# Centrifugation

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# Centrifugation

**Definition-** Biological centrifugation is a process that uses centrifugal force to separate and purify mixtures of biological particles in a liquid medium.

It is a key technique for isolating and analysing cells, subcellular fractions, supramolecular complexes and isolated macromolecules such as proteins or nucleic acids.

**Basic principle** The effect of sedimentation due to the influence of the Earth's gravitational field biological structures exhibit a drastic increase in sedimentation when they undergo acceleration in a centrifugal field. When designing a centrifugation protocol, it is important to keep in mind that:

- the more dense a biological structure is, the faster it sediments in a centrifugal field;
- the more massive a biological particle is, the faster it moves in a centrifugal field;

- the denser the biological buffer system is, the slower the particle will move in a centrifugal field.
- the greater the frictional coefficient is, the slower a particle will move.
- the greater the centrifugal force is, the faster the particle sediments.
- the sedimentation rate of a given particle will be zero when the density of the particle and the surrounding medium are equal.
- Biological particles moving through a viscous medium experience a frictional drag, whereby the frictional force acts in the opposite direction to sedimentation and equals the velocity of the particle multiplied by the frictional coefficient. The frictional coefficient depends on the size and shape of the biological particle.
- As the sample moves towards the bottom of a centrifuge tube in swing-out or fixed-angle rotors, its velocity will increase due to the increase in radial distance.

- the rate of sedimentation is dependent upon the applied centrifugal field , G, that is determined by the radial distance, r, of the particle from the axis of rotation (in cm) and the square of the angular velocity, , of the rotor (in radians per second):
- The average angular velocity of a rigid body that rotates about a fixed axis is defined as the ratio of the angular displacement
- The relative centrifugal field (g), RCF, which is the ratio of the centrifugal acceleration at a specified radius and the speed to the standard acceleration of gravity,

**Instrumentation**-

It consist of two component ,an electric motor to spin the sample and a rotor to hold the tubes

# Instrumentation

# Types of centifuge-

(1) Low speed centrifuge

(2) High speed centrifuge

(3) Ultra centrifuge

(4) Micro centrifuge

(5) Refrigerated centrifuge

# 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.
- Swinging bucket and fixed angle type of rotors can be used in these centrifuges.
- These are easy and compact centrifuges that are ideal for the analysis of blood samples and other biological samples.
- The low-speed centrifuge works on the same principle as all other centrifuges, but the application is limited to the separation of simpler solutions.


4000
99
406


4000
99
416

# 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.
- The high-speed centrifuges come with different adapters to accommodate the sample tubes of various sizes and volumes.
- All three types of rotors can be used for the centrifugation process in these centrifuges.


110,510
1:57
4
BECKMAN
COULTER
Avanti JXN-30

# Ultra centrifuge

- Ultracentrifuges are the centrifuges 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.
- 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.

Optima XPN

Optima XE

Optima MAX-XP

Optima MAX-TL

# 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.


4800
BECKMAN
COULTER
Microfuge 16
Centrifuge


BECKMAN
COULTER

# Refrigerated centrifuge

- 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.


64396
10
0
9
ENTER
PULSE
START
STOP
Allegra™ 64R Centrifuge
BECKMAN
COULTER

# 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.


Thermo
3000 10:00
SPEED
TIME
PULSE
OPEN
START
STOP

# Types of Rotor

- 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.

