# Centrifugation

Differential and density gradient centrifugation, sucrose density gradient, CsCl2 gradient, analytical centrifugation, ultracentrifugation

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# What is Centrifugation ?

- Centrifugation is a separation method of different components in an analyte mixture.

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- This method involves the rotation of the sample around a fixed axis, which causes the production of a centrifugal force.
- The centrifugal force causes the particles in the sample to move down through a liquid medium.
- This process causes the sedimentation of particles or cells with different sizes and densities at different rates.

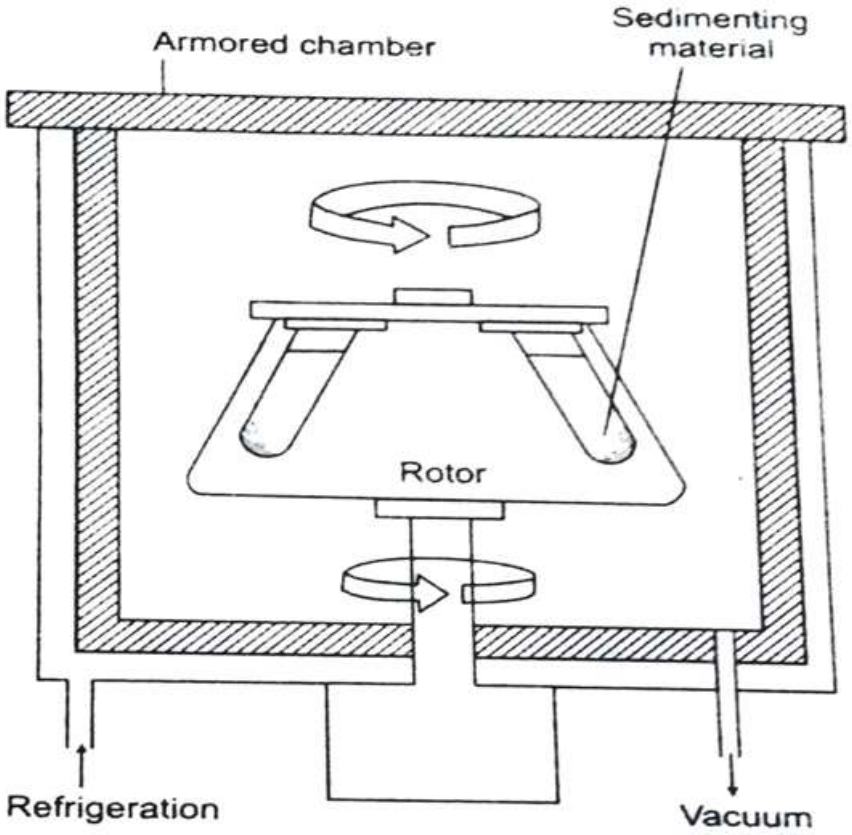



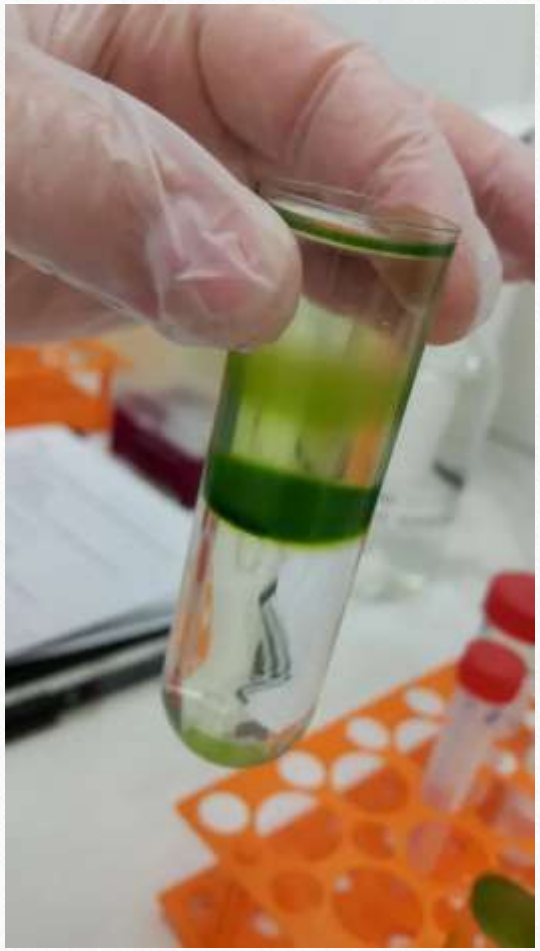

**Fig. 28.1** Inner details of a centrifuge.

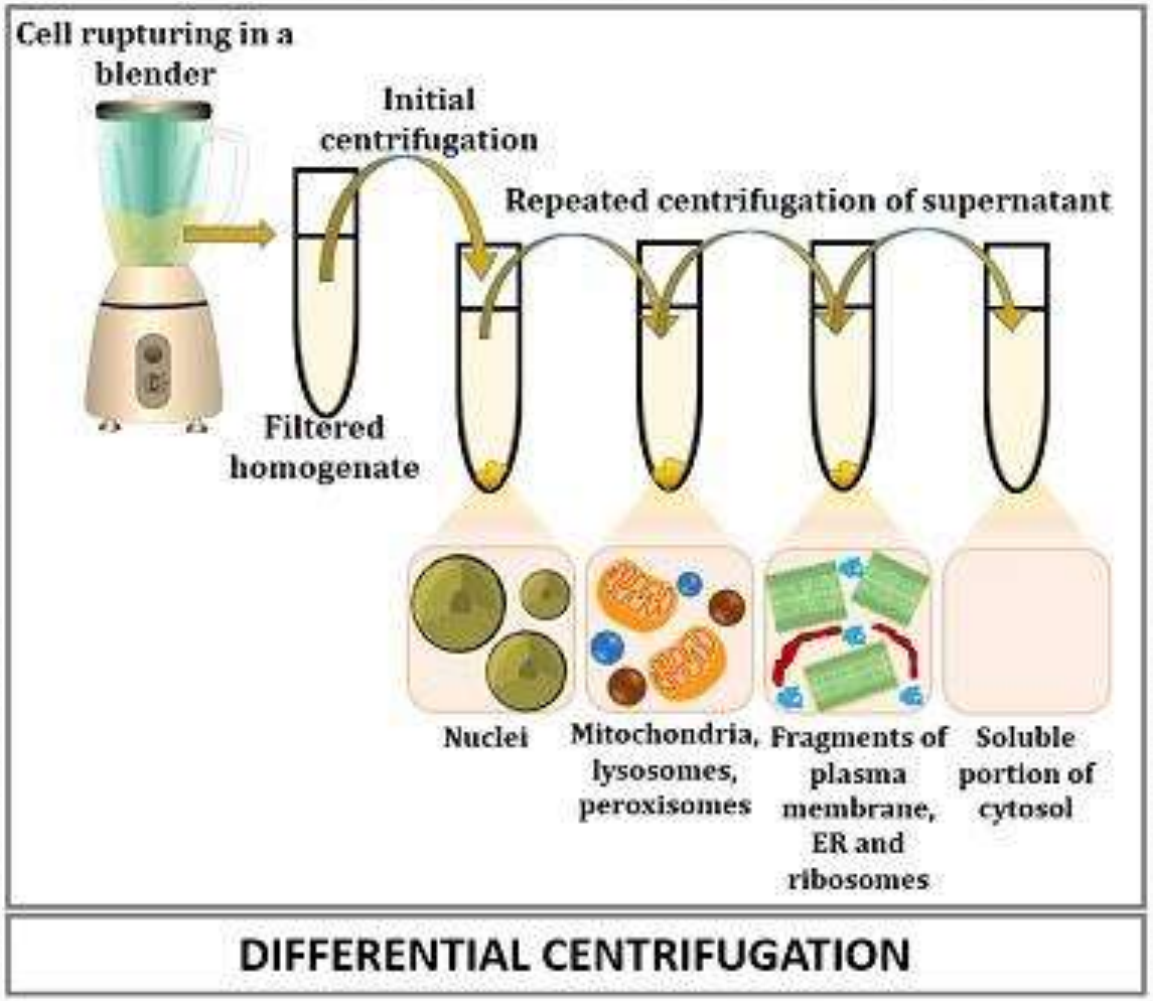
# Differential Centrifugation

- Differential centrifugation is an analytical technique in which we can separate the particles in a mixture depending on the size of the particle.
- It is the simplest form of centrifugation and also called **differential pelleting**.
- This method is important in separating the components in a cell.
- Particles having different sizes undergo sedimentation at different rates upon the centrifugation.
- In other words, large particles sediment faster than smaller particles. Moreover, the sedimentation rate can be increased by increasing the centrifugal force.
- Also known as **differential velocity centrifugation.**
- Differential centrifugation was first introduced by **Bensley and Hoerr** in 1934 who obtained a large granule fraction containing nuclei and mitochondria.

# Principle of Differential centrifugation

- 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.



