Centrifugation and Centrifuge

Separation of suspended particles from solution

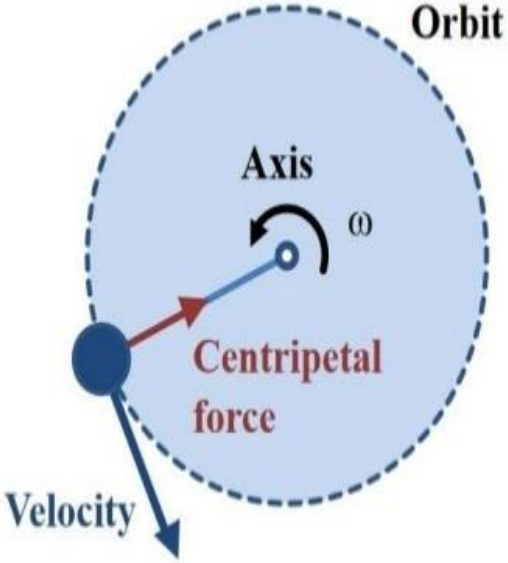
Centrifugation

- Centrifugation is the technique of separating components where the centrifugal force/ acceleration causes the denser molecules to move towards the periphery while the less dense particles move to the center
- The process of centrifugation relies on the perpendicular force created when a sample is rotated about a fixed point.
- The rate of centrifugation is dependent on the size and density of the particles present in the solution
- Centrifugation is a technique of separating substances which involves the application of centrifugal force
- The particles are separated from a solution according to their size, shape, density, the viscosity of the medium and rotor speed

Principle

- In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it floats to the top
- The greater the difference in density, the faster they move
- If there is no difference in density (isopycnic conditions), the particles stay steady
- A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward)
- The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction
- At the same time, objects that are less dense are displaced and move to the center

Centrifugal force



Principle

Relative Centrifugal Force (RCF)

- Relative centrifugal force is the measure of the strength of rotors of different types and sizes
- This is the force exerted on the contents of the rotor as a result of the rotation.
- RCF is the perpendicular force acting on the sample that is always relative to the gravity of the earth
- The RCF of the different centrifuge can be used for the comparison of rotors, allowing the selection of the best centrifuge for a particular function

The formula to calculate the relative centrifugal force (RCF) can be written as:

- RCF (g Force)= $1.118 \times 10^{-5} \times r \times (RPM)^2$
- where r is the radius of the rotor (in centimeters), and RPM is the speed of the rotor in rotation per minute

$$G = W^2 r$$

G= Centrifugal field

W= angular Velocity

R= radial distance of particle from axis of rotation

$$W = \frac{2\pi rev}{min}$$

$$G = \frac{4\pi r^2}{3600}$$

Centrifuge

- A centrifuge is a device used to separate components of a mixture on the basis of their size, density, the viscosity of the medium, and the rotor speed
- The centrifuge is commonly used in laboratories for the separation of biological molecules from a crude extract.
- In a centrifuge, the sample is kept in a rotor that is rotated about a fixed point (axis), resulting in strong force perpendicular to the axis
- There are different types of centrifuge used for the separation of different molecules, but they all work on the principle of sedimentation

Centrifuge and Centrifugation

Rotors:

in centrifuges are the motor devices that house the tubes with the samples

Centrifuge rotors are designed to generate rotation speed that can bring about the separation of components in a sample.

