Centrifugation

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Centrifugation

- Use of the centrifugal force for the separation of mixtures
- More-dense components migrate away from the axis of the centrifuge
- less-dense components of migrate towards the axis

DEFINATION

- "An apparatus that rotates at high speed and by centrifgal force separates substances of different densities."

BASIC PRINCIPLES OF CENTRIFUGATION

- A particle,whether it is precipitate,a macromolucle or cell organelle when rotated at high speed is subjected to a centrifugal force.
- Centrifugal force is defined as
- F=mw2r
- Where F=intensity of centrifugal force
- m=effective mass of sedimenting particle
- w=angular velocity of rotation
- r=distance of migrating particles from central axis of rotation

- A more common measurement of F,in terms of Gravitational force g,is Relative Centrifugal Force RCF, Is given as

$$RCF=(rpm)2(r)$$

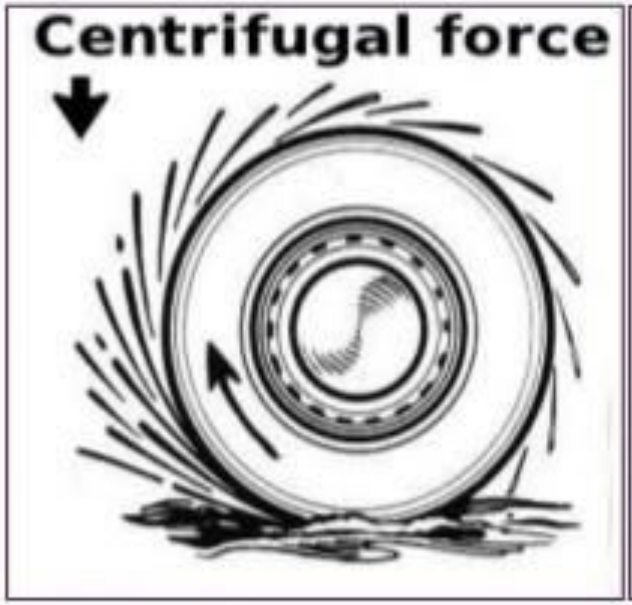
Thus this equation indicates that RCF varies with r,(the distance of the sedimenting particles from axis of rotation Thus it gives idea of only basic principle,it does not take into account other factors ie mass,shape,density of medium.

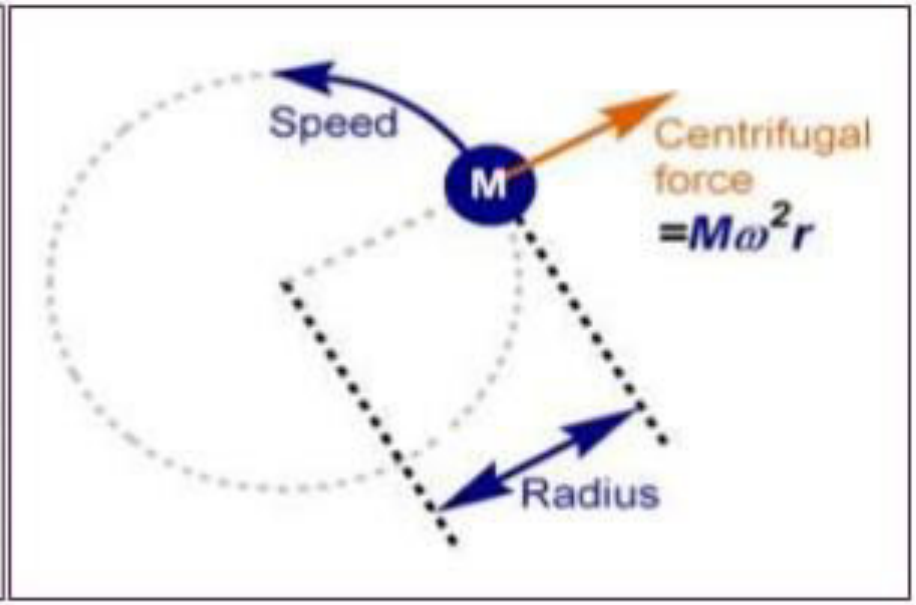
- Thus centrifugal force felt by particle is defined as

$$m=m0-movp$$

CALCULATION OF CENTRIFUGAL FORCE

- "A real or "reactive" centrifugal force occurs in reaction to a centripetal acceleration acting on a mass." So basically, it is the opposing force to Centripetal force.