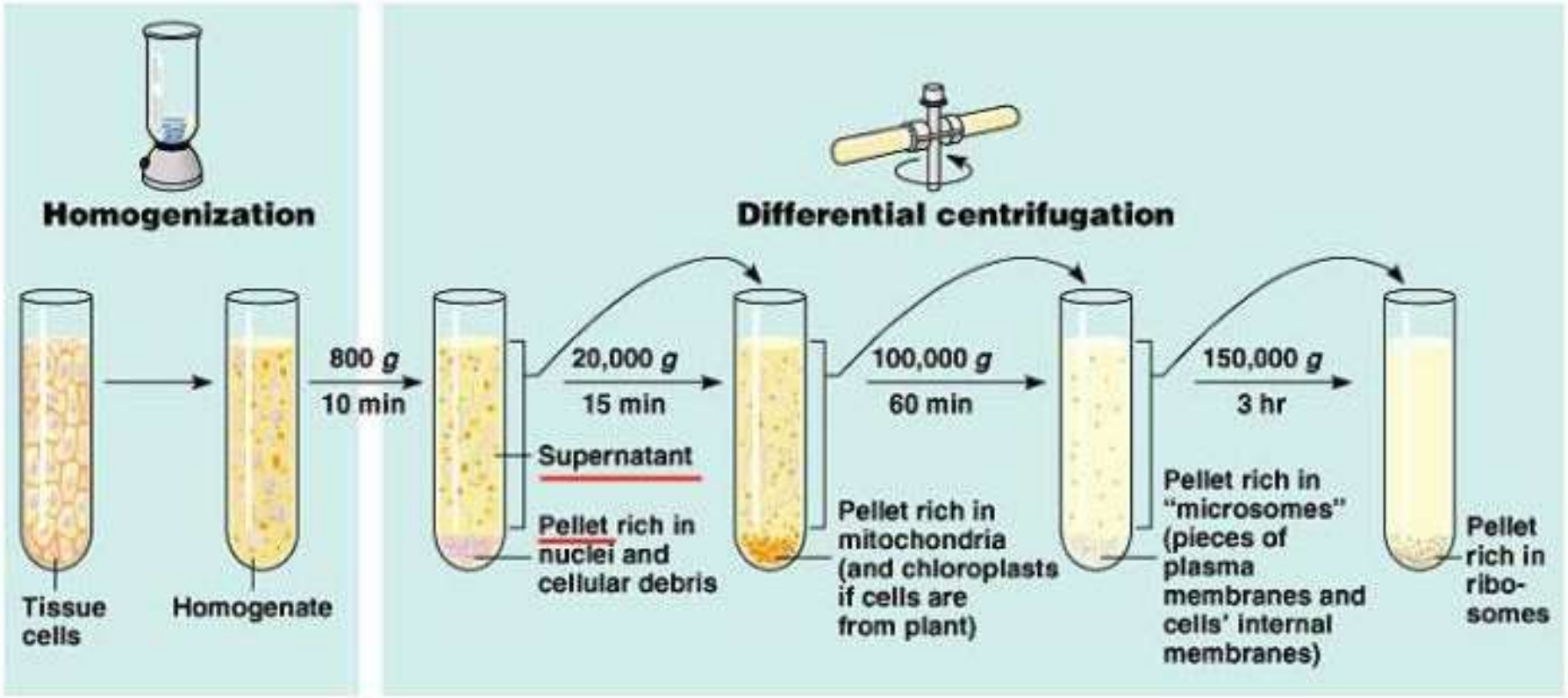



DIFFERENTIAL CENTRIFUGATION

## 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.
- The particles can then be identified by testing for indicators that are unique to the specific particles.

