E-Study material
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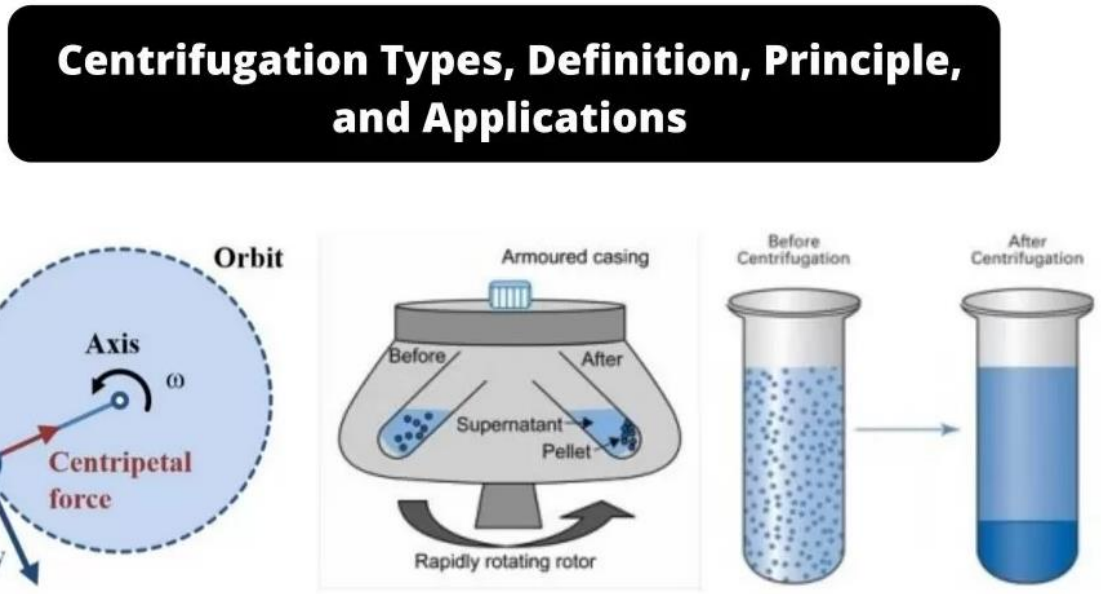
Centrifugation:

The separation of particles from a solution according to their size, shape, and density can be accomplished by the process of centrifugation, which is a method that is based on the utilization of the centrifugal force. The centrifugal force and acceleration is the fundamental mechanism that underlies the process of component extraction from suspension. Because of the acceleration and force, the molecules with a higher density will flow toward the periphery, while the particles with a lower density will move toward the center.



Definition of centrifugation:

Centrifugation is the method of separation of components. The force or acceleration of the centrifugal force makes the molecules with greater density move toward the outer edges, while the smaller particles are moved towards the center. Centrifugation is based on the perpendicular force that is generated by a sample being rotated around a fixed location. The speed of centrifugation will depend on dimension and density of particles within the sample.

Centrifuge definition:

A centrifuge is a machine designed to separate elements of a mix on the basis of their dimensions, density as well as the viscosity of medium, and also the rotor speed.

What is Relative Centrifugal Force (RCF):

Relative centrifugal force is a measurement for the rate of strength in rotors that are of different sizes and types. RCF is the force perpendicular to the surface applied to the sample, which is always in relation to the gravity of the earth.

The formula used to calculate the force of centrifugal relative (RCF) could be written as follows:

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.

Objectives of Centrifugation:

- To separate the liquids those are immiscible.
- Purify the substance by eliminating impurities that are present in the supernatant fluid.
- To separate crystallized drugs from the liquor that is their mother.

- To test the emulsions and suspensions to determine if they are creaming or sedimenting at a speed that is faster.

Working Principle:

In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it float to the top. When a solution of particles is allowed to stand, then the particles will tend to sediment according to various parameters (molecular mass, shape, and density) under the influence of gravity.