- The rate of change of angular displacement of the particle in a given time is called angular velocity.

- It is expressed as

$$\omega = \frac{d\theta}{dt}$$

- Where d θ is change in angular displacement, dt is change in time t.

CALCULATION OF ANGULAR VELOCITY

- The **angular velocity** is defined as the rate of change of angular displacement and is a vector quantity (more precisely, apseudovector) which specifies the angular speed (rotational speed) of an object and the axis about which the object is rotating.
- The SI unit of angular velocity is radians per second, although it may be measured in other units such as degrees per second, degrees per hour, etc. Angular velocity is usually represented by the symbol omega (**ω**, rarely **Ω**).

- **Angular Velocity Formula** is given by

$$\omega = \frac{\theta}{t}$$

- Where θ is angular displacement and t is the time taken.
- The linear Velocity and angular velocity is given by the formula

$$\omega = \frac{v}{r}$$

- Where V is the linear velocity r is the radius of circular path.
- Angular velocity is expressed in radian per second **(rad/s)**. Angular Velocity formula is used to calculate the angular velocity of any moving body.

CENTRIFUGE ROTOR

- A centrifuge rotor is the rotating unit of the centrifuge, which has fixed holes drilled at an angle. Test tubes are placed inside these holes and the rotor spins to aid in the separation of the materials.

TYPES OF ROTOR

swing-bucket Rotor

fixed-angle Rotor

vertical rotor

SWING-BUCKET ROTOR

- A swing-bucket rotor usually supports samples ranging in volume from 36 mL to 2.2 mL. Swing-buckets can support two types of separations: rate-zonal and Isopycnic.
- Swing-buckets are preferred for rate-zonal separations, because the distance between the outside of the meniscus and the outside of the bottom of the tube is long enough for separation to occur.

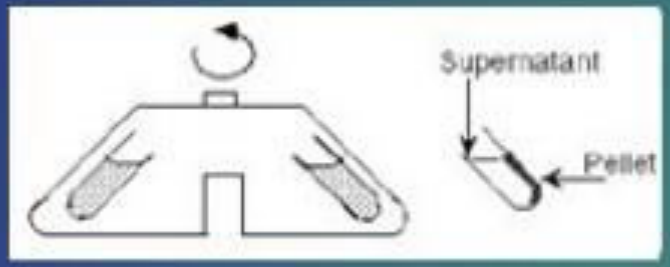
swing-bucket
Rotor

FIXED-ANGLE ROTOR

- Fixed-angle rotors are usually used for pelleting applications to either pellet particles from a suspension and remove the excess debris, or to collect the pellet. Rotor cavities range from 0.2 mL to 1 mL.
- The most important aspect in deciding to use a fixed-angle rotor is the K factor. The K factor indicates how efficient the rotor can pellet at maximum speed. The lower the K factor, the higher the pelleting efficiency.

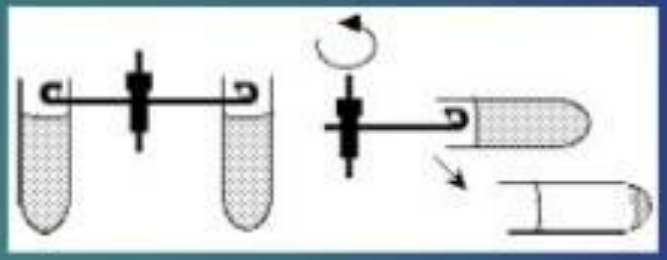
Centrifuge Rotors

- Fixed Angle Rotor



Sedimenting particles have only short distance to travel before pelleting. Shorter run time. The most widely used rotor type.

