CENTRIFUGE

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Centrifugation is a process used to separate particles from a solution based on their size, shape, density, medium viscosity and rotor speed using centrifugal force. The denser particles tend to move along the length of the tube to a greater radius of rotation, displacing the lighter particle to the proximal end. It is carried out with an apparatus called centrifuge. A centrifuge is a device for separation of microbes from the suspended fluid using centrifugal force or gravitational force. The word centrifugal force isoriginated from Latin word centrum, meaning "center", and fugere, means "to flee. The speed of centrifuge is denoted as rpm or revolutions per minute.

Figure 1: Centrifuge Machine

I. PRINCIPLE

The basic principle of centrifugation is depends on sedimentation. The movement of particles in a centrifugal field is called sedimentation and the particles settle down because of gravitation force. The sedimentation property of particles depends on a number of different factors including size, density and shape. According to sedimentation, the larger and denser particles settle first and small and lighter particle will float to the top. Density and size vary

significantly depending on the composition of the solution in which the particles are suspended. In centrifugation it is important to differentiate between the speeds of centrifugation Revolutions per minute (RPM) and the relative centrifugal force (RCF).

The particle (m) is acted on by three forces:

- FC - centrifugal force
- FB - buoyant force
- Ff - frictional force between the particle and the liquid.

Basis of Separation

- The more dense particle, the faster it sediments.
- The greater the friction coefficient is, the slower a particle will move.
- The longer the radius of rotation, the greater will be the centrifugal force.

Relative Centrifugal Force

- Particles suspended in a fluid move, under the influence of gravity towards the bottom of a vessel at a rate that depends on their size and density.
- Centrifugation utilizes centrifugal force which are greater that the gravitation force of earth to increase the rate of sedimentation of a particle.
- This is achieved by spinning the vessel containing the fluid and particles about an axis of rotation so that the particle experience centrifugal force act away from the axis.
- The force is measured in multiple of the gravitational force known as relative centrifugal field.
- The centrifugal force generated by a centrifuge can easily be calculated from the equation:

RCF (g Force) = 11.18 × R × (RPM/1000)^2
Where,
RPM = Revolutions per minute
RCF = Relative centrifugal force
R = distance from the centre of rotation in centimeters