**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.




## 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.

## Vertical rotors

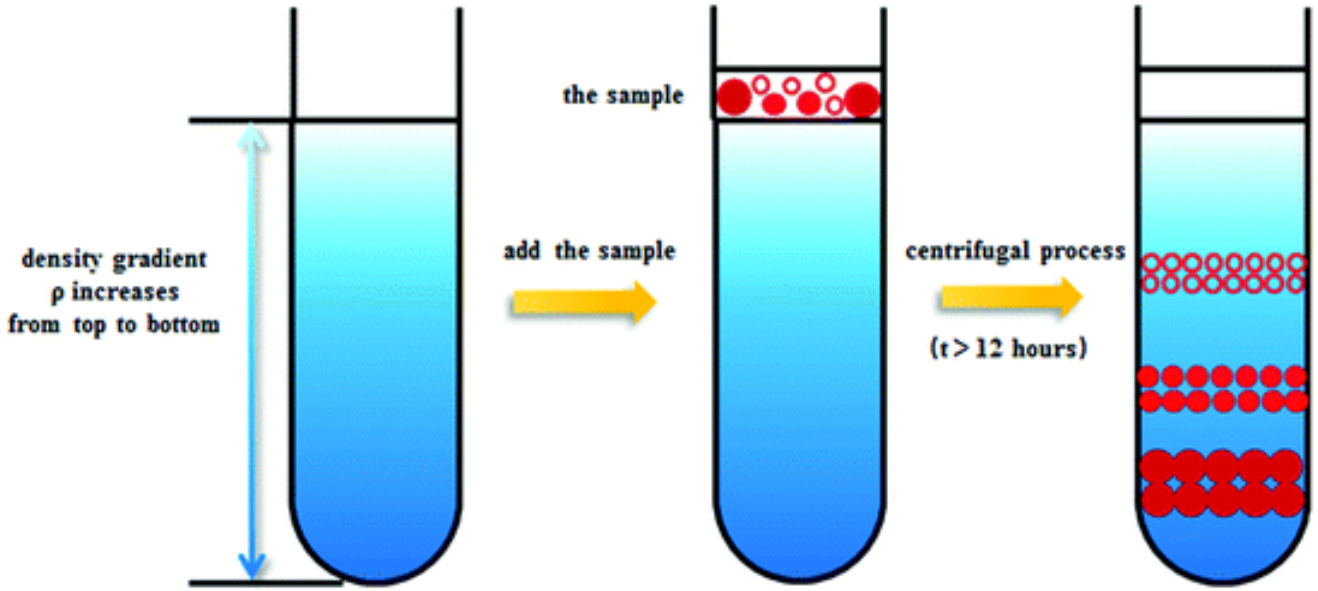
- Vertical rotors provide the shortest pathlength, fastest run time, and the highest resolution of all the rotors.
- In vertical rotors, the tubes are vertical during the operation of the centrifuge.
- The yield of the rotor is not as ideal as the position of the tube doesn't align with the direction 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.

# Density gradient centrifugation

Density gradient centrifugation is the separation of molecules where the separation is based on the density of the molecules as they pass through a density gradient under a centrifugal force.

## Principle

- Density gradient centrifugation is based on the principle that molecules settle down under a centrifugal force until they reach a medium with the density the same as theirs.
- In this case, a medium with a density gradient is employed, which either has to decrease density or increasing density.
- Molecules in a sample move through the medium as the sample is rotated creating a centrifugal force.
- The more dense molecules begin to move towards the bottom as they move through the density gradient.
- The molecules then become suspended at a point in which the density of the particles equals the surrounding medium.
- In this way, molecules with different densities are separated at different layers which can then be recovered by various processes.


density gradient
ρ increases
from top to bottom
add the sample
the sample
centrifugal process
(t > 12 hours)

## Steps of Density gradient centrifugation

- A density gradient of a medium is created by gently laying the lower concentration over the higher concentrations in a centrifuge tube.
- The sample is then placed over the gradient, and the tubes are placed in an ultracentrifuge.
- The particles travel through the gradient until they reach a point at which their density matches the density of the surrounding medium.
- The fractions are removed and separated, obtaining the particles as isolated units.

# Uses of Density gradient centrifugation

- Density gradient centrifugation can be applied for the purification of large volumes of biomolecules.
- It can even be used for the purification of different viruses which aids their further studies.
- This technique can be used both as a separation technique and the technique for the determination of densities of various particles.

# Differential centrifugation

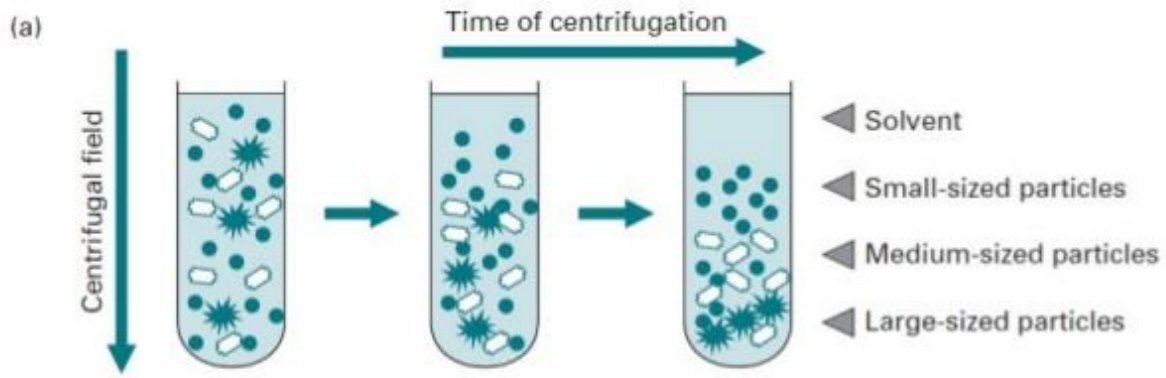
- Differential centrifugation is a type of centrifugation process in which components are separately settled down a centrifuge tube by applying a series of increasing centrifugal force.