- Swinging Bucket Rotor

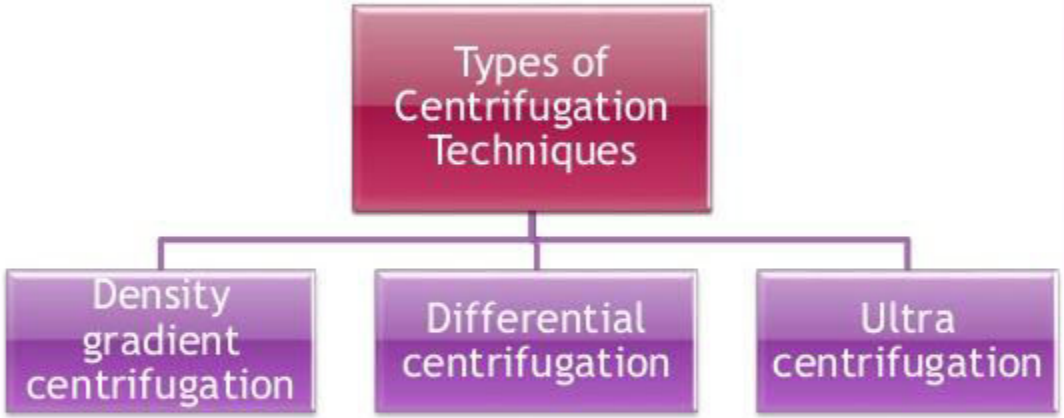


Longer distance of travel may allow better separation, such as in density gradient centrifugation. Easier to withdraw supernatant without disturbing pellet.

Fixed-angle Rotor

Vertical Rotor

Classification


Types of Centrifugation Techniques
Density gradient centrifugation
Differential centrifugation
Ultra centrifugation

DENSITY GRADIENT CENTRIFUGATION

- It allow separation of many or all components in a mixture and allows for measurement to be made
- There are two forms of Density gradient centrifugation :

Rate zonal centrifugation

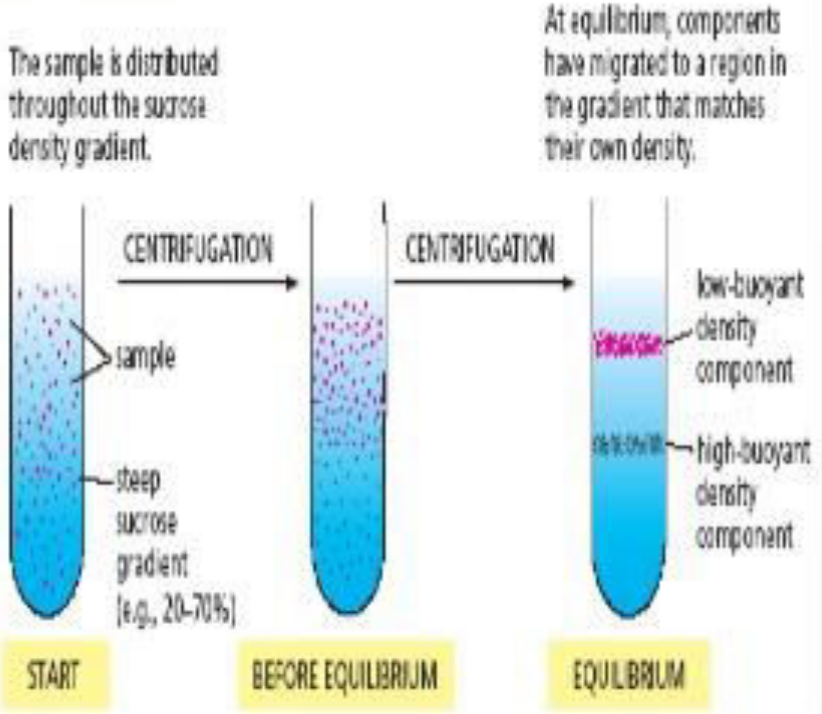
Isopycnic or sedimentation equilibrium centrifugation