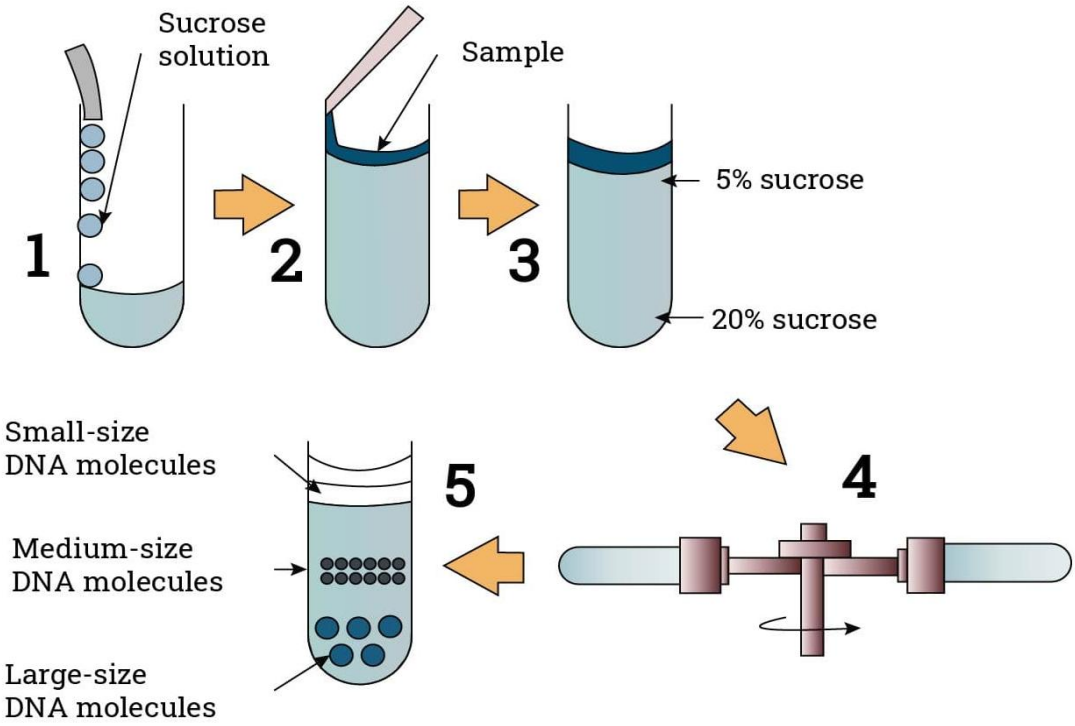


Figure 2: Working Mechanism of Centrifuge

II. SEDIMENTATION RATE

Asolution is an intimate mixture of one or more solutes and a solvent. When the solution is allowed to stand, the solute settles at the bottom. The settling of the solutes in a solvent is called sedimentation. The sedimentation is accelerated by rotation of the solution in a centrifuge. The speed at which a solute sediments, is depending on the mass of the molecule, the speed of rotation, density, viscosity and temperature of the medium and shape of the solute.

Sedimentation depends on the following factors are given below-

- The density of the particle
- Gravitational force
- The viscosity of the medium
- Particle size

The rate of sedimentation is dependent upon the applied centrifugal field (G), that is determine by the radial distance (r), of the particle from the axis of rotation, and the square of the angular velocity of the rotor.

$G = \omega^2 r$

- Angular velocity, ω (in radians/sec)
- Radial distance, r (in cm)
- In a suspension of biological particles, the rate of sedimentation is dependent not only upon the applied centrifugal field but also on the nature of the particle i.e. density and radius and also viscosity of the surrounding medium. Stokes law describe these relationships for the sedimentation of a rigid spherical particle:

$$v = \frac{2r^2}{9} \frac{(p_p - p_m)}{n} x g$$

III. SEDIMENTATION COEFFICIENT

The ratio of the velocity to the centrifugal acceleration is called sedimentation co-efficient (S). The sedimentation co-efficient of biological macromolecule are relatively small and are usually expressed as Svedberg unit, S.

$$\boldsymbol{s = Vt/a}$$

where,
Vt = Velocity,
a= acceleration.
Here, acceleration is due to centrifugal force

$$\boldsymbol{a = \omega^2 r}$$

Where,
ω = angular velocity,
r = distance of the particle from rotor axis.
Substituting 'a' in sedimentation co-efficient (s)

$$\boldsymbol{S = \frac{Vt}{a} = Vt/\omega 2r}$$

The sedimentation co-efficient of proteins falls in the range between 10^{-13} S and 200×10^{-13} S.

IV. PARTS OF CENTRIFUGE

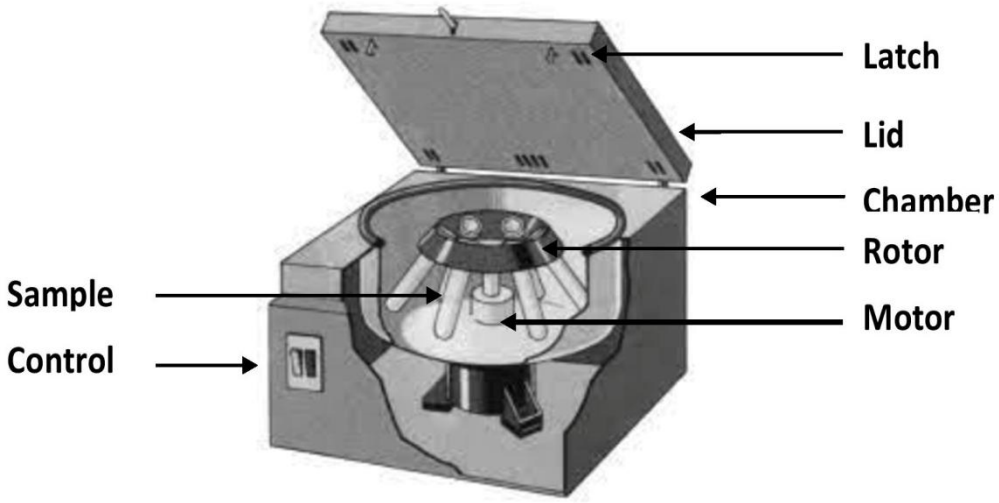


Figure 3: Parts of Centrifuge

1. **Motor:** The motor makes the rotor to spin around the axis of the centrifuge.

2. **Rotor:** The rotor is fitted with a horizontal rod or disc containing sample holder. The rotor may be angle type or swinging bucket type.

