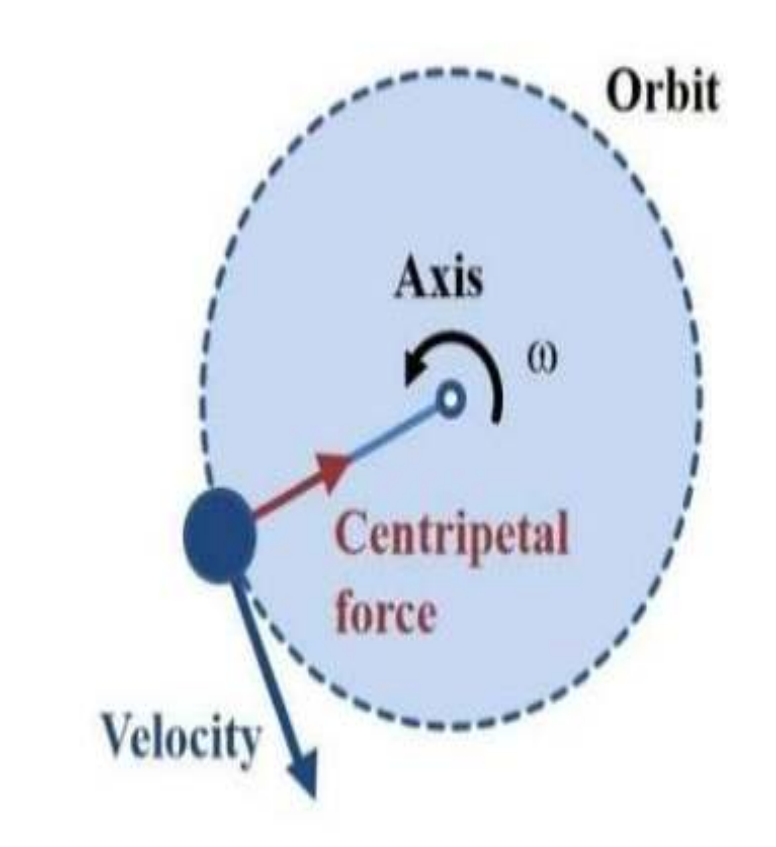
Centrifugation- Principle, Types and Applications