Greater the difference in density, the faster they move. If there is no difference in density (isopycnic conditions), the particles stay steady. To take advantage of differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful "centrifugal force" provided by a centrifuge. Particles differing in their size, density, shape can be separated as they sediment at different rates (sedimentation rate) in a centrifugal force. Sedimentation rate is directly proportional to the applied centrifugal field. (Wilson K. and Walker J. (2000) Practical Biochemistry; Principles and techniques, Cambridge University Press, 5th ed, Chapter 5, pp 263-310).

The rate of sedimentation depends upon the applied centrifugal field (G) being directed radialy out wards. It is determined by square of angular velocity of rotor (ω, in radians S-1) and the radial distance (r, in centimeters) of particular from the axis of rotation.

$G=\omega 2r$

Effective radial dimension of a given particle will change according to its position in the sample container and will vary between rmin and rmax.

Centrifugal field generated is proportional to ω2r, particle away from the axis of rotation will experience a greater field. The relative centrifugal field

is calculated from average radius of rotation (rav) of a column of liquid in a tube (i.e. the distance from the centre of rotation to the middle of liquid column in the centrifugation tube).

In a partially filled container of a swinging bucket and fixed angle rotor, the r min is effectively increased and therefore particles will have reduced path length to travel hence quicker will be the sedimentation.

Types of Centrifugation:

1. Analytical Centrifugation

Analytical centrifugation is an approach to separation which is where the particles within the sample are separated based on their density as well as the force generated by centrifugal forces. The process of analytical ultracentrifugation (AUC) is a flexible and durable method to perform qualitative analysis of macromolecules in solution.

Principle of Analytical Centrifugation:

Analytical centrifugation works on the idea that particles with greater density than other particles settle down more quickly. The larger molecules are more able to move when they exert centrifugal force than smaller ones. Analytical ultracentrifugation to determine of the molecular weight of a macromolecule could be carried out using a sedimentation speed method or a sedimentation equilibrium technique. The properties of macromolecules' hydrodynamics are outlined in terms of their coefficients of sedimentation. They are determined by the speed at which the boundary of concentration for specific biomolecule moves in the field of gravitational force.

The sedimentation coefficient may be used to identify the changes in structure and size of macromolecules due to changes in the experimental conditions. There are three optical methods accessible for the ultracentrifuge for analytical purposes (absorbance as well as interference

and fluorescence) which permit exact and precise monitoring of sedimentation in real time.

The steps of Analytical Centrifugation:

Small samples (20-120 millimeters) are collected in analytical cells and placed in the ultracentrifuge.

The ultracentrifuge is operated to create a centrifugal force that creates a movement of biomolecules distributed randomly throughout the solvent, radiating away from the center of rotation.

The distance of particles from their center are calculated using the Schlieren optical system.

The graph can be drawn using the concentration of solutes against the squared radial separation from the centre of rotation upon the molecular mass measured.

Uses of Analytical Centrifugation:

1. Analytical centrifugation may be employed to determine the quality of macromolecules.
2. It is also used to study modifications in the molecular mass and supramolecular structures.
3. Additionally, it permits the calculation of the molecular mass for solutes in their original state.

2. Density gradient centrifugation:

Density gradient centrifugation refers to an approach to separation between molecules in which the separation is determined by the concentration of molecules as they move through a density gradient under the influence of a centrifugal force.

Principle of Density gradient centrifugation:

Density gradient centrifugation relies on the idea that molecules settle under the force of centrifugal forces until they find the same density in a medium similar to their own. In this instance the medium has the density gradient is utilized that needs to reduce density or increase density. Molecules within a sample travel through the medium while the sample rotates producing an axial force.

The molecules that are denser start to shift towards the bottom of the gradient as they travel through the densities gradient. The molecules are then suspended at a level at which the density of particles is greater than that of the medium. These way molecules of different density are separated into various layers. They can be recovered through various methods.