3. **Containers:** A variety of containers are present in the rotors such ascuvettes, centrifuge tubes, test tubes, blood bagsetc.

4. **Control Panel:** It is used to regulate many factors, including rotating speed and temperature.

5. **Latch:** The latch keeps the lid closed while running.

6. **Lid:** To prevent mishaps, the centrifuge spin only when will the lid is locked.

Type of Rotor

1. **Fixed Angle Rotors:** Fixed angle rotor is ideal for pelleting during the differential separation of biological components whose sedimentation rate are significantly different. Centrifugation tubes are held at a fixed angle of between $14^{\cdot}$ To $40^{\cdot}$ to the vertical in this type of rotor.

2. **Vertical Rotors:** Centrifuge tubes are held parallel to the axis of rotation in and are restrained in the rotor cavities by screws. Vertical rotors sample are not separated down the length of the centrifuge tubes but across the diameter of the tube. Vertical rotors are divided into two types, true vertical rotor and near vertical rotor. Near vertical rotor exhibits a reduced angle of 7. -10. Results in much shorter run times as compared to fixed angle rotor.

3. **Swinging Bucket Rotors/ Horizontal Rotors:** They are loaded in a vertical position and during the initial acceleration phase, rotor buckets swing out horizontally and then position themselves at the rotor body for support.

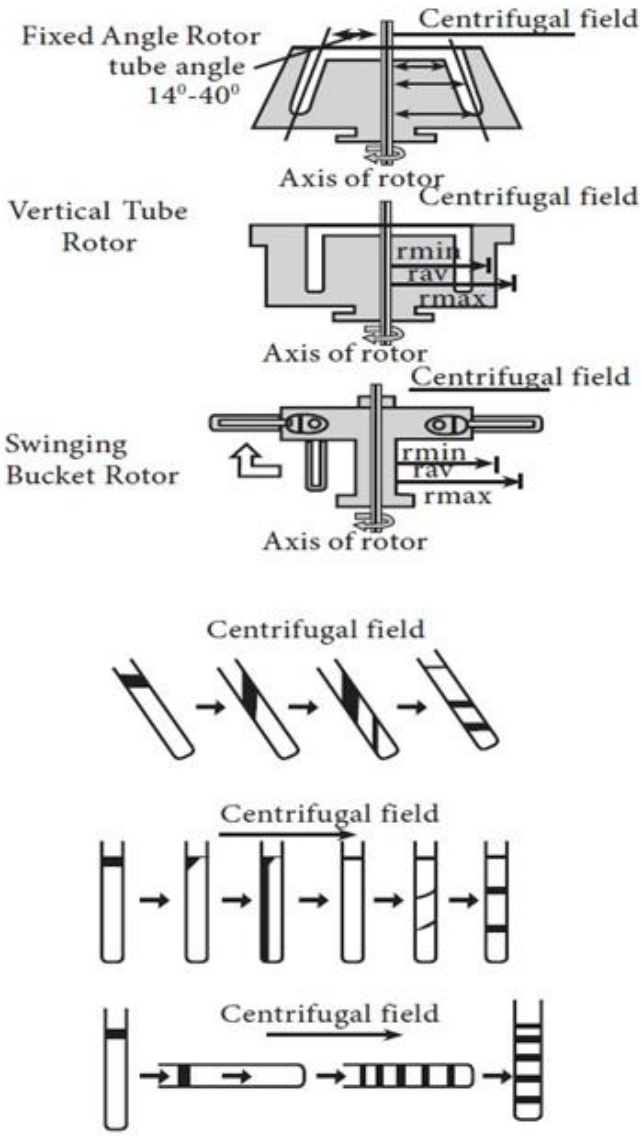
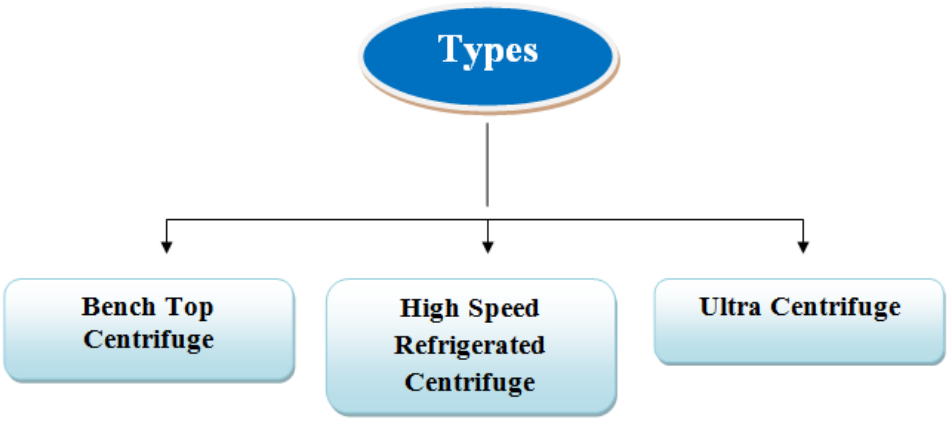