PG Sem II
Paper CC6
Unit IIA

Definition of Centrifugation



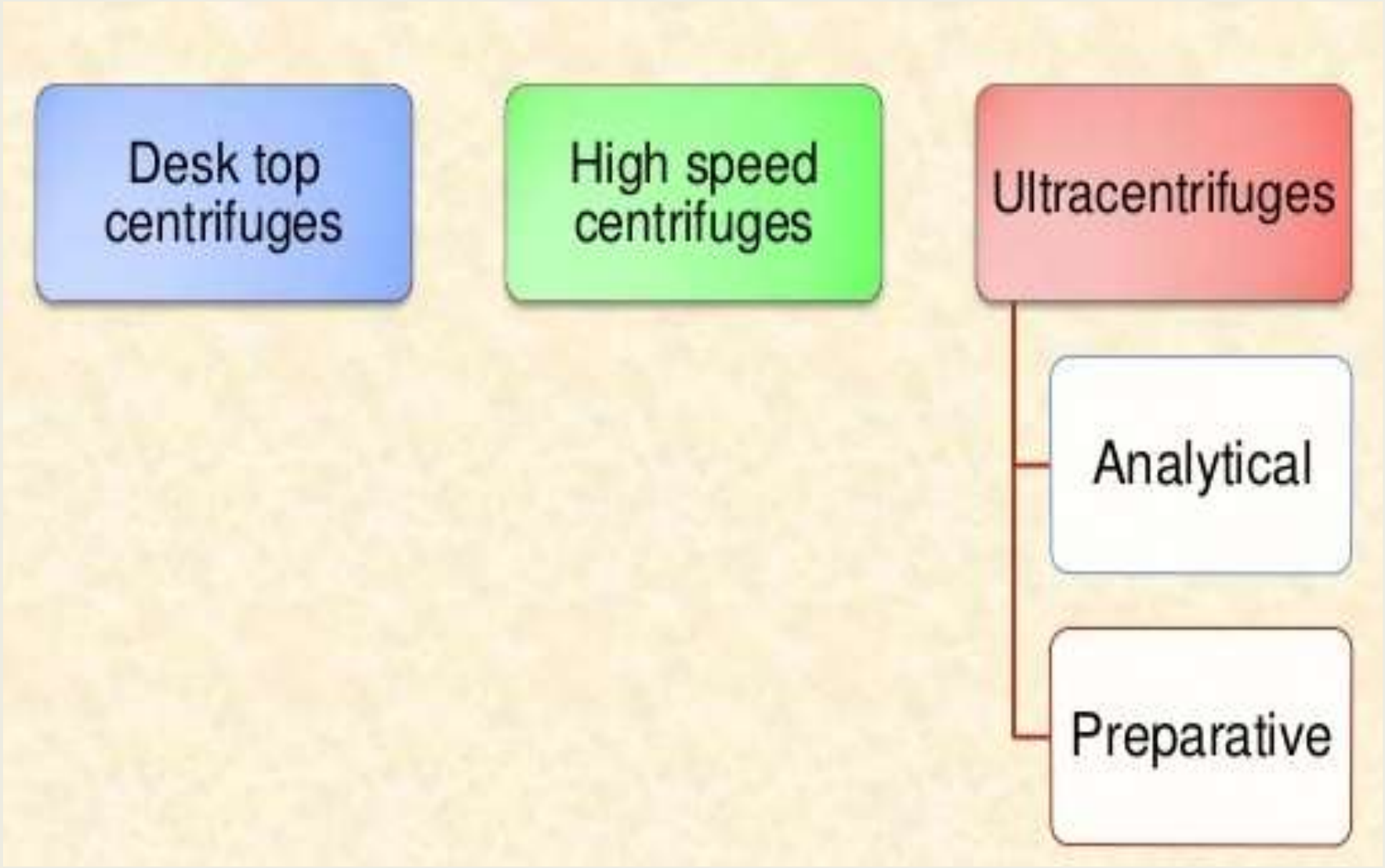
- Centrifugation is a technique of separating substances which involves the application of centrifugal force.The particles are separated from a solution according to their size, shape, density, the viscosity of the medium and rotor speed.

Principle of Centrifugation

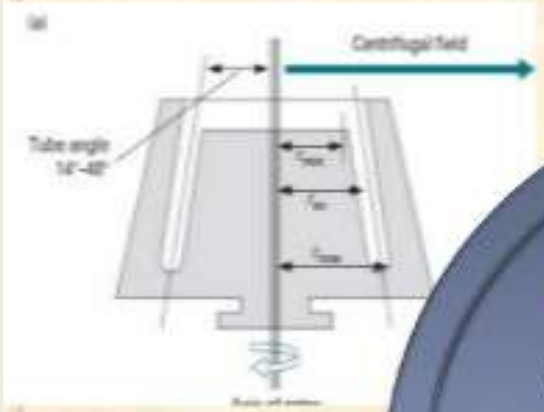
- In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it floats to the top.
- The greater the difference in density, the faster they move. If there is no difference in density (isopycnic conditions), the particles stay steady.
- To take advantage of even tiny 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.
- 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.
- In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low- density substances rise to the top.