Steps of Density gradient centrifugation:

A density gradient in the medium is produced by gently spreading the concentrations with lower levels over more concentrated ones in the centrifuge tube.

The sample is placed over the gradient after which the tubes will be then placed inside an ultracentrifuge.

The particles move along their gradients until they arrive at a point where their density is equal to with the denseness of medium around them.

The fragments are then removed and separated, giving particles that are isolated.

Uses of Density gradient centrifugation:

1. Density gradient centrifugation may be used to purify huge quantities of biomolecules.
2. It could even be utilized to purify various viruses, which can aid in their future research.
3. This method can be utilized to separate particles as well as a method to determine the densities of different particles.

Examples of centrifugation using a density gradient:

1. This technique was utilized for the long-running experiment which demonstrated that DNA was semi-conservative employing different nitrogen isotopes.
2. Another instance is the use of this method for the removal of the microsomal fraction in muscle homogenates and then the segregation of membrane vesicles that have different density.

3. Differential centrifugation:

Differential centrifugation is one type of centrifugation procedure where components are separated and placed into the centrifuge tube using an increasing centrifugal forces.

Principle of Differential centrifugation:

Differential centrifugation is based on the variations in the rate of sedimentation of biological particles that vary in dimensions and densities. When the greater force of centrifugal forces is applied, the initial sedimentation of the bigger molecules begins. The particles continue to settle, based on the speed and length of the individual centrifugation processes as well as the density and relative size of the particles.

The biggest category of particles form in a small pellet at the inside of the tube leaving smaller-sized structures in the supernatant. So, larger molecules are able to settle rapidly and have lower centrifugal forces , while smaller molecules require longer time and have higher forces. When particles that are not as dense in comparison to the surrounding medium they tend to flounder instead of sinking.

Steps of Differential centrifugation:

A homogenized sample is created the buffer that contains it.

The sample is then put inside the centrifuge tube which is run at the same centrifugal force for the specified amount of time at a specific temperature.

After this process the pellet will have formed in the middle of the tube. It is then separated by the supernatant.

The supernatant is then added to the new centrifuge tube, where it is then centrifuged at a new speed for a specific period of period of time and at a specific temperature.

In addition, the supernatant gets separate from the pellets that have formed.

This process continues until the components are isolated from one the other.

The particles are identified by looking for specific indicators that are specific to the particular particle.

Uses of Differential centrifugation:

1. Differential centrifugation is a common method to separate membranes and organelles of cells within the cell.
2. It is also used to separate the nucleus.
3. Because this method is able to separate particles based on the size it can be used to purify extracts that have larger impurities.

4. Sucrose gradient centrifugation:

Sucrose gradient centrifugation is one kind of density gradient centrifugation in which it is made from sucrose by altering the sucrose concentration.

Principle of Sucrose gradient centrifugation:

Sucrose gradient centrifugation works on the idea that molecules will settle under the force of a centrifugal until they find an environment with a density identical to their own. In this instance the medium has sucrose gradient is used or has smaller density towards the top, or a greater density on the lower end. Molecules within a sample are moved through the medium when the sample rotates producing an axial force.

The heavier molecules start moving toward the bottom as they traverse the densities gradient. The molecules are then suspended at a point which the density of particles is greater than that of the medium. In this manner molecules of various densities are separated in various layers. They can be recovered using different methods.

Steps of Sucrose gradient centrifugation:

A sucrose density gradient is created by gently placing the sucrose with a lower concentration over the higher concentrations of sucrose in the centrifuge tube.

The sample is placed on top of the gradient after which the tubes will be then placed inside an ultracentrifuge.

The particles move through this gradient till they arrive at a point where their density is equal to with the denseness of medium around them.

The particles are separated and separated, giving the particles in separate units.

Uses of Sucrose gradient centrifugation:

Sucrose gradient centrifugation can be described as a efficient method to separate of macromolecules such as DNA and the RNA.

It has also been utilized to study protein complexes as well as to measure the density and the size of other macromolecules.

