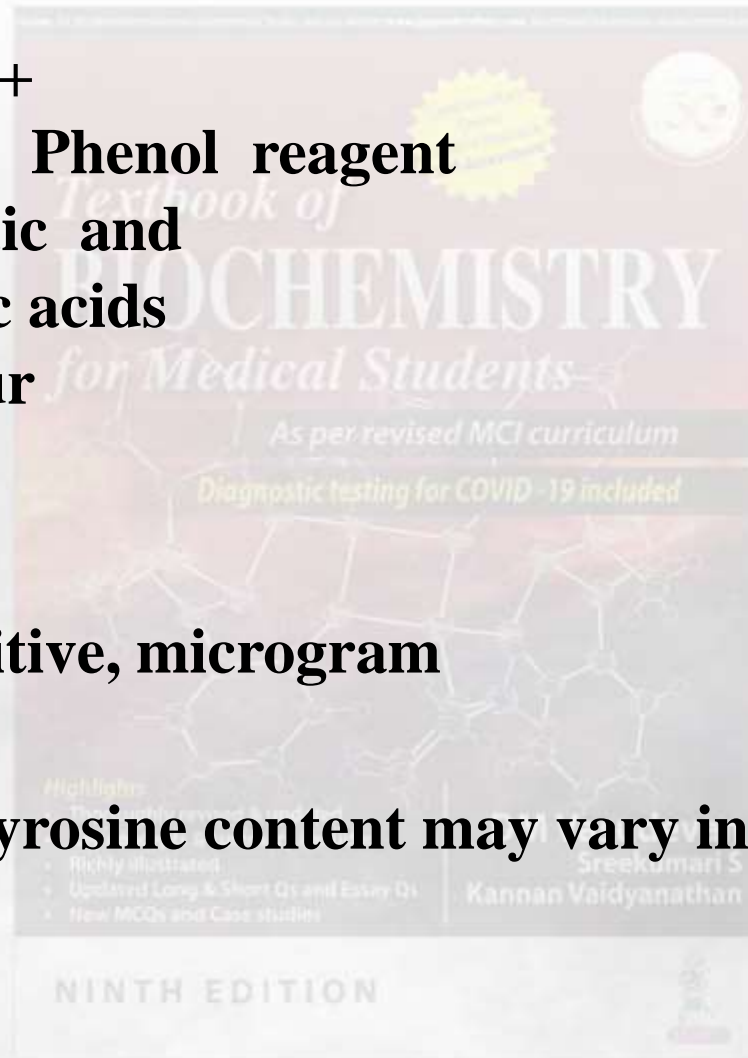   Simple; suitable for lab

Disadvantage: Less sensitive

Protein (Y+ W) +
Folin-Ciacalteou Phenol reagent
Phospho molybdic and
Phospho tungstic acids
➡️   Blue colour

Advantage: sensitive, microgram

Disadvantage: Tyrosine content may vary in test and standard

Spectrophotometer

**Proteins absorb
Ultra violet light at 280 nm**

Advantage

> **Accurate**
> **Simple**
> **Protein is not wasted**
> **Highly sensitive; microgram**

Disadvantage

> **Costly**

Nephelometry

Antigen-Antibody complex
Scattering of light by colloids

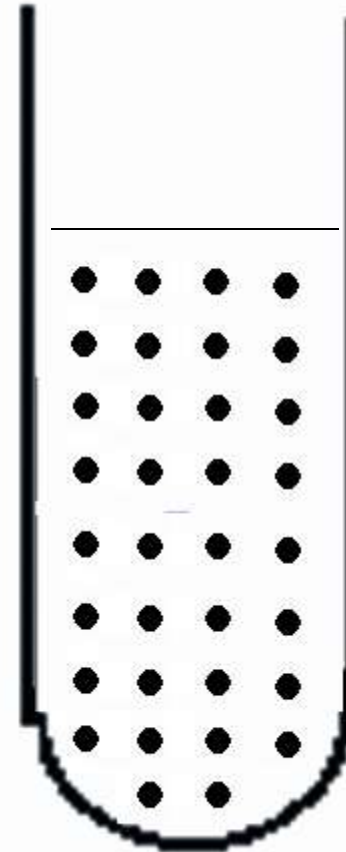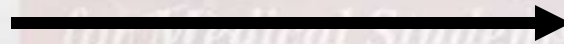**Advantage: very rapid; automated**
Disadvantage: costly