Types of Centrifuge



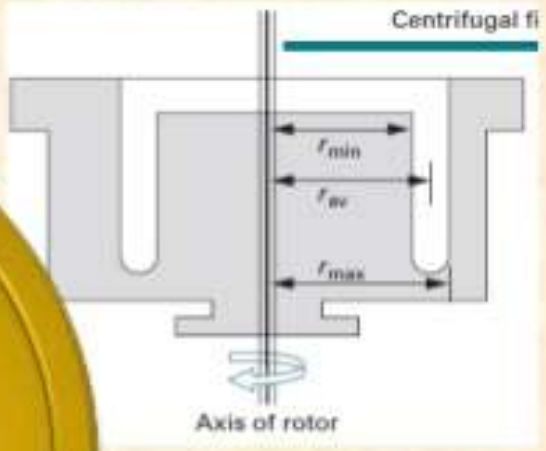
Types of rotor

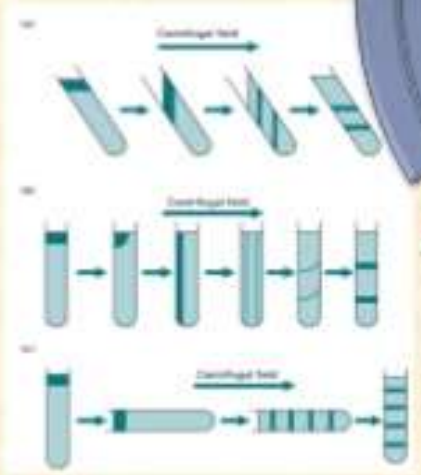


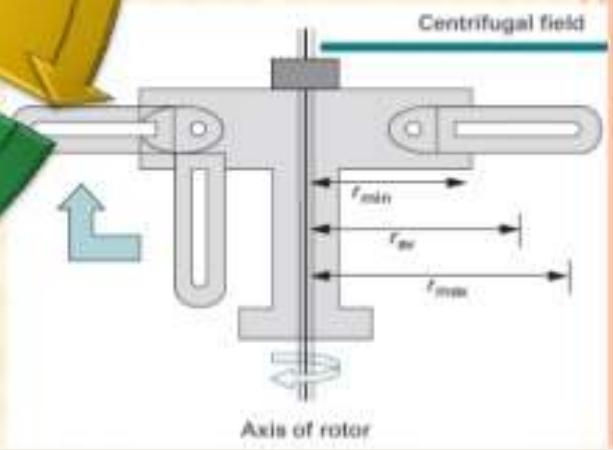
Fixed angle rotors

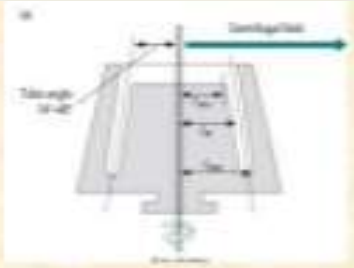
Vertical tube rotors

Swinging-bucket rotors

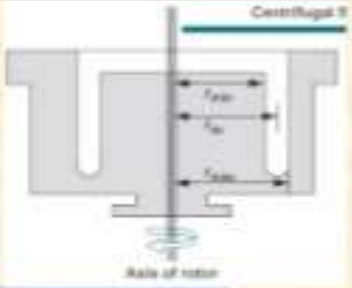








Types of rotor



Fixed angle rotors

- Tubes are held at angle of 14 to 40^0 to the vertical.
- Particles move radially outwards, travel a short distance.
- Useful for differential centrifugation
- Reorientation of the tube occurs during acceleration and deceleration of the rotor.