**Principle**

- Differential centrifugation is based upon the differences in the sedimentation rate of biological particles of different size and density.
- As the increasing centrifugal force is applied, initial sedimentation of the larger molecules takes place.
- Further particles settle down depending upon the speed and time of individual centrifugation steps and the density and relative size of the particles.
- The largest class of particles forms a pellet on the bottom of the centrifuge tube, leaving smaller-sized structures within the supernatant.
- Thus, larger molecules sediment quickly and at lower centrifugal forces whereas the smaller molecules take longer time and higher forces.
- In the case of particles that are less dense than the medium, the particles will float instead of settling.

## Preparative Centrifugation Types

# 1.Differential centrifugation




Based on the differences in the **sedimentation rate of the biological particles** of different size, shape and density

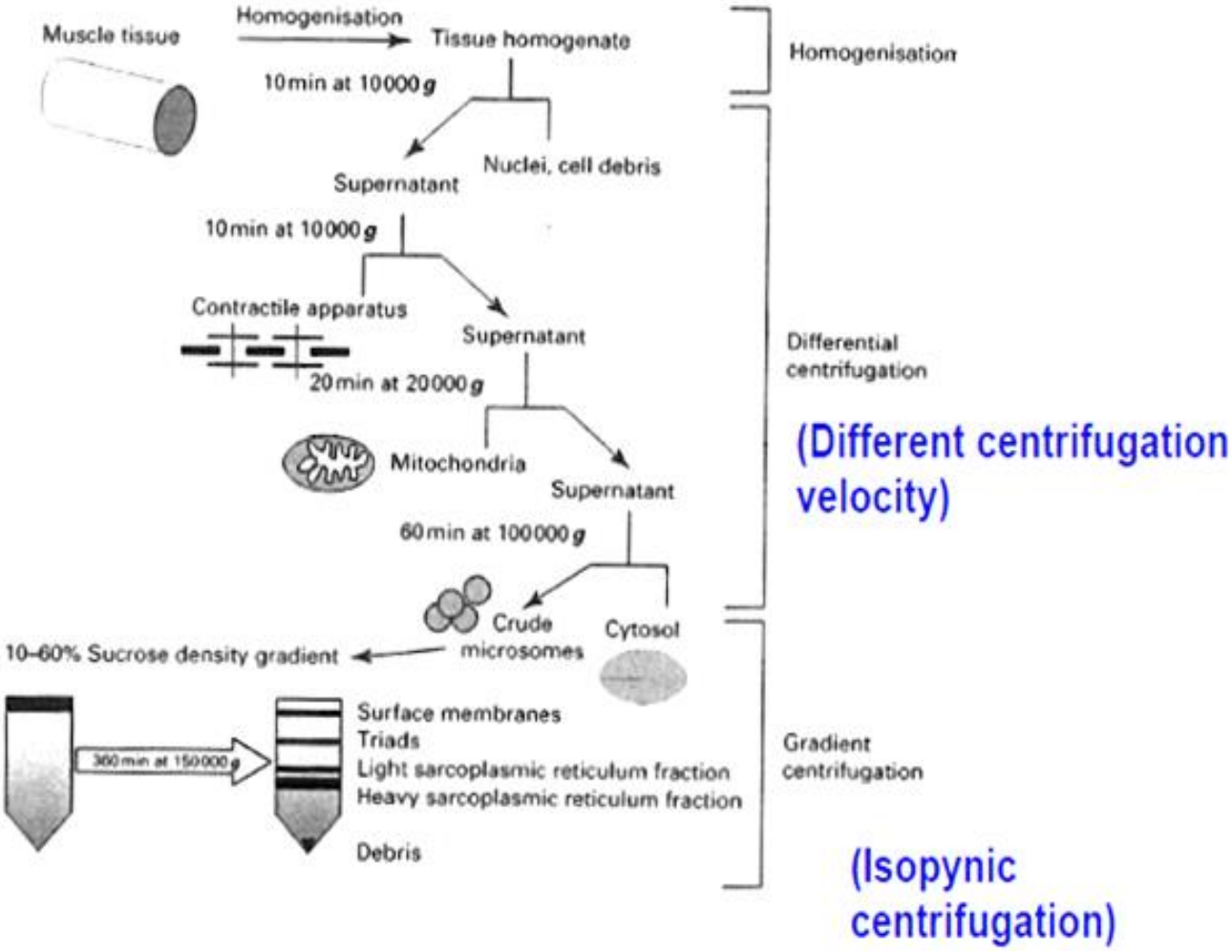
## Steps of Differential centrifugation

- The sample solution is homogenized in the medium containing buffer.
- The sample is then placed in the centrifuge tube, which is operated at a particular centrifugal force for a specific time at a particular temperature.
- By the end of this operation, a pellet will be formed at the bottom of the tube, which is separated from the supernatant.
- The supernatant is added to a new centrifuge tube where it is centrifuged at another speed for a particular time and particular temperature.
- Again, the supernatant is separated from the pellets formed.
- These steps are continued until all particles are separated from each other.