There are three main types of rotors used in a centrifuge, which are:

1. Fixed angle rotors

- These rotors hold the sample tubes at an angle of 45° in relation to the axis of the rotor
- In this type of rotor, the particles strike the opposite side of the tube where the particles finally slide down and are collected at the bottom
- These are faster than other types of rotors as the pathlength of the tubes increases
- However, as the direction of the force is different from the position of the tube, some particles might remain at the sides of the tubes

1. Fixed angle rotors

2. Swinging bucket rotors/ Horizontal rotors

- Swinging bucket rotors hold the tubes at an angle of 90° as the rotor swings as the process is started
- In this rotor, the tubes are suspended in the racks that allow the tubes to be moved enough to acquire the horizontal position
- In this type of rotors, the particles are present along the direction or the path of the force that allows the particles to be moved away from the rotor towards the bottom of the tubes
- Because the tubes remain horizontal, the supernatant remains as a flat surface allowing the deposited particles to be separated from the supernatant

2. Swinging bucket rotors/ Horizontal rotors

3. Vertical rotors

- Vertical rotors provide the shortest pathlength, fastest run time, and the highest resolution of all the rotors
- In vertical rotors, the tubes are vertical during the operation of the centrifuge
- The yield of the rotor is not as ideal as the position of the tube doesn’t align with the direction of the centrifugal force
- As a result, instead of settling down, particles tend o spread towards the outer wall of the tubes
- These are commonly used in isopycnic and density gradient centrifugation

3. Vertical rotors

Types of Centrifuge

1. Benchtop centrifuge

- Benchtop centrifuge is a compact centrifuge that is commonly used in clinical and research laboratories
- It is driven by an electric motor where the tubes are rotated about a fixed axis, resulting in force perpendicular to the tubes
- Because these are very compact, they are useful in smaller laboratories with smaller spaces
- Different variations of benchtop centrifuges are available in the market for various purposes
- A benchtop centrifuge has a rotor with racks for the sample tubes and a lid that closes the working unit of the centrifuge

2. LOW-SPEED CENTRIFUGE

- **Low-speed centrifuges are the traditional centrifuges that are commonly used in laboratories for the routine separation of particles.**
- **These centrifuges operate at the maximum speed of 4000-5000 rpm.**
- **These are usually operated under room temperature as they are not provided with a system for controlling the speed or temperature of the operation.**
- **Two types of rotors are used,Fixed angle and Swinging bucket**
- **It is used for sedimentation of red blood cells until the particles are tightly packed into a pellet and supernatant is separated by decantation**
- **These are easy and compact centrifuges that are ideal for the analysis of blood samples and other biological samples**

3. High-speed centrifuge

- High-speed centrifuge, as the name suggests, is the centrifuge that can be operated at somewhat larger speeds
- The speed of the high-speed centrifuge can range from 15,000 to 30,000 rpm.
- The high-speed centrifuge is commonly used in more sophisticated laboratories with the biochemical application and requires a high speed of operations
- High-speed centrifuges are provided with a system for controlling the speed and temperature of the process, which is necessary for the analysis of sensitive biological molecules
- High-speed centrifuges are used in more sophisticated biochemical applications, higher speeds and temperature control of the rotor chamber are essential
- The operator of this instrument can carefully control speed and temperature which is required for sensitive biological samples

All three types of rotors are available for high-speed centrifugation- Fixed angle, Swinging bucket and Vertical rotors

4. Microcentrifuge

- Microcentrifuges are the centrifuges used for the separation of samples with smaller volumes ranging from 0.5 to 2 µl.
- Microcentrifuges are usually operated at a speed of about 12,000-13,000 rpm
- This is used for the molecular separation of cell organelles like nuclei and DNA and phenol extraction
- Microcentrifuges, also termed, microfuge, use sample tubes that are smaller in size when compared to the standard test tubes used in larger centrifuges
- Some microcentrifuges come with adapters that facilitate the use of larger tubes along with the smaller ones
- Microcentrifuges with temperature controls are available for the operation of temperature-sensitive samples

5. Refrigerated centrifuges

- Refrigerated centrifuges are the centrifuges that are provided with temperature control ranging from -20°C to -30°C
- A different variation of centrifuges is available that has the system of temperature control which is essential for various processes requiring lower temperatures
- Refrigerated centrifuges have a temperature control unit in addition to the rotors and racks for the sample tubes
- These centrifuges provide the RCF of up to 60,000 xg that is ideal for the separation of various biological molecules
- These are typically used for collecting substances that separate rapidly like yeast cells, chloroplasts, and erythrocytes
- The chamber of refrigerated centrifuge is sealed off from the outside to meet the conditions of the operations