Figure 4: Types of Rotors

V. TYPES OF CENTRIFUGE



1. **Benchtop Centrifuge:** Simple bench top centrifuge vary in design and are used mainly to collect small amount of biological material such as blood cells. Modern benchtop centrifuge is a low speed (3000- 7000 x g) centrifuge that may or may not be have a cooling system.

2. **High Speed Refrigerated Centrifuges:** High speed centrifuge are absolutely essential for the sedimentation of protein precipitates, large intact organelles, cellular debris, derived from tissue homogenization and microorganism. They operate at maximum centrifugal field of approximately 1,00,000 g. Such centrifugal force is not sufficient to sediment smaller vesicles and ribosomes, but can be employed to separate nuclei, mitochondria.

3. **Ultra Centrifuge:** In ultracentrifuge the sample rotates at a high speed upto 60,000 rpm. The speed of motor is control with a speed control drive attach to the motor. It has an automatic system to slow down the speed as soon as it finds over speed in a motor. There is an infrared radiometric sensor which measure and control the temperature of the rotor.

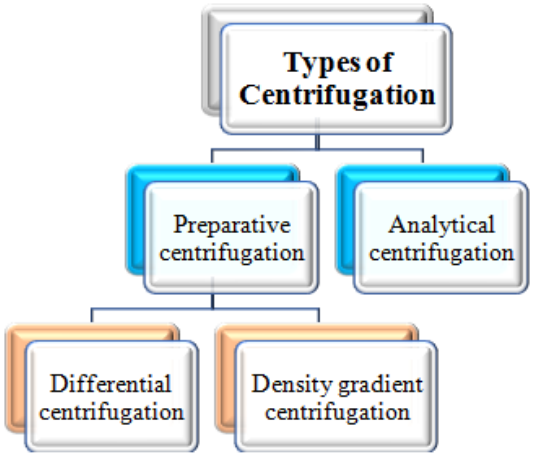
VI. TYPES OF CENTRIFUGATION

Centrifugation can be divided into two types:

1. **Preparative Centrifugation:** The preparative-scale separation procedure is simple as it requires the placing of the sample in the tube, inserting the tube in the rotor and spinning the sample for a fixed period. These results in two phases, pellet (the particles which is settled at the bottom of the tube) and the supernatant. This technique is also called Velocity sedimentation

centrifugation. It is primarily used for separation and purification of sample for further analysis.

Preparative-scale separation makes use of specific method of separation, such as differential centrifugation and density gradient centrifugation.



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a. **Differential Centrifugation:** It consists of successive centrifugation at increasing rotor speeds. Separation of particles in a homogenous medium by centrifuging that solution at different speeds at different times is called differential centrifugation. It is followed when the solution contains different particles whose molecular masses are enough to sediment that at different speeds. During the centrifugation the media components are kept constant.