4. **Ultracentrifugation:**

Ultracentrifuges are the highly sophisticated and advanced types of centrifugesthat operate at extremely high speeds. These centrifuges are large in size and separate samples in large batches and in a continuous flow system. They help in separation of smaller molecules like ribosomes, proteins, and viruses. They help in separation of molecules that cannot be separated with

other centrifuges. The speed of these centrifuges ranges from 60,000 to 150,000 rpm. Most of the ultracentrifuges are refrigerated because this helps in balancing heat produced during intense spinning. The working of this centrifuge is based on the principle of sedimentation. The denser particles settle down fast under the influence of gravity. The molecules get separated on the basis of their buoyant density. The band of highest buoyant density is at the bottom while one with lowest buoyant density is at the top. The separation is independent of size and shape. A high concentration of sucrose or cesium chloride is used in this type of centrifugation. Each component moves down and reaches a position where the density of the solution is equal to its own buoyant density. The denser molecules move outwards to the periphery of the tube whereas the less dense molecules get inclined towards the center of the tube. Ultracentrifuges can also be used for the determination of properties such as size, shape, and density of macromolecules. Ultracentrifuges are used for separation of cell organelles like mitochondria, ribosomes and viruses. The macromolecular conformational changes due to changes in pH, temperature, and other environmental factors can be detected using this instrument. The densities of various macromolecules can also be determined using this type of centrifuge. The purification of various biological crude extracts can also be made using this instrument.

Besides these, some centrifuges are also available that possess temperature control system required for certain special processes. Refrigerated centrifuges provide temperature control ranging from -20° C to -30° C. Microcentrifuges are the centrifuges used for the separation of samples with smaller volumes ranging from 0.5 to 2 µL. They usually operate at a speed of about 12,000-

13,000 rpm and are used for the molecular separation of cell organelles like nuclei and DNA and phenol extraction. Microcentrifuges are also called microfuges.

Applications of Centrifugation:

Separations in Laboratory: There are many laboratory-scale centrifuges that can be used to separate suspended liquids or immiscible liquids in biology, chemistry, and clinical medicine.

Separation of Isotope: There are other centrifuges that separate isotopes. The first being the Zippe type centrifuge. These centrifuges can be used in nuclear power or nuclear weapon programs.

Industrial centrifugal separator: This coolant filtration system is used to separate particles from liquids like grinding coolant or machining coolant. It is used to separate non-ferrous particles such as silicon, glass and ceramic. It does not use any consumable parts such as filter bags. This saves the earth.

Geotechnical centrifuge modeling: Geotechnical Centrifuge Modelling is used to test models that involve soils. To scale models, centrifuge acceleration is used to create prototype scale stresses. Problems like building foundations, earth dams and tunnels, as well as slope stability.

Materials Synthesis: The high gravity conditions created by the centrifuge can be used in the chemical industry, casting and material synthesis. Gravitational conditions can greatly affect convection and mass transfers. Researchers found that high-gravity levels can have a significant impact on the product's phase composition and morphology.

Commercial applications:

Hand-washed clothes can be dried in a standalone centrifuge, usually with a water outlet.

To get rid of water from laundry loads, washing machines can be used as centrifuges.

In Mission: SPACE at Epcot, Walt Disney World, centrifuges are used to propel riders. This attraction uses a combination of a centerifuge and motion simulator to give the illusion of going into space.

Centrifuges use centrifugal acceleration in soil mechanics to match soil stresses in scale models to real-life conditions.

Many large industrial centrifuges can be used to dry sludges in wastewater and water treatment. The resultant dry product is commonly called cake. After

most solids have been removed, the centrifuge leaves behind what is known as centrate.

For the removal of solids from drilling fluid, large industrial centrifuges can also be used by the oil industry.

Some companies use disc-stack centrifuges to separate bitumen from water and other solids.

Centrifuges can be used to separate cream from milk (remove fat). See Separator (milk).

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