# Differential centrifugation



# 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.

# Density Gradient Centrifugation

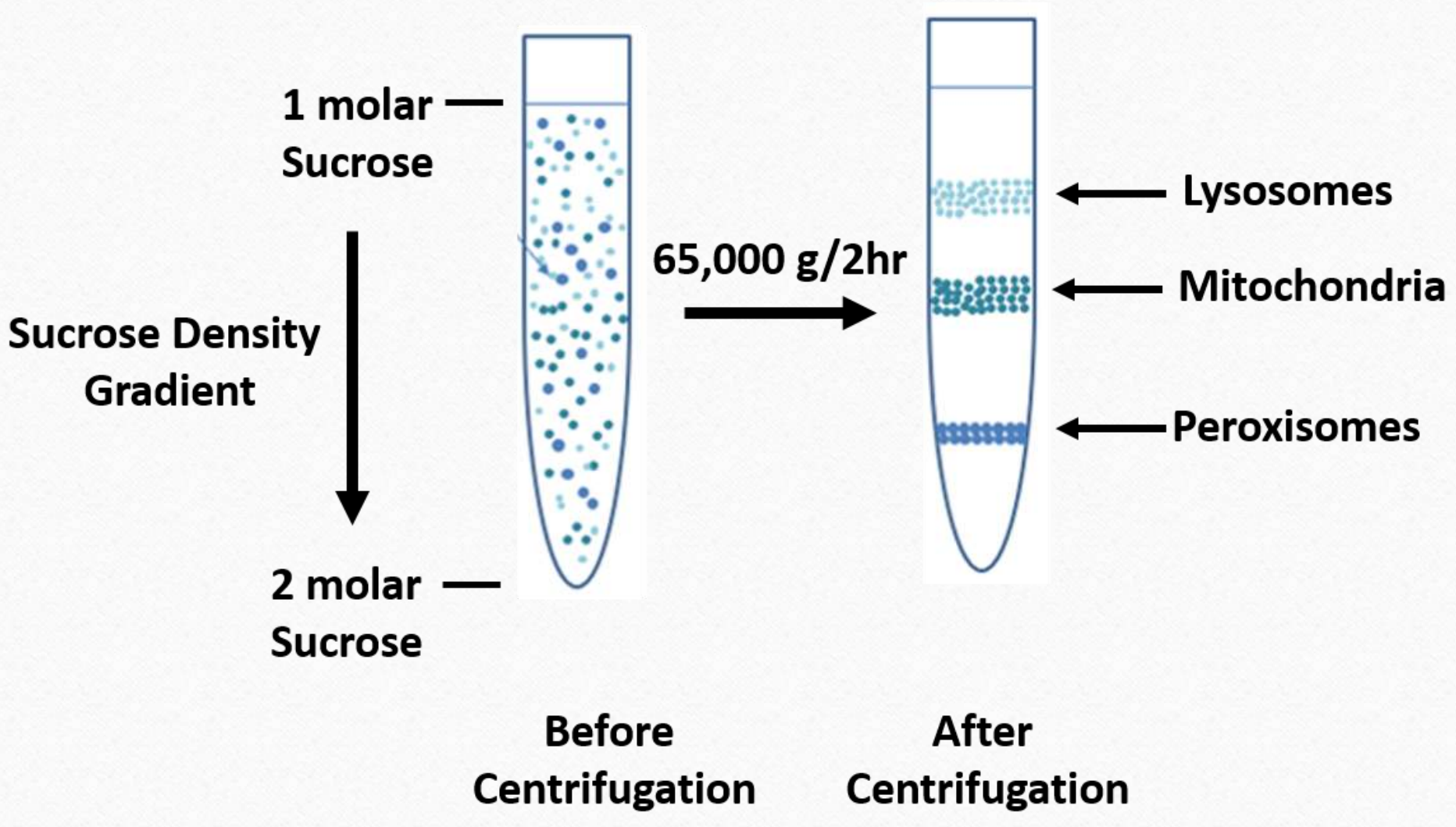
- Density gradient centrifugation is an analytical technique in which we can separate the particles in the analyte mixture based on the density of the particle.
- In this method, the substances are concentrated in a solution of caesium salts or in sucrose.
- The method involves the fractionation of particles based on buoyancy density.

(Buoyancy:the ability or tendency of something to float in water or other fluid/ the power of a liquid to keep something afloat)

- The density gradient in this method is the Caesium salt or the sucrose medium.
- Density gradient centrifugation, developed by **Brakke** (1951, 1960).

# Principle of Density gradient centrifugation

- 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.


1 molar
Sucrose
Sucrose Density
Gradient
2 molar
Sucrose
65,000 g/2hr
Lysosomes
Mitochondria
Peroxisomes
Before
Centrifugation
After
Centrifugation

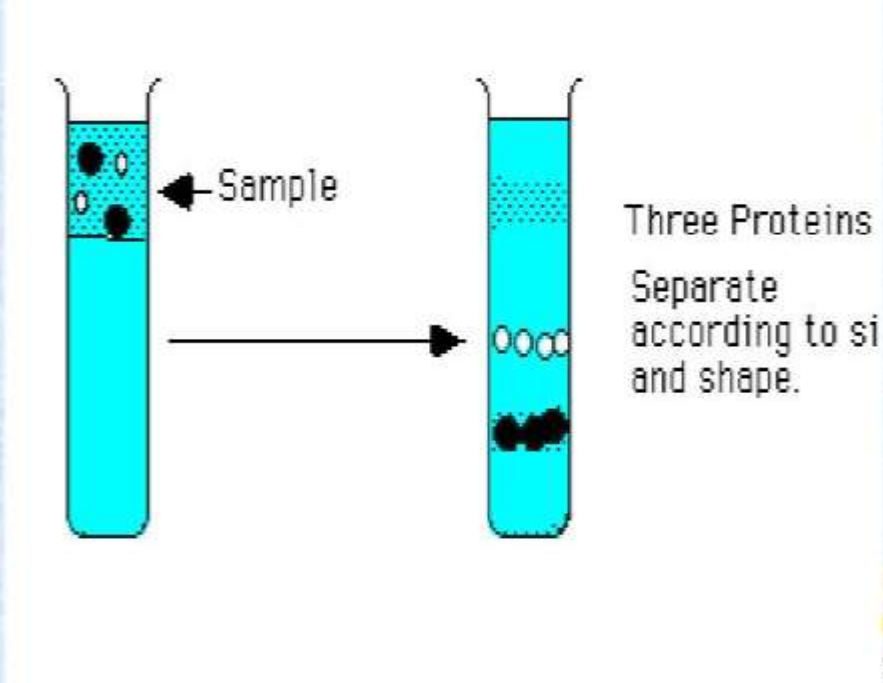
# Steps of Density gradient centrifugation