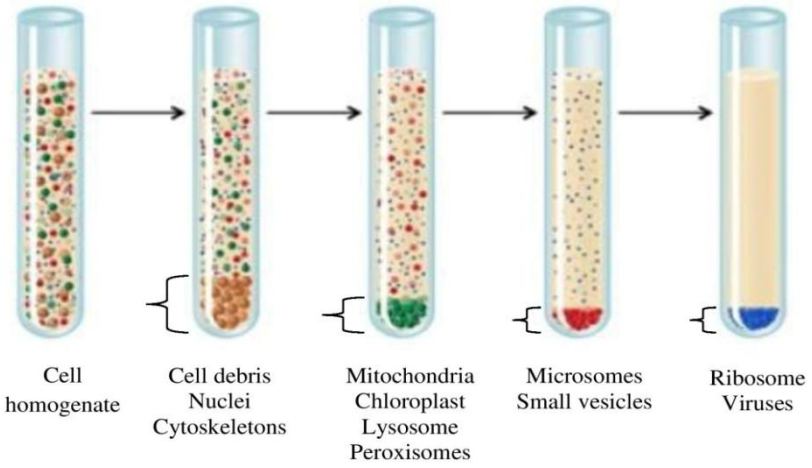

Figure 5: Saparation of Biological Fluid Using Differential Centrifugation

Example

- A tissue homogenate which contains the whole cells, nuclei, cytoskeletons, plasma membrane, mitochondria, lysosomes, peroxisomes, microsomes, endoplamic reticulum, small vesicles, large molecules like ribosomes and protein can be separated by differential centrifugation.
- After a centrifuge run at low speed at 1000 g for 10 min, the heavier particles like nuclei, whole cell, cell debris, plasma membrane will settle down at the bottom of the tube forming pellet.
- The supernatant thus obtained can be subjected to medium speed centrifugation at 20,000 g for 20 min. Subcellular organelles like mitochondria, lysosomes, peroxisomes will settle as pellet at the bottom of the tube. The supernatant again can be run on a high speed centrifugation at 80,000 g for 1 h, thus settling down miscrosomes and small vesicles as pellet.
- The supernatant can be further centrifuged to separate out organelles like ribosome from the soluble protein. Thus differential centrifugation is used to fractionate cell homogenates into their components.
- The tissue homogenate contains many sub-cellular organelles which differ in size and therefore sediments at different rates. Each pellet is a mixture of different sub-cellular organelles.
- Therefore, the differential centrifugation is a rough fractionation of the cytoplasmic contents which can be further purified by density gradient centrifugation.

Drawback

- Poor yield
- Impure preparation

Applications

- Used to study sub cellular organelles.
- It used for low resolution separation of nucleus.
- Used for purification of extracts.

b. Density Gradient Centrifugation: Density gradient centrifugation can be divided as Zonal centrifugation (separation is depending on size) and Isopycnic centrifugation (separation is depending on density). The centrifugation of a sample in a density gradient solution along the gradient of increasing order of density of sucrose or cesium chloride is called density gradient centrifugation. It is followed when the particles to be separated are more or less similar in their size but have different density. If particles have different densities they are collected at their equilibrium densities when the solutions is centrifuges along the density gradient of increasing order of sucrose concentration. The particles sink in the solution of particular density, if the density of the particle is then that of the band of sucrose solution. They float on a particular sucrose density, if their density is lesser than the band of sucrose solution.

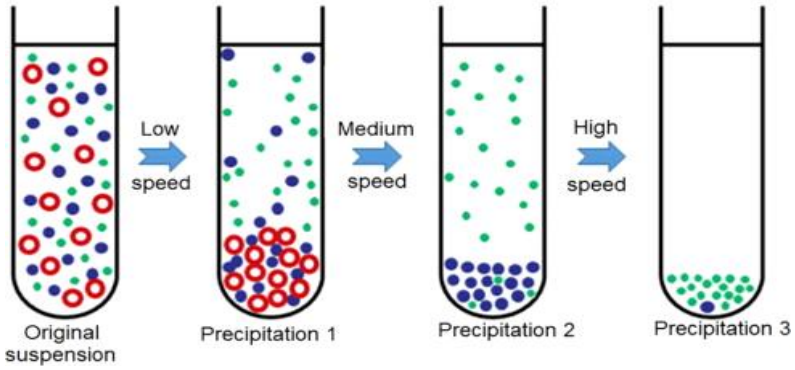

Figure 6: Density Gradient Centrifugation

Application

- Large quantities of biomolecules can be purifying by Density gradient centrifugation.

- It is used for the purification of particles separated by differential centrifugation
- Density of cells in suspension and pollen grains can be measured with Density gradient centrifugation
- Density gradient centrifugation is used for the isolation of plant and animal viruses.

VII. RATE ZONAL CENTRIFUGE

- In zonal centrifugation, the particles of interest are placed on top of the gradient medium and centrifuge in an ultracentrifuge. Solutions of sucrose or glycerol or cesium chloride or cesium sulfate are the high density solutions which are used to make the gradient medium.
- The molecules are separated on the basis of sedimentation coefficient. Particles with larger mass and more compact structure have high sedimentation coefficient and they form band at the bottom. Since each organelle has different ratios of lipid and protein content, there is a difference in their buoyant densities.
- These organelles are separated by centrifugation through a column of solvent with graded density of sucrose solution. The sucrose solution is most concentrated at the bottom of the tubes and decrease in concentration or density towards the top of the tube. So, various organelles move down the tube to an equilibrium position where their density is equal to that of the sucrose at that position.