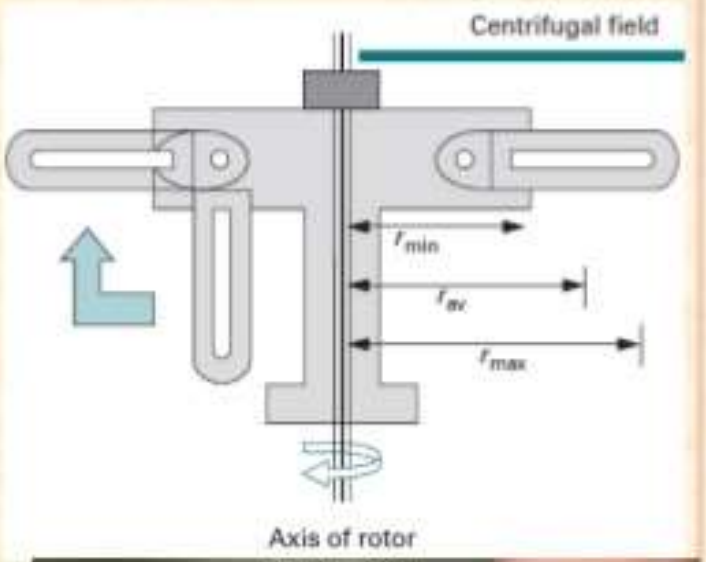
Vertical tube rotors

- Held vertical parallel to rotor axis.
- Particles move short distance.
- Time of separation is shorter.
- Disadvantage: pellet may fall back into solution at end of centrifugation.

Types of rotor

Swinging-bucket rotors

- Sing out to horizontal position when rotor accelerates.
- Longer distance of travel may allow better separation, such as in density gradient centrifugation.
- Easier to withdraw supernatant without disturbing pellet.
- Normally used for density-gradient centrifugation.



LOW-SPEED CENTRIFUGE

1) Most laboratories have a standard low-speed centrifuge used for routine sedimentation of heavy particles
2) The low-speed centrifuge has a maximum speed of 4000-5000rpm
3) These instruments usually operate at room temperatures with no means of temperature control.
4) Two types of rotors are used in it,

- Fixed angle
- Swinging bucket.

5) It is used for sedimentation of red blood cells until the particles are tightly packed into a pellet and supernatant is separated by decantation.

HIGH-SPEED CENTRIFUGES

- High-speed centrifuges are used in more sophisticated biochemical applications, higher speeds and temperature control of the rotor chamber are essential.
- The high-speed centrifuge has a maximum speed of 15,000 – 20,000 RPM
- The operator of this instrument can carefully control speed and temperature which is required for sensitive biological samples.
- Three types of rotors are available for high-speed centrifugation-
- Fixed angle
- Swinging bucket
- Vertical rotor

High speed centrifuges

- Maximum speed of 25000rpm, providing 90000g centrifugal forces.
- Equipped with refrigeration to remove heat generated.
- Temperature maintained at 0-4^{0}C by means of thermocouple.
- Used to collect microorganism, cell debris, cells, large cellular organelles, precipitates of chemical reactions.
- Also useful in isolating the sub-cellular organelles(nuclei, mitochondria, lysosomes)

