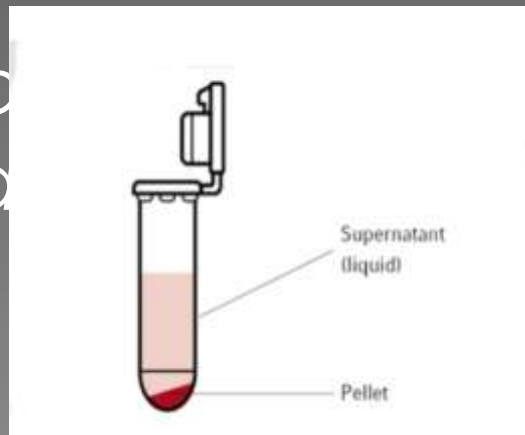


CENTRIFUGATION

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INTRODUCTION

- Centrifuge is a machine , that applies cetrifugal force to separate fluid of different densities or liquids from solids.
- Separation of compounds depends on migration.
- The separated components are supernatant and pellet.



PRINCIPLES OF CENTRIFUGATION

- *The centrifuge works using sedimentation principle.*
- *The sedimentation principle is , centrifugal force acting on a particle in a solution depends upon a density between particle and medium.*
- *The centrifugal acceleration causes , denser objects to move outward in radial direction.*
- *Objects that are less dense are displaced and move to the center.*

SEDIMENTATION COEFFICIENT AND SEDIMENTATION VELOCITY

SEDIMENTATION VELOCITY

- Sedimentation velocity is an analytical ultracentrifugation method that measures the rate at which molecules move in response to centrifugal force generated in a centrifuge.*
- The centrifugal force acting on particle produces an acceleration.*

SEDIMENTATION COEFFICIENT

- The sedimentation coefficient of particle characterizes its sedimentation during centrifugation .*
- It depends on molecular weight and molecular shape.*

ROTORS OF CENTRIFUGE

TYPES OF ROTOR

There are three types of rotor. They are,

- *Fixed angle rotor.*
- *Vertical rotor.*
- *Swinging bucket rotor.*

FIXED ANGLE ROTOR

- The fixed angle rotor holds the tube at 45° .
- During centrifugation, the densest particles in the solution will separate towards the angle of the centripetal force.
- Dense particles form a concentrated pellet at the bottom of the tube.



VERTICAL ROTOR

- In the vertical rotors , the tubes are vertical during the run.
- The short path length and low k factors of these rotors mean that particle has only a short distance to travel to pellet, reducing application



SWINGING BUCKET ROTOR

- *It is the rotor, in which tubes are located in bucket and those buckets are not rigidly attached certain angle.*
- *The rotor allows the tube to change angle during the run.*
- *These rot*
zonal sep



to rate

ULTRACENTRIFUGE

- *The ultracentrifuge is centrifuge optimized for spinning rotor at very high speeds , capable of generating acceleration as high as 1 000 000g.*
- *The principle is, it spins liquid samples at high speeds and thus creates a strong centripetal force which cause pelleting.*
- *It is used in molecular biology , biochemistry and cell biology.*
- *Ultracentrifuge is of two types they are Preparative and Analytical.*

TYPES OF ULTRACENTRIFUGE

PREPARATIVE

- *It is a high velocity centrifuge used in the separation of small submicroscopic particle.*
- *It is used in pelleting small materials such as membranes, organelles, viruses etc.,*

ANALYTICAL

- *It is a high velocity centrifuge used in the analytical process.*
- *It is used to determine the mass and shape of macromolecules such as protein complexes and rate of sedimentation of molecules.*

PREPARATIVE

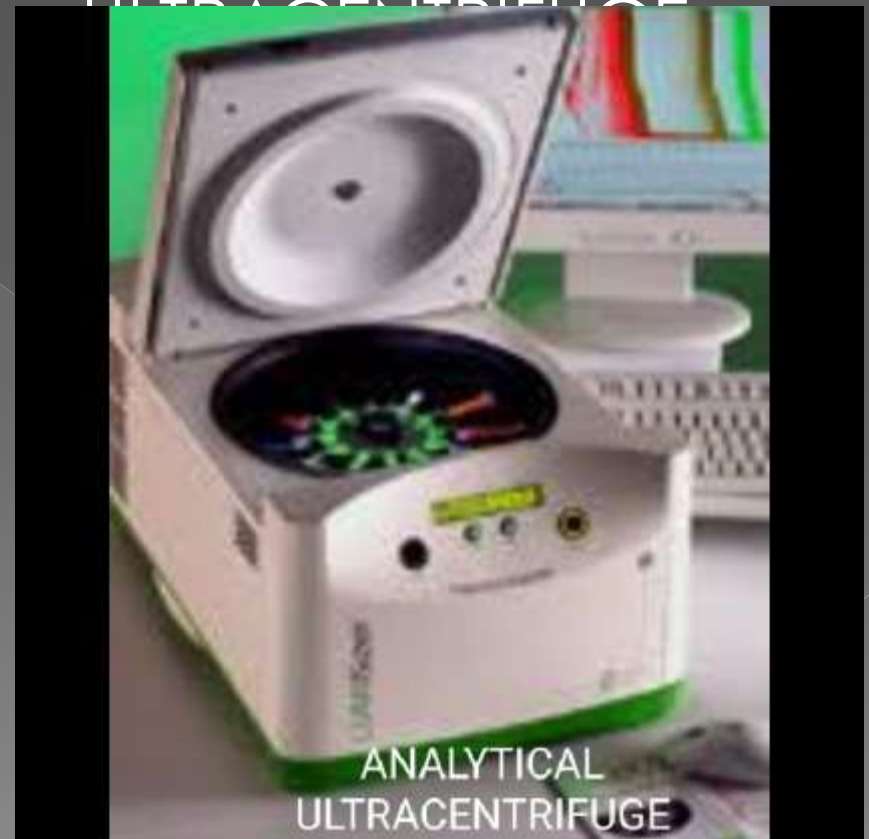
ULTRACENTRIFUGE



PREPARATIVE
ULTRACENTRIFUGE

ANALYTICAL

ULTRACENTRIFUGE



ANALYTICAL
ULTRACENTRIFUGE

HIGH SPEED CENTRIFUGATION

- High speed centrifuges uses 60,000 *g g- force .
- It can spun the sample at > 5000 rpm.
- Used in the separation of the nucleus , mitochondrial, etc.,