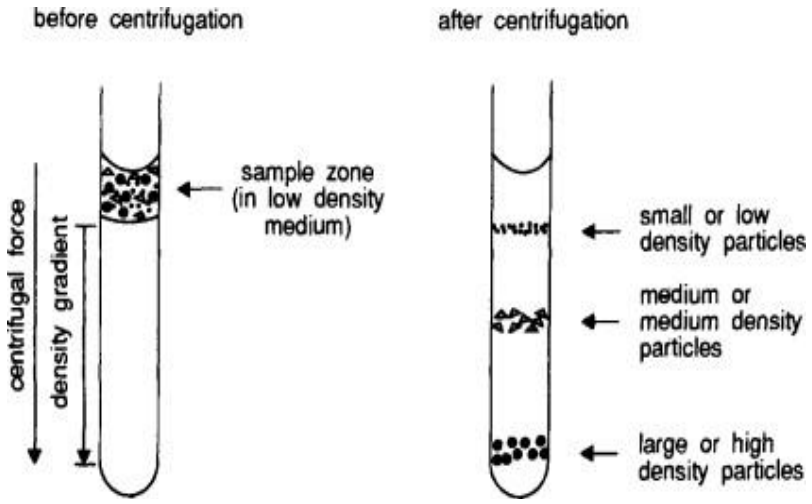

Figure 7: Rate Zonal Centrifuge

Application

- Rate zonal centrifugation is widely employed in the isolation and separation of nucleic acids, such as DNA and RNA.
- Purification of Proteins.
- Fractionation of Cell Components.
- Viral Particle Analysis.

VIII. ISOPYCNIC CENTRIFUGATION

- It is also known as equilibrium density gradient centrifugation.
- It separates particles solely on the basis of buoyant density and is independent of shape and size of particles.
- This technique is used to separate particle of similar size but different density.
- Lengthening the centrifugation time will have no effect, because the distribution of the molecule is at equilibrium.

Application

- It is commonly used to separate and purify circular and linear forms of DNA.
- By the incorporation of heavy isotopes, separations can be improved (e.g15N).
- It can use to purify viruses
- It can also purify human plasma lipoproteins.

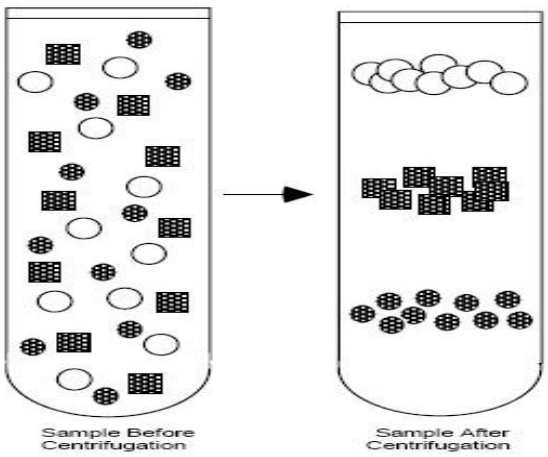

Figure 8: Isopycnic Centrifugation

3. **Analytical Centrifugation:** A typical analytical centrifuge can generate a centrifugal field of 2,50,000 g. Within these extremely high gravitational field the centrifuge cell has to allow light passage through the sample for proper measurement of the concentration distribution. The availability of high intensity xenon flash lamps and the advance in instrumental sensitivity and wavelength range has made the accurate measurement of highly dilute protein sample possible below 230 nm.