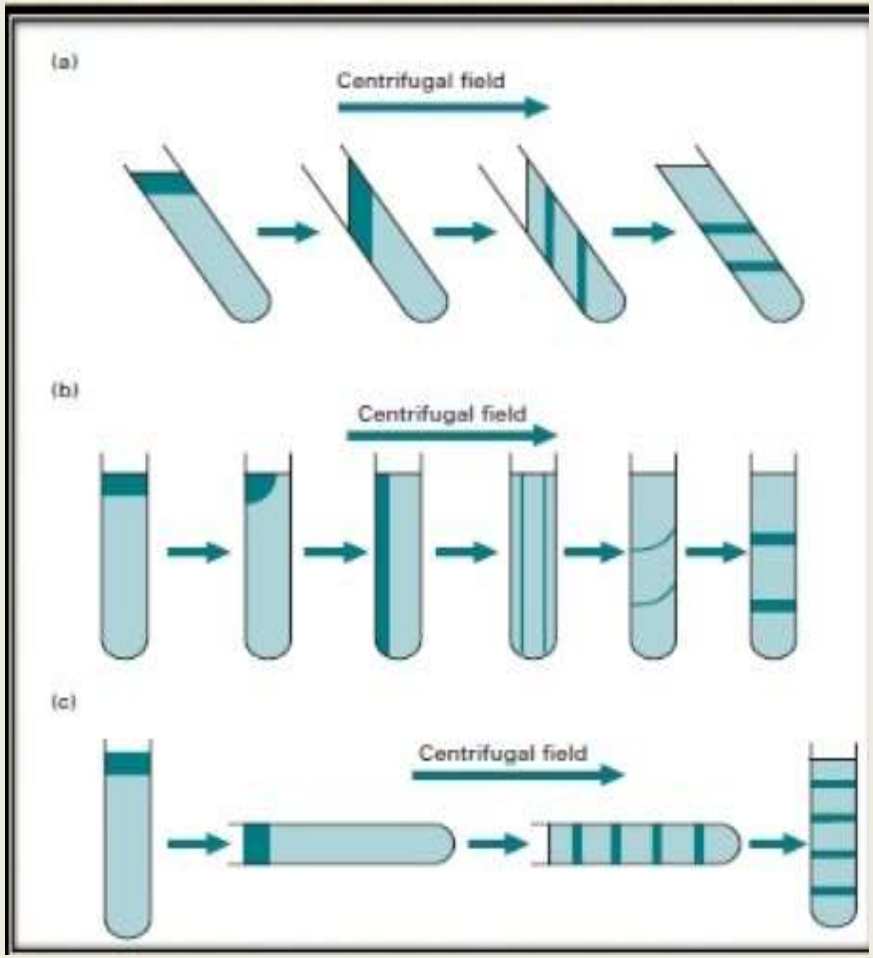
ULTRACENTRIFUGES

- It is the most sophisticated instrument.
- Ultracentrifuge has a maximum speed of 65,000 RPM (100,000's x g).
- Intense heat is generated due to high speed thus the spinning chambers must be refrigerated and kept at a high vacuum.
- It is used for both preparative work and analytical work

Ultracentrifuges

- Operate at speed of 75,000rpm, providing the centrifugal force of 500,000g.
- Rotor chamber is sealed and evacuated by pump to attain vacuum.
- Refrigeration system (temp 0-4^{0}C).
- Rotor chamber is always enclosed in a heavy armor plate.
- Centrifugation for isolation and purification of components is known as preparatory centrifugation, while that carried out with a desire for characterization is known as analytical centrifugation.

Types of Centrifugation



Preparative centrifugation

- Is concerned with the actual isolation of biological material for subsequent biochemical investigations.
- Divided into two main techniques depending on suspension medium in which separation occur.
  - Homogenous medium – differential centrifugation
  - Density gradient medium – density gradient centrifugation

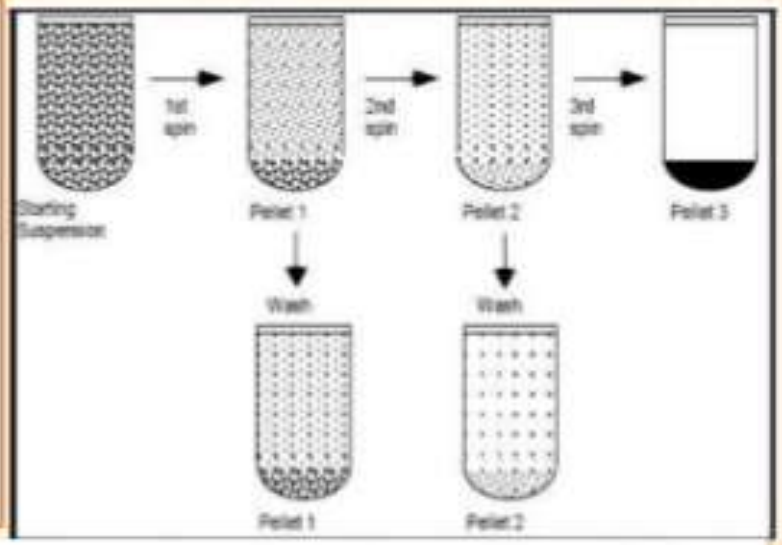
1. Differential centrifugation

- Separation is achieved based in the size of particles in differential centrifugation.
- Commonly used in simple pelleting and obtaining the partially pure separation of subcellular organelles and macromolecules.
- Used for study of subcellular organelle, tissues or cells (first disrupted to study internal content)

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.