Incident
Laser

Scattered light
at 60 degree

Detector
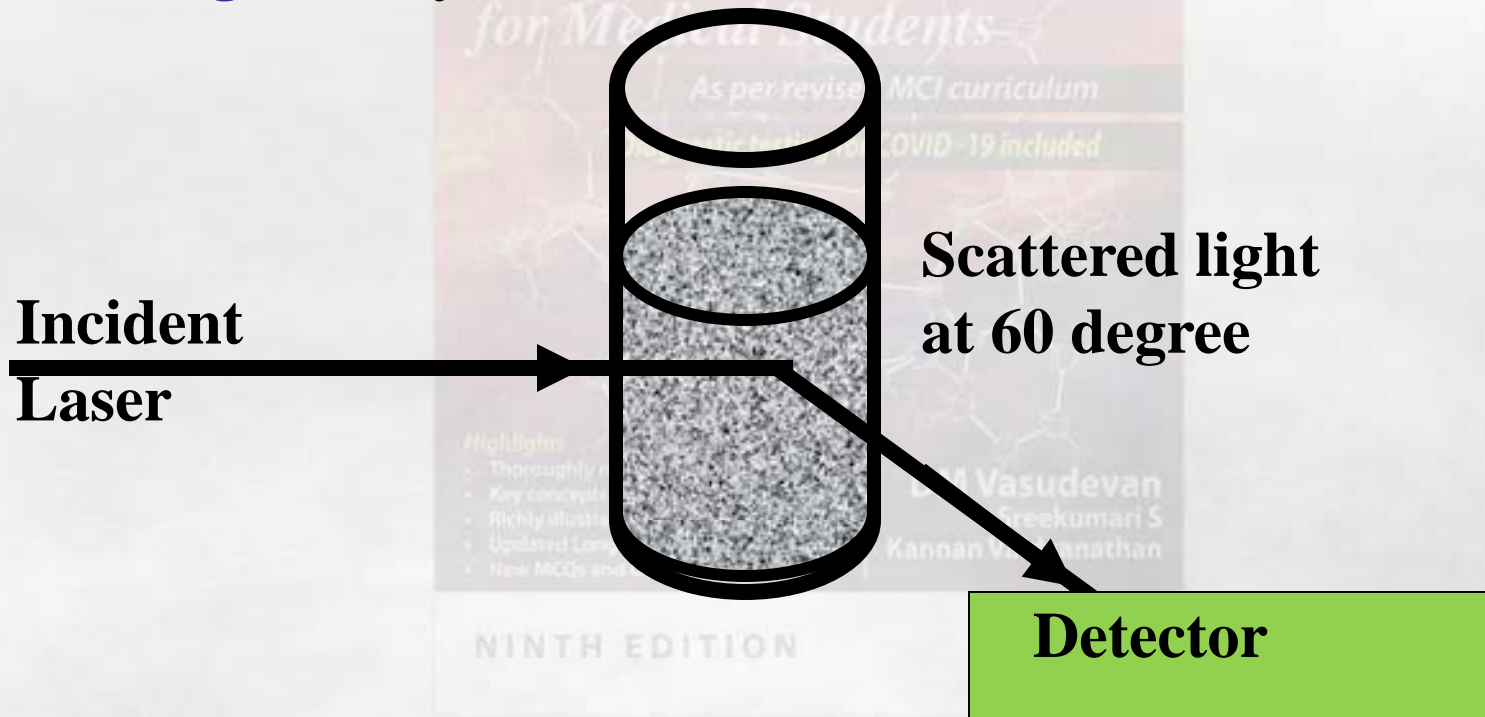
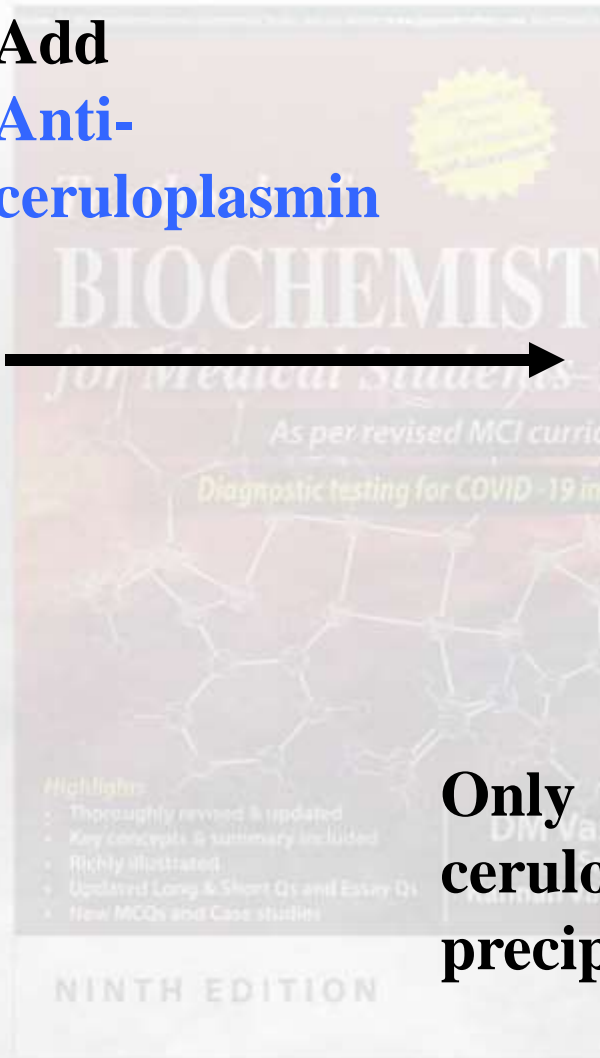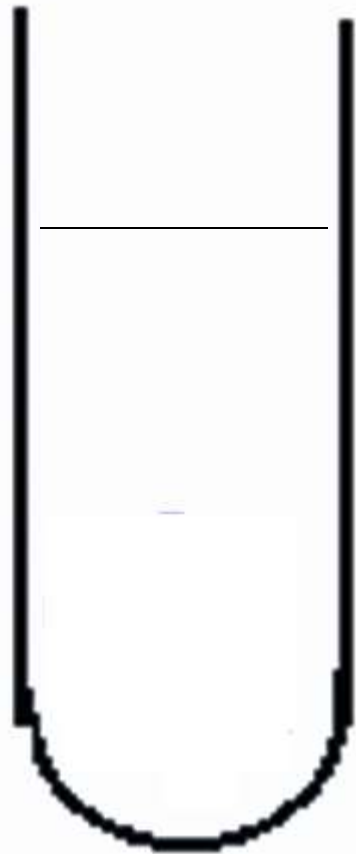| NEPHELOMETRY	TURBIDIMETRY
Fine particles	Course particles
Laser	Ordinary light
Emergent light 60 degree angle	Emergent light 180 degree
Sensitive	Less sensitive
Costly	Cheap