LOW SPEED CENTRIFUGATION

- Low speed floor- standing centrifuges uses the g- force less than 10,000 rcf.
- It can spun the sample at < 5000 rpm.
- Used in cell culture or blood etc.,

HIGH SPEED CENTRIFUGATION

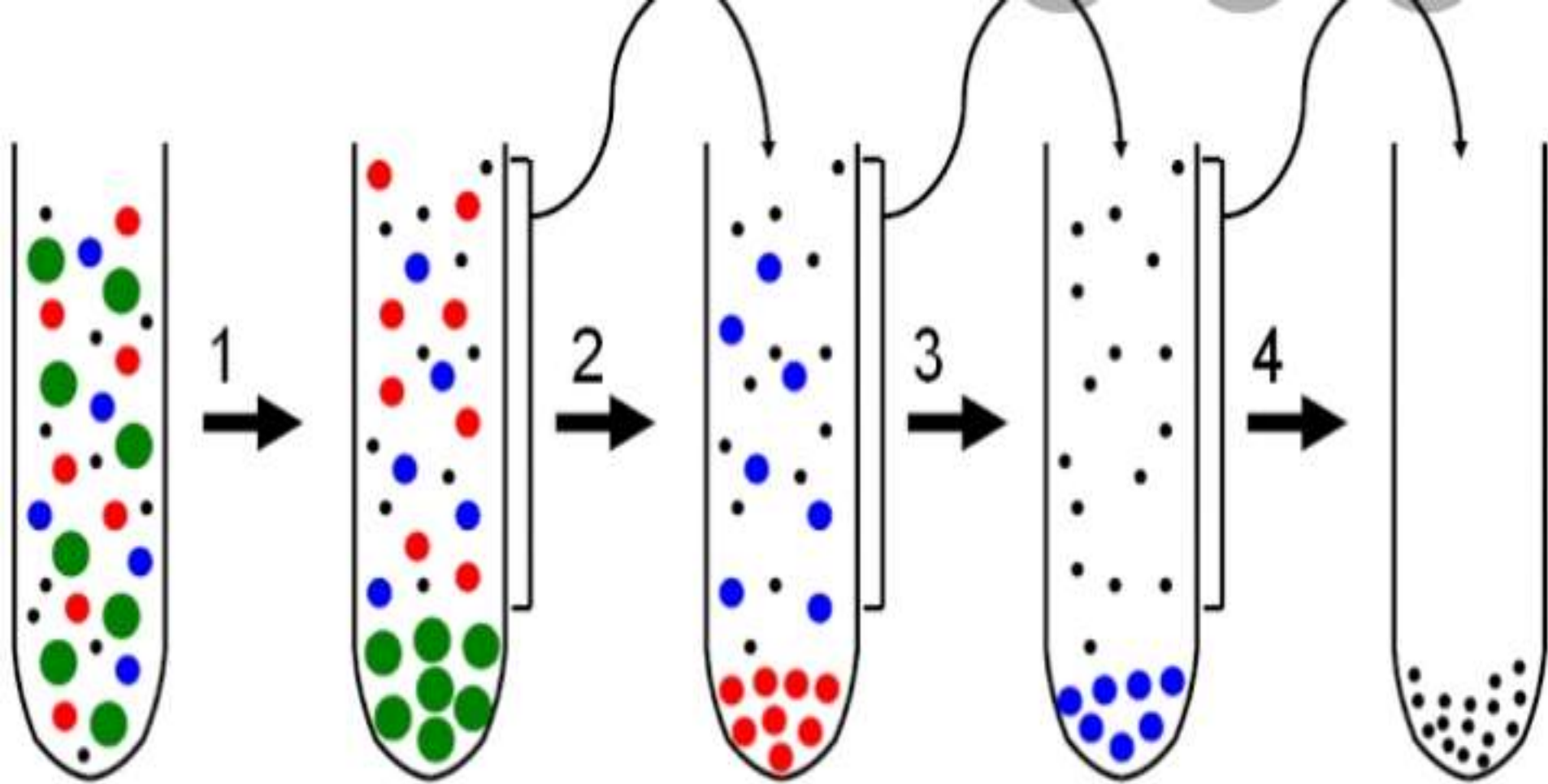


LOW SPEED CENTRIFUGATION



DIFFERENTIAL CENTRIFUGATION

- *It is used to separate organelles and other sub-cellular particles on the basis of the sedimentation.*
- *Differential centrifugation works by a stepwise increase in centrifugation speed.*
- *In this centrifugation, the separation occurs based on the size of the particles in differential centrifugation.*
- *Its application is , it is used in the biochemistry and cell biology to separate organelles and sub- cellular particles.*



DIFFERENTIAL CENTRIFUGATION

DENSITY GRADIENT CENTRIFUGATION

- *Density gradient centrifugation refers to separation method where the substances concentrated in solution of cesium salts or sucrose.*
- *The separation occurs based on the density of the particles in density gradient centrifugation .*
- *A homogenized solution is used as sample in density gradient centrifugation*
- *It is widely used to fractionate cells, viral particles lysosome etc.,*