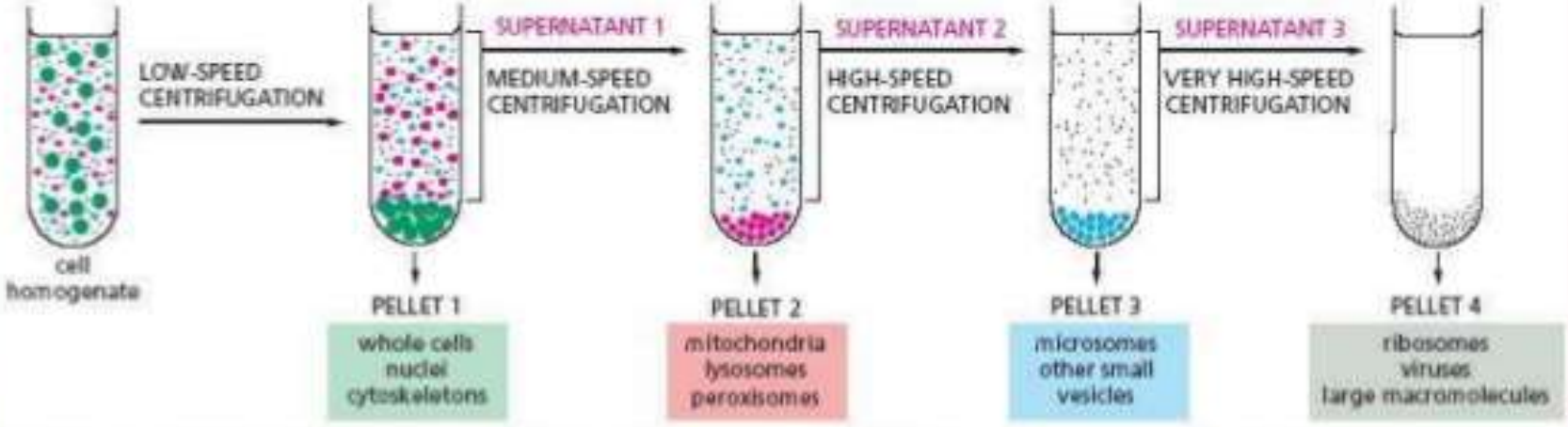
- During centrifugation, larger particles sediment faster than the smaller ones.
- At a series of progressive higher g-force generate partially purified organelles.



DIFFERENTIAL CENTRIFUGATION

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

Centrifugation separates cell components on the basis of size and density. The larger and denser components experience the greatest centrifugal force and move most rapidly. They sediment to form a pellet at the bottom of the tube, while smaller, less dense components remain in suspension above, a portion called the supernatant.