Immuno Turbidimetry
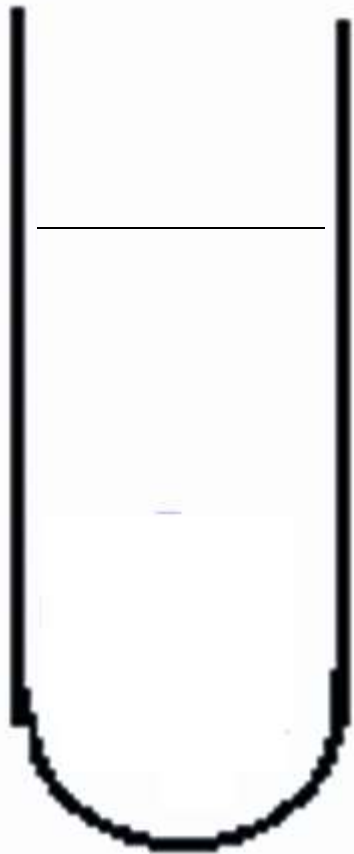
Add **Anti-ceruloplasmin**

Serum

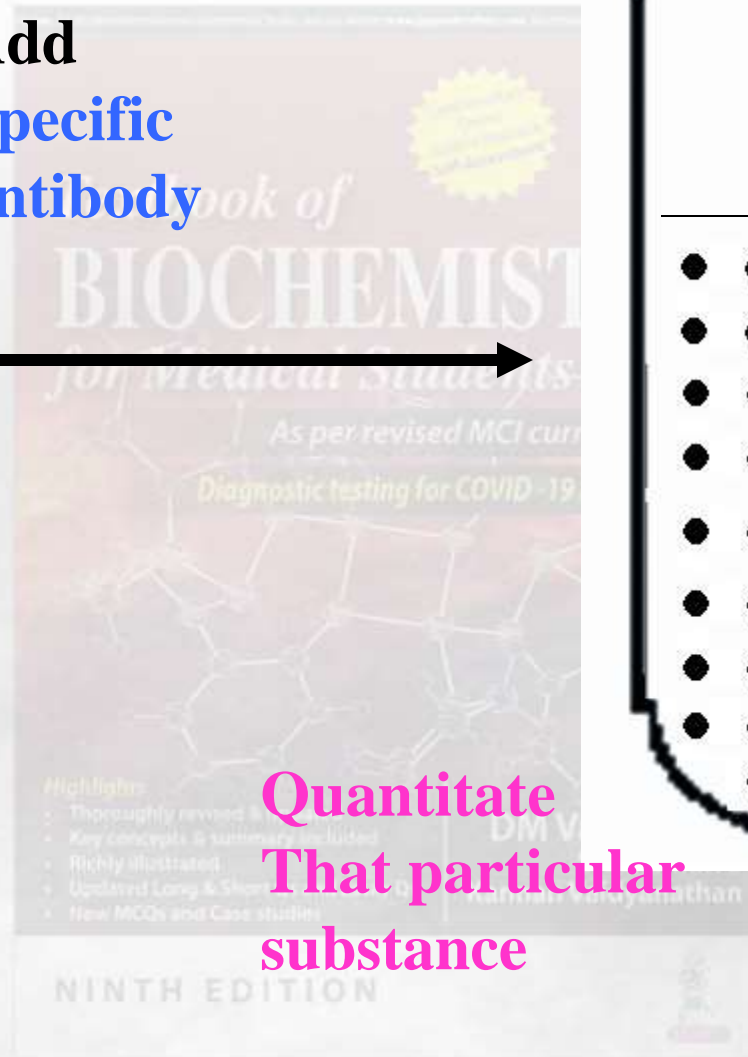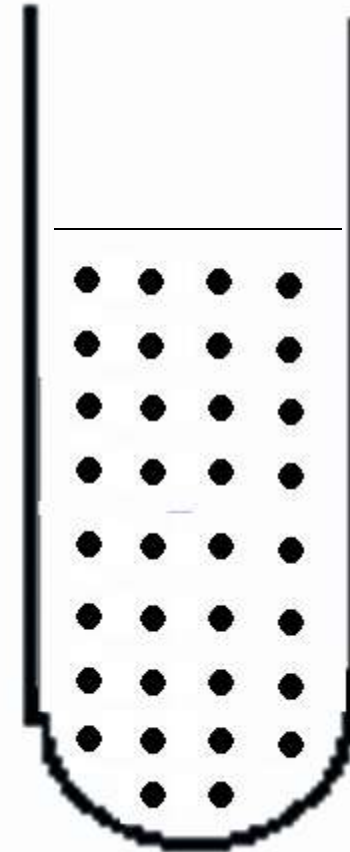Only ceruloplasmin precipitated