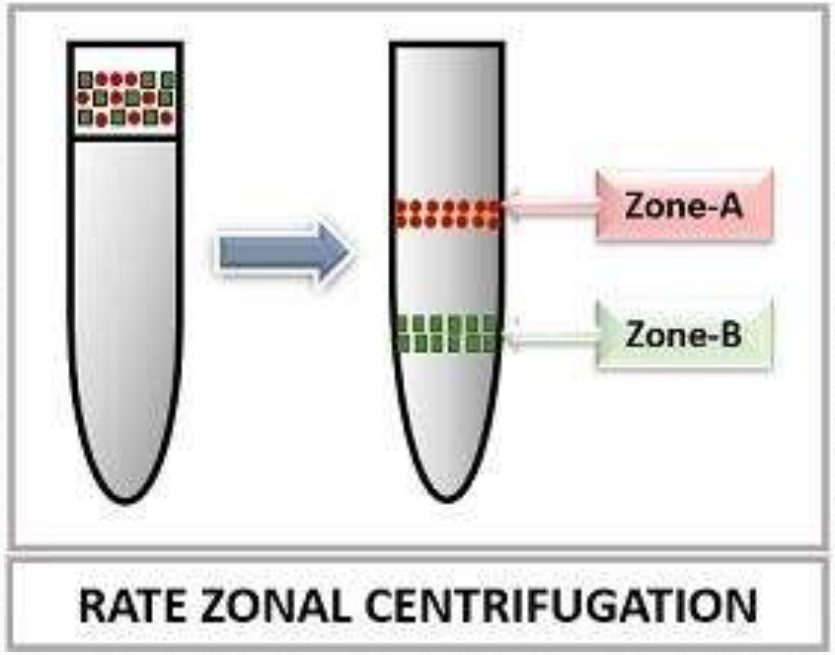
- A density gradient of a medium is created by gently laying the lower concentration over the higher concentrations in a centrifuge tube.
- The sample is then placed over the gradient, and the tubes are placed in an ultracentrifuge.
- The particles travel through the gradient until they reach a point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

- There are two types of density gradient centrifugation:
  - rate-zonal centrifugation
  - isopycnic centrifugation.

## Rate-zonal centrifugation

- During the rate-zonal centrifugation, the sample is layered as a narrow zone on top of a density gradient.
- Particles move at different rates under the centrifugal force on the basis of their density.
- The actual sedimentation rate primarily depends upon the size and mass of the particles.
- As the density of the particles is higher than the density gradient, all particles produce pellets.

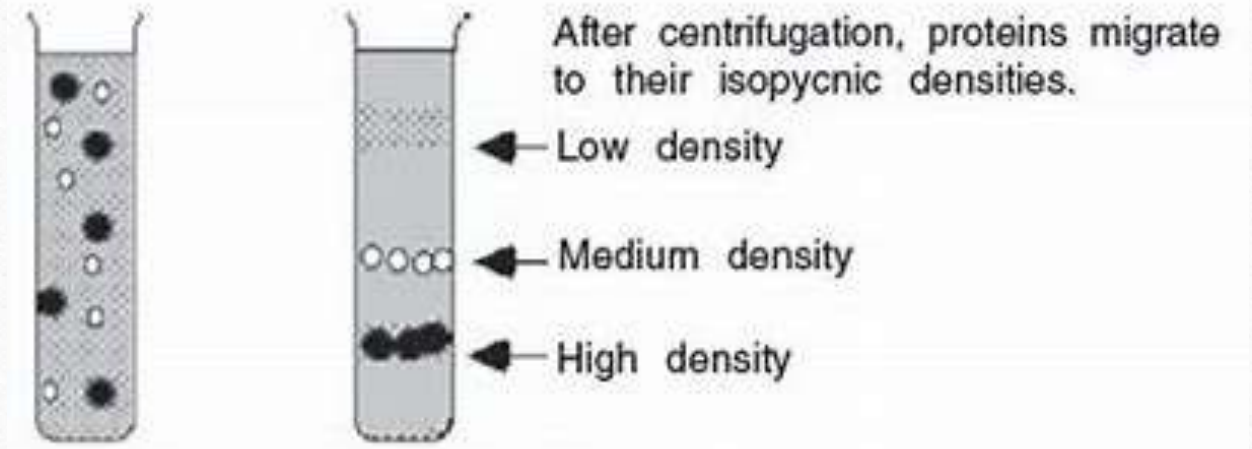

Sample
Three Proteins
Separate
according to si
and shape.