6. Ultracentrifuges

- **Ultracentrifuge is the most sophisticated instrument that operate at extremely high speeds that allow the separation of much smaller molecules like ribosomes, proteins, and viruses**
- **It is the most sophisticated type of centrifuge that allows the separation of molecules that cannot be separated with other centrifuges**
- **Refrigeration systems are present in such centrifuges that help to balance the heat produced due to the intense spinning**
- **The speed of these centrifuges can reach as high as 150,000 rpm with maximum speed of 65,000 RPM (100,000's x g)**
- **It can be used for both preparative and analytical works**
- **Ultracentrifuges can separate molecules in large batches and in a continuous flow system**
- **In addition to separation, ultracentrifuges can also be used for the determination of properties of macromolecules like the size, shape, and density.**

Types of Centrifugation

1. Differential Pelleting (differential centrifugation)

- It is the most common type of centrifugation employed.
- Tissue such as the liver is homogenized at 32 degrees in a sucrose solution that contains buffer.
- The homogenate is then placed in a centrifuge and spun at constant centrifugal force at a constant temperature.
- After some time a sediment forms at the bottom of a centrifuge called pellet and an overlying solution called supernatant.
- The overlying solution is then placed in another centrifuge tube which is then rotated at higher speeds in progressing steps.

2. Density Gradient Centrifugation

- **This type of centrifugation is mainly used to purify viruses, ribosomes, membranes, etc.**
- **A sucrose density gradient is created by gently overlaying lower concentrations of sucrose on higher concentrations in centrifuge tubes**
- **The particles of interest are placed on top of the gradient and centrifuge in ultracentrifuges.**
- **The particles travel through the gradient until they reach a point at which their density matches the density of surrounding sucrose.**
- **The fraction is removed and analyzed.**

3. Rate-Zonal Density-Gradient Centrifugation

- Zonal centrifugation is also known as band or gradient centrifugation
- It relies on the concept of sedimentation coefficient (i.e. movement of sediment through the liquid medium)
- In this technique, a density gradient is created in a test tube with sucrose and high density at the bottom.
- The sample of protein is placed on the top of the gradient and then centrifuged.
- With centrifugation, faster-sedimenting particles in sample move ahead of slower ones i.e. sample separated as zones in the gradient.
- The protein sediment according to their sedimentation coefficient and the fractions are collected by creating a hole at the bottom of the tube.

4. Isopynic Centrifugation

- **The sample is loaded into the tube with the gradient-forming solution (on top of or below pre-formed gradient, or mixed in with self-forming gradient)**
- **The solution of the biological sample and cesium salt is uniformly distributed in a centrifuge tube and rotated in an ultracentrifuge.**
- **Under the influence of centrifugal force, the cesium salts redistribute to form a density gradient from top to bottom.**
- **Particles move to point where their buoyant density equals that part of gradient and form bands. This is to say the sample molecules move to the region where their density equals the density of gradient.**
- **It is a "true" equilibrium procedure since depends on bouyant densities, not velocities**
- **Eg: CsCl, NaI gradients for macromolecules and nucleotides – "self-forming" gradients under centrifugal force.**

Applications of Centrifugation

- To separate two miscible substances
- To analyze the hydrodynamic properties of macromolecules
- Purification of mammalian cells
- Fractionation of subcellular organelles (including membranes/membrane fractions) Fractionation of membrane vesicles
- Separating chalk powder from water
- Removing fat from milk to produce skimmed milk
- Separating particles from an air-flow using cyclonic separation
- The clarification and stabilization of wine
- Separation of urine components and blood components in forensic and research laboratories
- Aids in the separation of proteins using purification techniques such as salting out, e.g. ammonium sulfate precipitation.