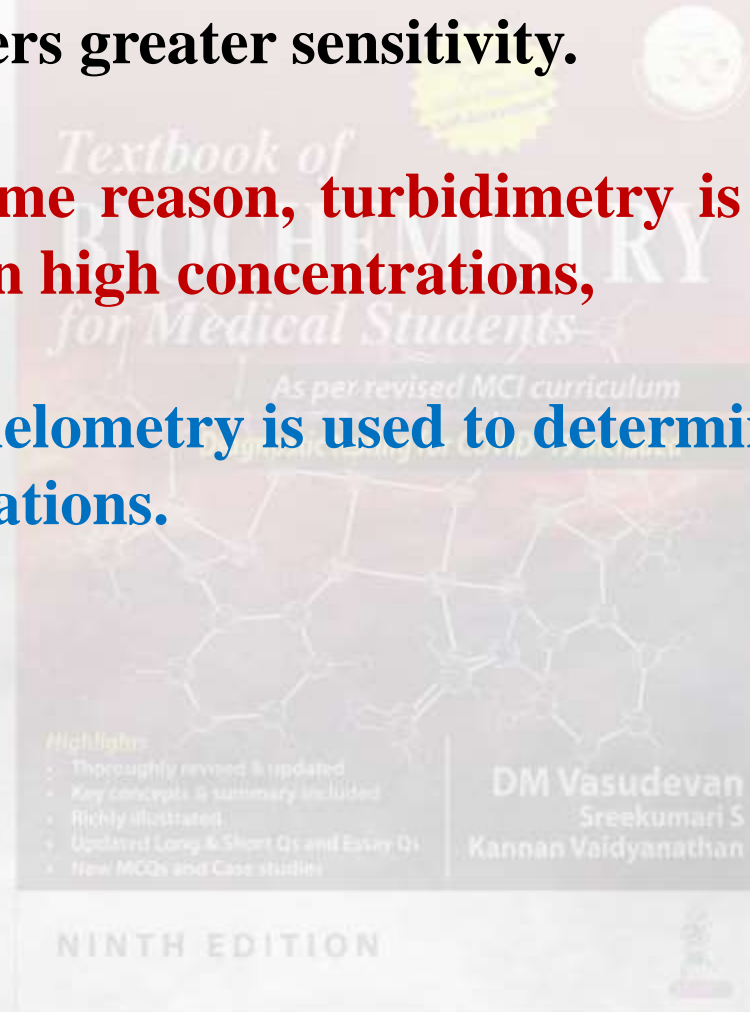# Immuno Turbidimetry

Serum

Add Specific antibody

Quantitate That particular substance

Turbidimetry offers high linearity and nephelometry offers greater sensitivity.

For the same reason, turbidimetry is used to determine proteins present in high concentrations,