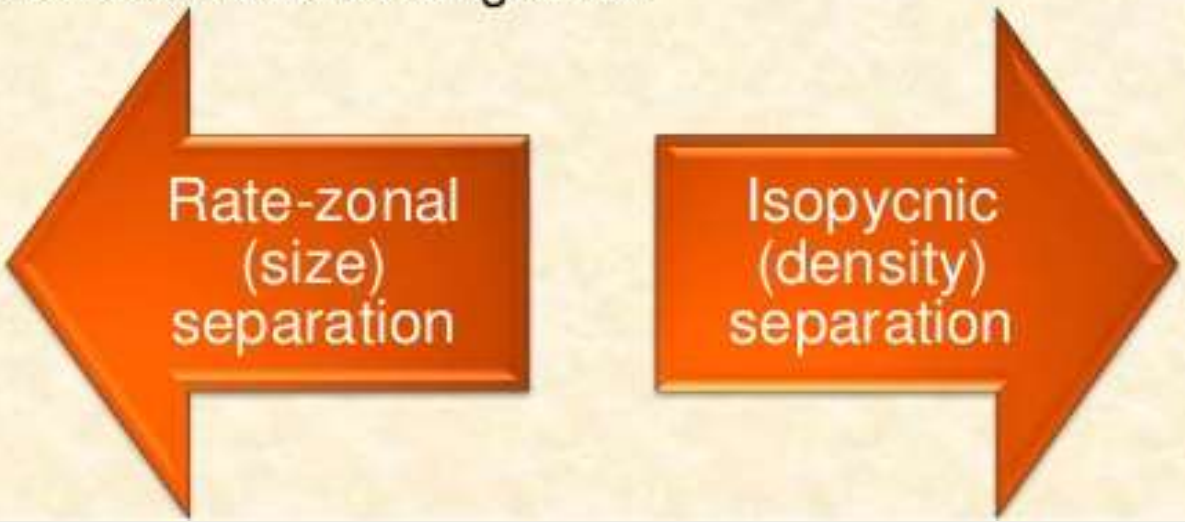
- Inspite of its reduced yield differential centrifugation remains probably the most commonly used method for isolation of intracellular organelle from tissue homogenates because of its;
  - relative ease
  - Convenience
  - Time economy
- Drawback is its poor yield and fact that preparation obtained never pure.

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.

2. Density gradient centrifugation

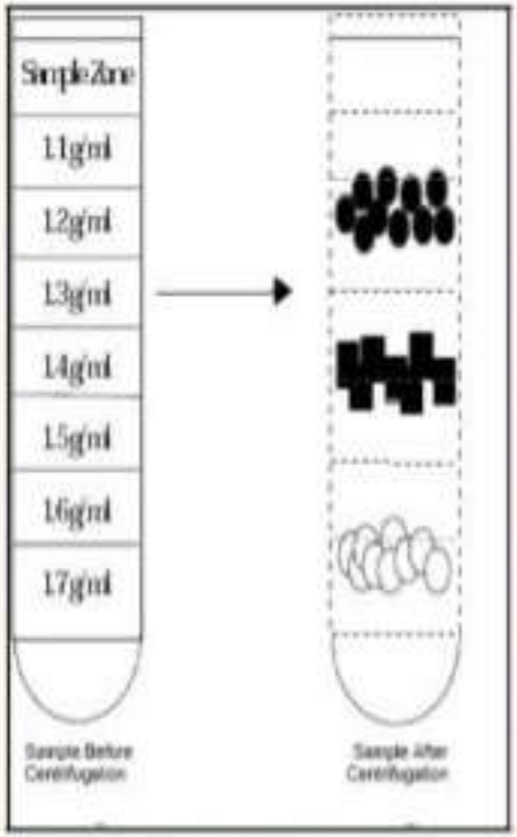
- It is the preferred method to purify subcellular organelles and macromolecules.
- Density gradient can be generated by placing layer after layer of gradient media such as sucrose in tube, with heaviest layer at the bottom and lightest at the top in either.
- Classified into two categories:



- Gradient material used are:
  - Sucrose (66%, 5^0C)
  - Silica sols
  - Glycerol
  - CsCl
  - Cs Acetate
  - Ficol (high molecular wgt sucrose polymer & epichlorhydrin)
  - Sorbitol
  - Polyvinylpyrrolidone