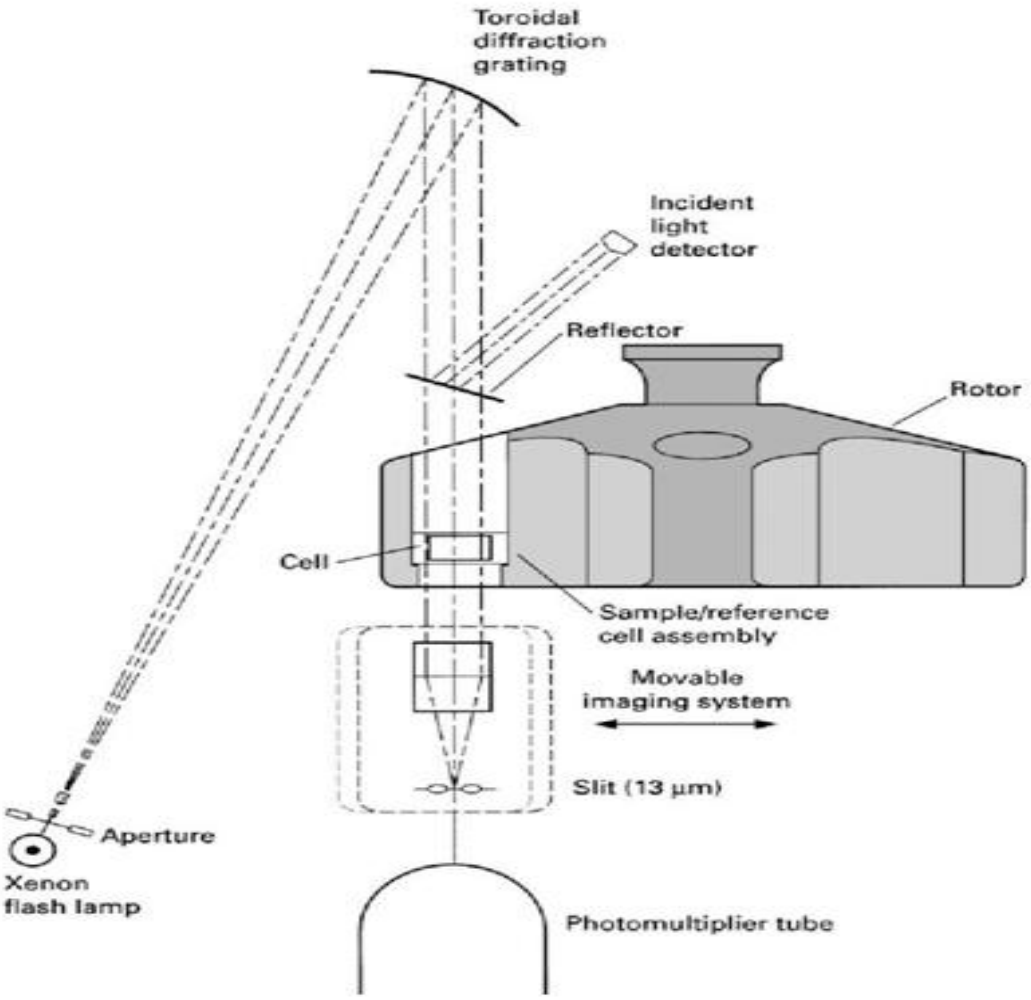

Figure 9: Working of Analytical Centrifuge

IX. APPLICATIONS OF ANALYTICAL CENTRIFUGATION

- Analytical centrifugation may be employed to determine the quality of macromolecules.
- The hydrodynamic properties of macromolecules are described by their sedimentation coefficients and can be determined from the rate that a

concentration boundary of the particular biomolecules moves in the gravitational field.
- It is also used to detect changes in conformations.
- Ligand-binding study.

Merits

- Small amount of sample is required
- Continuous monitoring of process.

X. APPLICATIONS OF CENTRIFUGE IN DIFFERENT AREAS

1. Industries

    - Centrifuge is commonly used in food industry to separate cream (fat) from milk.
    - Centrifuges are commonly used in water treatment plant to treat waste water.

2. Material Synthesis: For casting and material synthesis in chemical industry the centrifuge is used.

3. In Biological laboratory

    - Use for the removal of cellular debris from blood to prepare cell free plasma serum Concentrate cellular elements and other components for microscopic analysis
    - It can be used to separate protein bound or antibody bound ligand from free ligand in immunological assay.
    - It uses to separate lipid components such as chylomicrons from other components of plasma.
    - Separation of sub cellular organelles, RNA, DNA. for the determination of purity and shape of the biomolecules.

4. There are some centrifuges that are used to separate isotopes such as Zippe type centrifuges.

References

[1] Image Credit –

https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.chanitmedical.com%2Fproduct%2Fcentrifuge-for laboratory%2F&psig=AOvVaw0_g7zXzU3fZ3gr17XA8BON&ust=1719656397615000&source=images&cd=vfe&opi=89978449&ved=0CBEQjRxqFwoTCLDJibyJ_oYDFQAAAAAdAAAAABAE

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