while nephelometry is used to determine proteins present in lower concentrations.
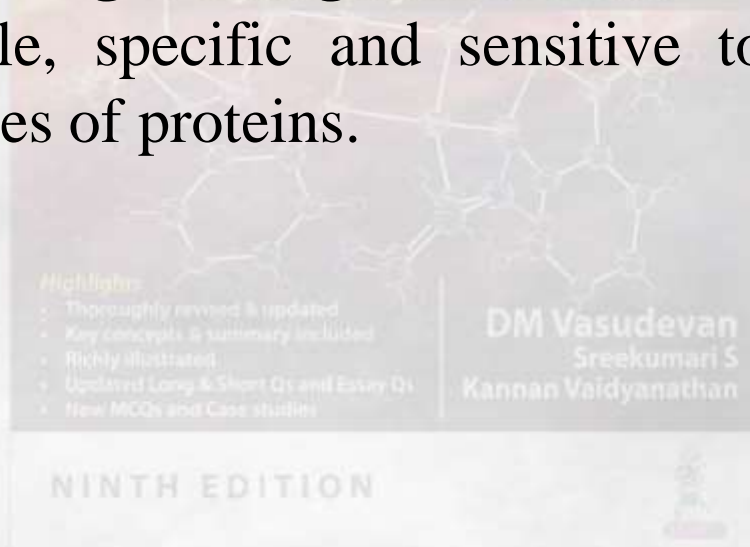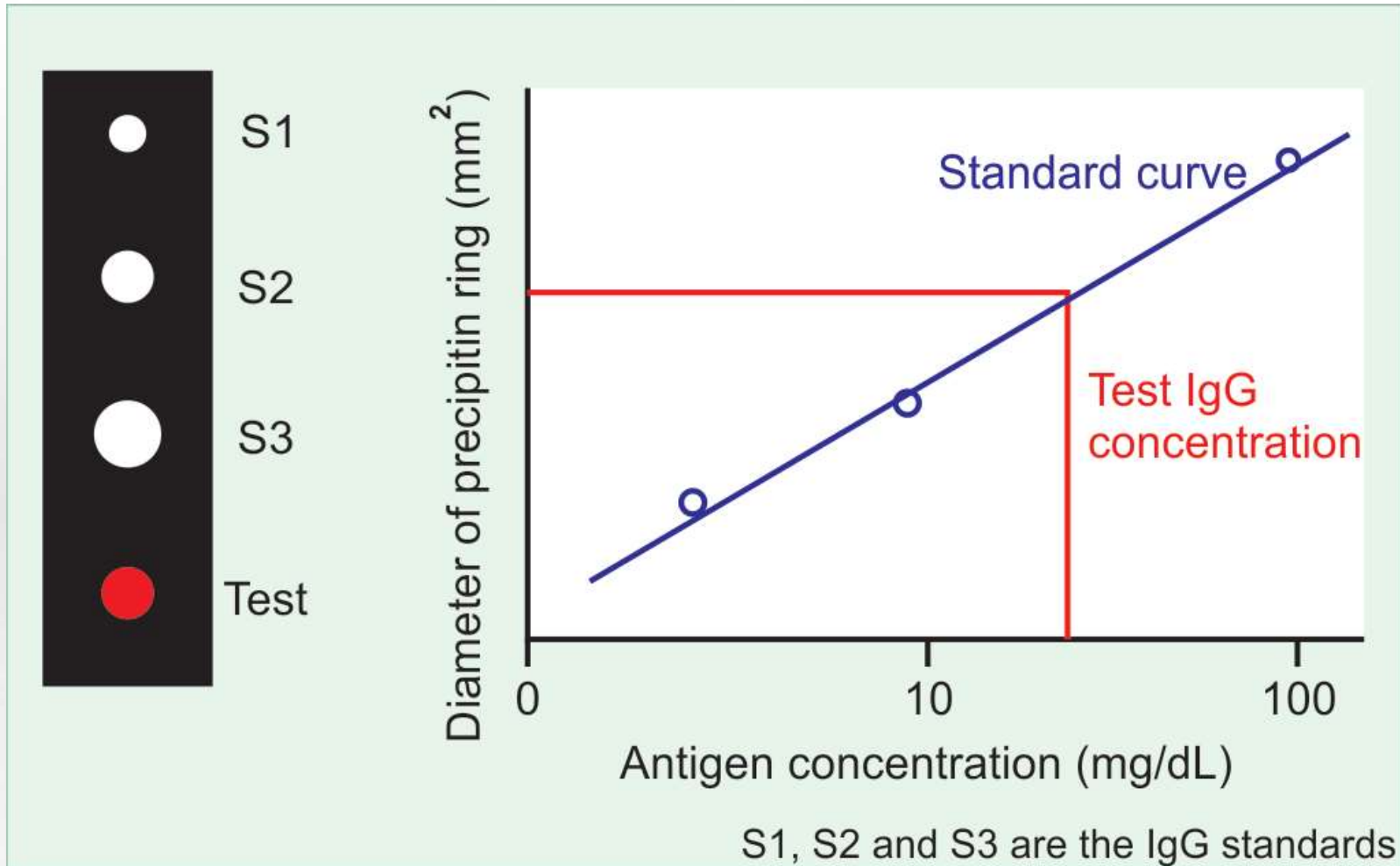
Radial Immunodiffusion (Mancini's Technique)

Specific antiserum is incorporated in the liquid agar, and allowed to solidify. Small wells are cut in the agar, and antigen (protein solution, patient's sera) is added in the well. The plate is incubated at 4°C for 1 to 3 days. A white ring of precipitate is seen, where equimolecular concentration (1:1 ratio) of antigen and antibody is attained. **The diameter of the precipitation ring will be proportional to the log of antigen concentration**.

Advantage: Simple, specific and sensitive to quantitate mg or microgram quantities of proteins.

Radial immunodiffusion