## Uses of Differential centrifugation

- Differential centrifugation is commonly used for the separation of cell organelles and membranes found in the cell.
- It can also be used for low-resolution separation of the nucleus.
- As this technique separates particles based on their sizes, this can be used for the purification of extracts containing larger-sized impurities.

# Subcellular Fractionation




(Different centrifugation velocity)

(Isopynic centrifugation)

# Care and maintinance

- Corrosion and degradation due to biological buffer systems used within rotors or contamination of the interior or exterior of the centrifuge via spillage may seriously affect the lifetime of this equipment.
- Another important point is the proper balancing of centrifuge tubes.
- In order to avoid damaging the protective layers of rotors, such as polyurethane paint or aluminium oxide, care should be taken in the cleaning of the rotor exterior. Coarse brushes that may scratch the finish should not be used and only non-corrosive detergents employed. Corrosion may be triggered by longterm exposure of rotors to alkaline solutions, acidic buffers, aggressive detergents or salt. Thus, rotors should be thoroughly washed with distilled or deionised water after every run.
- To avoid damage to the hinge pins of swinging-bucket rotors, they should be dried with tissue paper following removal of biological buffers and washing with water.

# Isopycnic centrifugation

- Isopycnic centrifugation is a type of centrifugation where the particles in a sample are separated on the basis of their densities as centrifugal force is applied to the sample.

## Principle

- Isopycnic centrifugation is also termed the equilibrium centrifugation as the separation of particles takes place solely on the basis of their densities and not on their sizes.
- The particles move towards the bottom, and the movement is based on the size of the particles. And, the flow ceases once the density of the particle becomes equal to the density of the surrounding medium.
- The density in the gradient increases as we move down the tube towards the bottom. As a result, the particles with higher densities settle down at the bottom, followed by less dense particles that form bands above the denser particles.
- It is considered as a true equilibrium as this depends directly on the buoyant densities and not the sizes of the particles.

**Steps of Isopycnic centrifugation**

- A gradient prepared with an increasing density towards the bottom of the tube is prepared. A pre-performed gradient can also be used.
- The solution of the biological sample and salt is uniformly distributed in the centrifuge tube and placed inside the centrifuge.
- Once the centrifuge is operated, a density gradient of the salt is formed in the tube.
- The particles move down the tube and settle down as they reach the region with their respective densities.
- The particles are then separated and identified using different other processes.

**Uses of Isopycnic centrifugation**

- Isopycnic centrifugation can be applied for the purification of large volumes of biomolecules.
- This technique can be used as a technique for the determination of densities of various particles.

# Analytical centrifugation

- Analytical centrifugation is a separation method where the particles in a sample are separated on the basis of their density and the centrifugal force they experience.

**Principle**

- Analytical centrifugation is based on the principle that particles that are denser than others settle down faster. Similarly, the larger molecules move more quickly in the centrifugal force than the smaller ones.
- Analytical ultracentrifugation for the determination of the relative molecular mass of a macromolecule can be performed by a sedimentation velocity approach or sedimentation equilibrium methodology.
- The hydrodynamic properties of macromolecules are described by their sedimentation coefficients. They can be determined from the rate that a concentration boundary of the particular biomolecules moves in the gravitational field.
- The sedimentation coefficient can be used to characterize changes in the size and shape of macromolecules with changing experimental conditions.

**Steps of Analytical Centrifugation**

- Small sample sizes (20-120 mm3) are taken in analytical cells to be placed inside the ultracentrifuge.
- The ultracentrifuge is then operated so that the centrifugal force causes a migration of the randomly distributed biomolecules through the solvent radially outwards from the center of rotation.
- The distance of the molecules from the center is determined through the Schlieren optical system.
- A graph is drawn from the solute concentration versus the squared radial distance from the center of rotation, based on which the molecular mass is determined.

**Uses of Analytical Centrifugation**

- Analytical centrifugation can be used for the determination of the purity of macromolecules.
- It can also be used for the examination of changes in the molecular mass of supramolecular complexes.
- Besides, it allows the determination of the relative molecular mass of solutes in their native state.