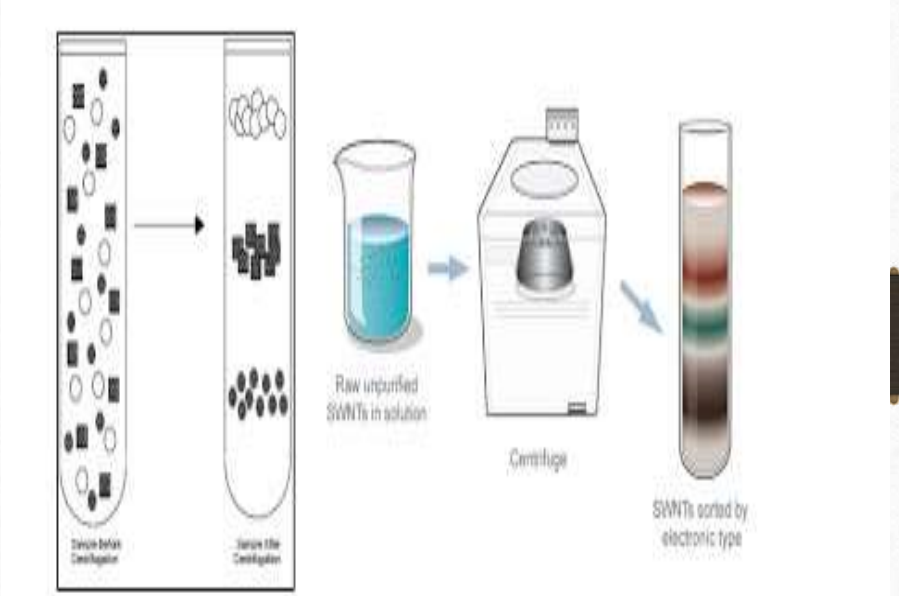

Zone-A
Zone-B
RATE ZONAL CENTRIFUGATION

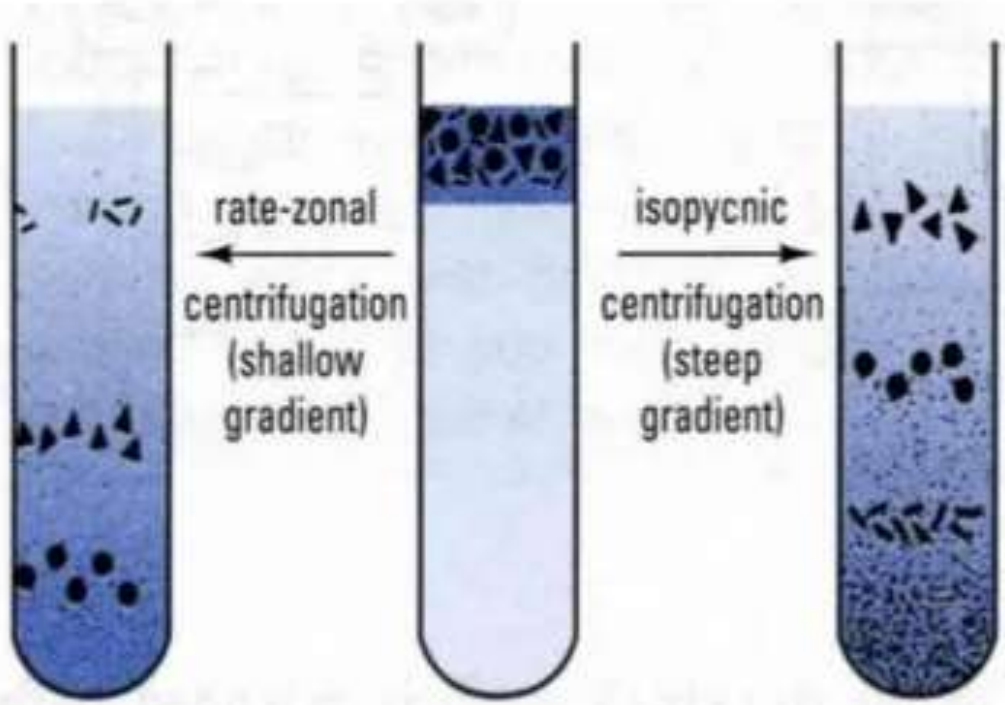
## Iopycnic centrifugation

- Isopycnic centrifugation is the second type of density gradient centrifugation method.
- It starts with a homogeneous solution.
- Under the centrifugal force, the particles in the analyte mixture move until the density of the particles is similar to the density gradient.