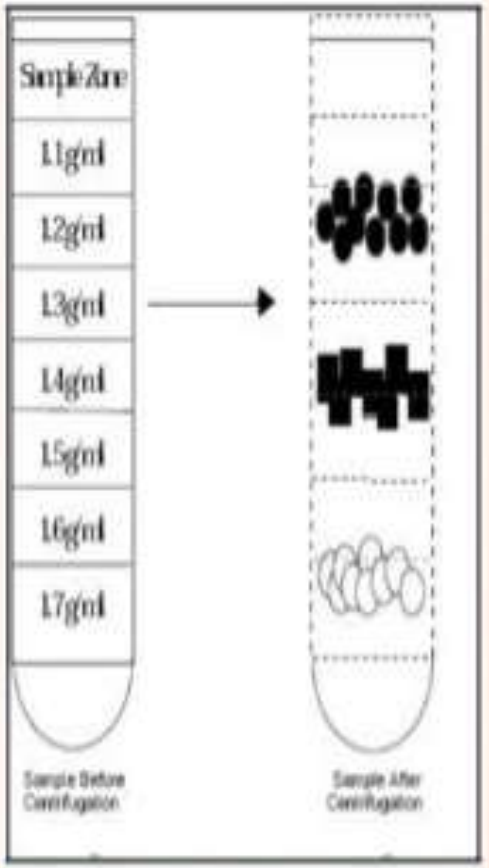
2.1 Rate zonal centrifugation

- Gradient centrifugation.
- Take advantage of particle size and mass instead of particle density for sedimentation.
- Ex: for common application include separation of cellular organelle such as endosomes or proteins (such as antibodies)



2.1 Rate zonal centrifugation

- Criteria for successful rate-zonal centrifugation:
  - Density of sample solution must be less than that of the lowest density portion of the gradient.
  - Density of sample particle must be greater than that of highest density portion of the gradient.
  - Path length of gradient must be sufficient for the separation to occur.
  - Time is important, if you perform too long runs, particles may all pellet at the bottom of the tube.



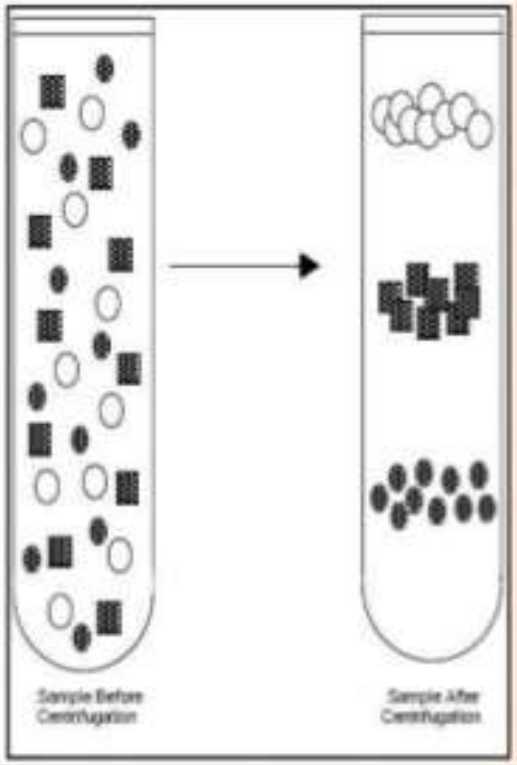
Rate-Zonal Density-Gradient Centrifugation

- 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.

2.2 Isopycnic centrifugation

- Particle of a particular density will sink during centrifugation until a position is reaches where the density of the surrounding solution is exactly the same as the density of the particle.
‘
- Once quasi-equilibrium is reached, the length of centrifugation doesnot have any influence on the migration of particle.
- Ex: separation of Nucleic acid in CsCl (Caseium chloride) gradient.

Figure 3. ISOPYCNIC (DENSITY) SEPARATION



Isopycnic 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.

	Rate-Zonal	Isopycnic
Synonym	S-zonal, sedimentation velocity	Density equilibrium, sedimentation equilibrium
Gradient	•Shallow, •Maximum gradient density less than the least dense sedimenting specie, •Gradient continuous.	•Steep, •Maximum gradient density greater than that of the most dense sedimenting specie, •Continuous or discontinuous gradients.
Centrifuga-tion	•Incomplete sedimentation, •Low speed, •Short time	•Complete sedimentation till equilibrium is achieved, •High speed, • Long time.
Separation	RNA- DNA hybrids, ribosomal subunits, etc.,	DNA, plasma lipoproteins, lysosomes, mitochondria, peroxisomes, etc.,

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