  Therefore, we can name this technique as equilibrium centrifugation as well.


After centrifugation, proteins migrate to their isopycnic densities.
Low density
Medium density
High density
he sample is evenly distributed throughout the centrifuge tube
entrifugation.


Raw unpurified SWNTs in solution
Centrifuge
SWNTs sorted by electronic type




Rate zonal centrifugation

Isopycnic Centrifugation

# 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.

This method was used in the famous experiment, which proved that DNA is semi-conservative by using different isotopes of nitrogen.

# Difference Between Differential and Density Gradient Centrifugation

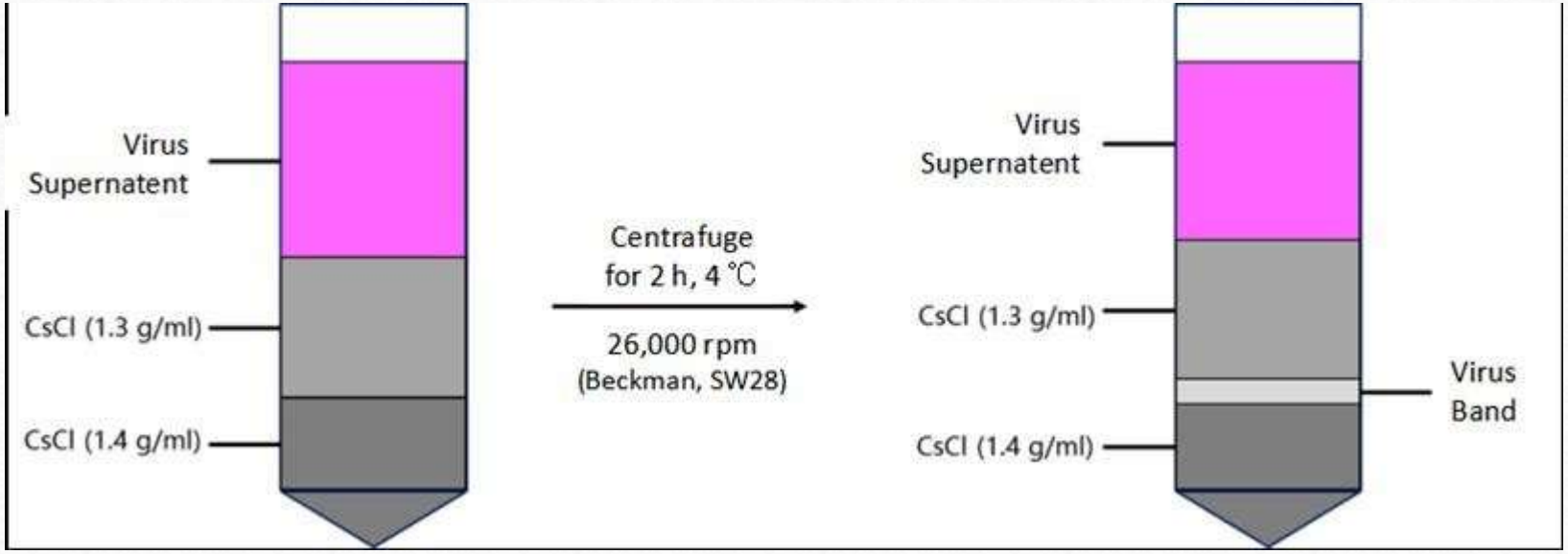
| Characters | Differential Centrifugation | Density Gradient Centrifugation |
|---|---|---|
| Separation | The separation occurs based on the size of the particles in differential centrifugation. | The separation occurs based on the density of the particles in density gradient centrifugation. |
| Types of Molecules | Differential centrifugation is used to separate cells, organelles or macromolecules. | Density gradient centrifugation is used to separate molecules or particles. |
| Type of Sample | A homogenized organ is used as the sample in differential centrifugation. | A homogenized solution is used as the sample in density gradient centrifugation. |
| Sugar Solution | No sugar solution is used in the differential centrifugation. | A sucrose or any sugar solution is used in the density gradient centrifugation. |
| Easiness of the Method | Differential centrifugation is easy to use. | Density gradient centrifugation is difficult to use. |
| Contamination | No contamination occurs in differential centrifugation. | Some contamination is always possible in density gradient centrifugation. |

# Cesium chloride (CsCl) gradient centrifugation

- This technique was discovered by Meselson and Stahl.
- It is the **density gradient centrifugation that utilises CsCl as the medium**.
- A gradient of densities from lowest to highest is formed and components sediment in the layer that corresponds to their densities.
- A CsCl solution is set up in a centrifuge tube. The CsCl forms a concentration gradient within the tube when centrifuged at high speed, with more concentrated CsCl towards the base.
- Because cesium is a heavy element, a cesium salt solution is much denser than the density of most salt solutions and the cesium salt solution did not affect viruses or DNA, it is used in centrigugation.

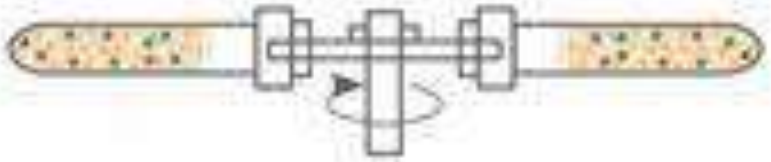
## Uses:

- Cesium chloride gradient centrifugation is the most widely used method for purification of recombinant adenovirus.
- Since CsCl has a density almost equivalent to that of DNA, it helps in isolating DNA from a sample containing DNA, RNA, and proteins.
- Caesium chloride is widely used for separating various types of DNA.



# CsCl density gradient centrifugation

Sample + CsCl + EtBr



Centrifugation

Gradient is formed

# Analytical Centrifugation

- Analytical centrifugation refers to a high-velocity centrifuge used in the analytical processes.
- 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.
- Analytical ultracentrifugation (AUC) is a versatile and robust method for the quantitative analysis of macromolecules in solution.
- Analytical centrifugation is used to determine the mass and shape of macromolecule_such as protein complexes and rate of sedimentation of molecules.

## Principle of Analytical Centrifugation

- 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.

## Facts about Analytical centrifugation :

- Analytical centrifugation refers to a high-velocity centrifuge used in the analytical processes.
- Analytical centrifugation is used in analytical procedures.
- Analytical centrifugation is used to determine the mass and shape of macromolecules such as protein complexes and rate of sedimentation of molecules.
- Only small sample volumes (less than 1 mL) can be processed by analytical centrifugation.
- Relatively pure samples are used in the analytical centrifugation.
- A precise sedimentation coefficient and molecular weight are used in analytical centrifugation.
- The progress of the analytical centrifugation can be observed visually during analytical centrifugation.
- As high speed generates more heat, a cooling arrangement should be used in the analytical centrifugation

## Uses of Analytical Centrifugation

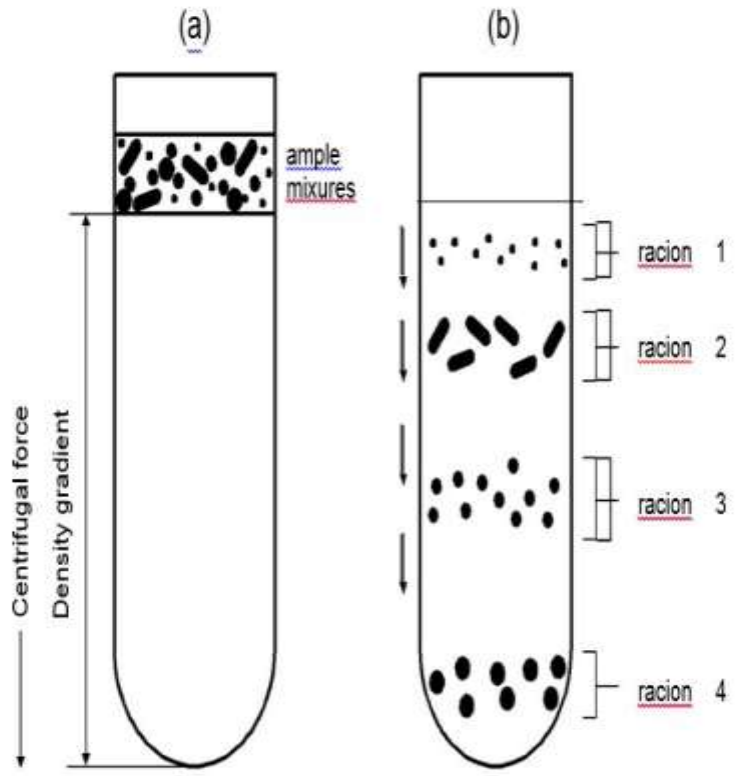
•Analytical centrifugation can be used for the determination of the purity of macromolecules.

•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.

•Analytical centrifugation is used to characterize particle properties such as molecular weight, diffusion and sedimentation coefficients etc.

•Analytical ultracentrifuge is most commonly used for the determination of properties of biomolecules like proteins and nucleic acids..


(a)
(b)
ample
mixures
racion 1
racion 2
racion 3
racion 4
Centrifugal force
Density gradient

# THANK YOU