DENSITY GRADIENT CENTRIFUGATION

- DENSITY GRADIENT CENTRIFUGATION
- 1)This type of centrifugation is mainly used to purify viruses,ribosomes,membranese etc.
- b)A sucrose density gradient is created by gently overlaying lower concentrations of sucrose on higher concentrations in centrifuge tubes
- c)the particles of interest are placed on top of the gradient and centrifuge in ultra centrifuges.
- d)the particles travel through the gradient until they reach a point at which their density matches with the density of surrounding sucrose.,the the fraction is removed and analyzed.
-

EQUILIBRIUM SEDIMENTATION

The ultracentrifuge can also be used to separate cell components on the basis of their buoyant density, independently of their size or shape. The sample is usually either layered on top of, or dispersed within, a steep density gradient that contains a very high concentration of sucrose or cesium chloride. Each subcellular component will move up or down when centrifuged until it reaches a position where its density matches its surroundings and then will move no further. A series of distinct bands will eventually be produced, with those nearest the bottom of the tube containing the components of highest buoyant density. The method is also called density gradient centrifugation.

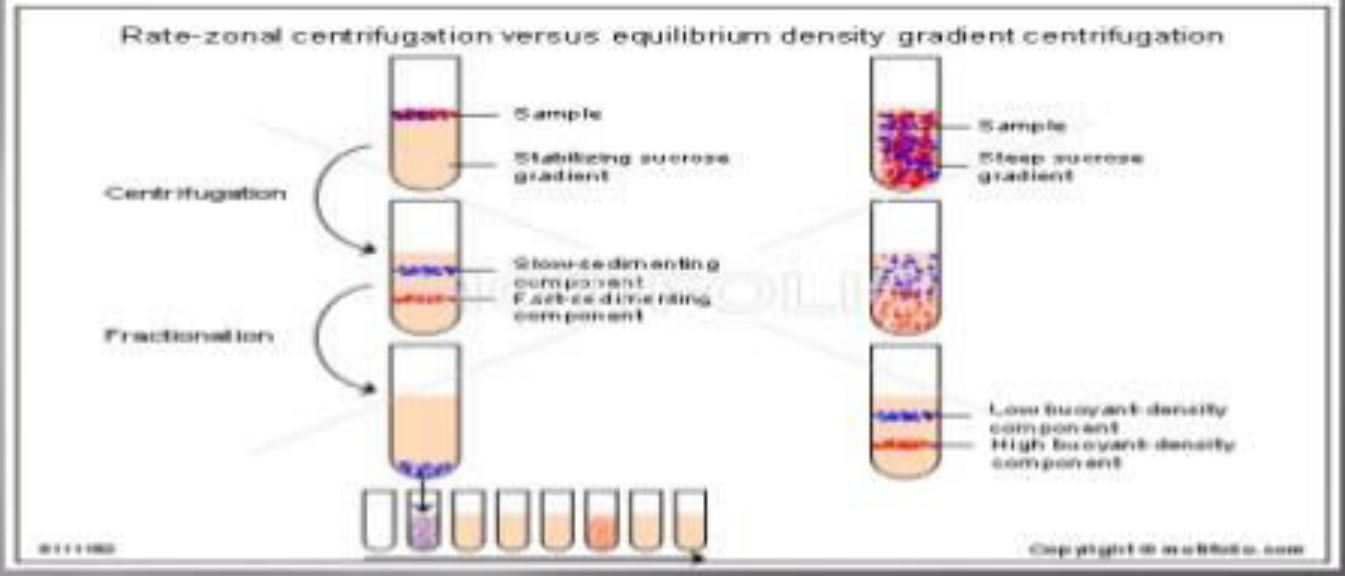


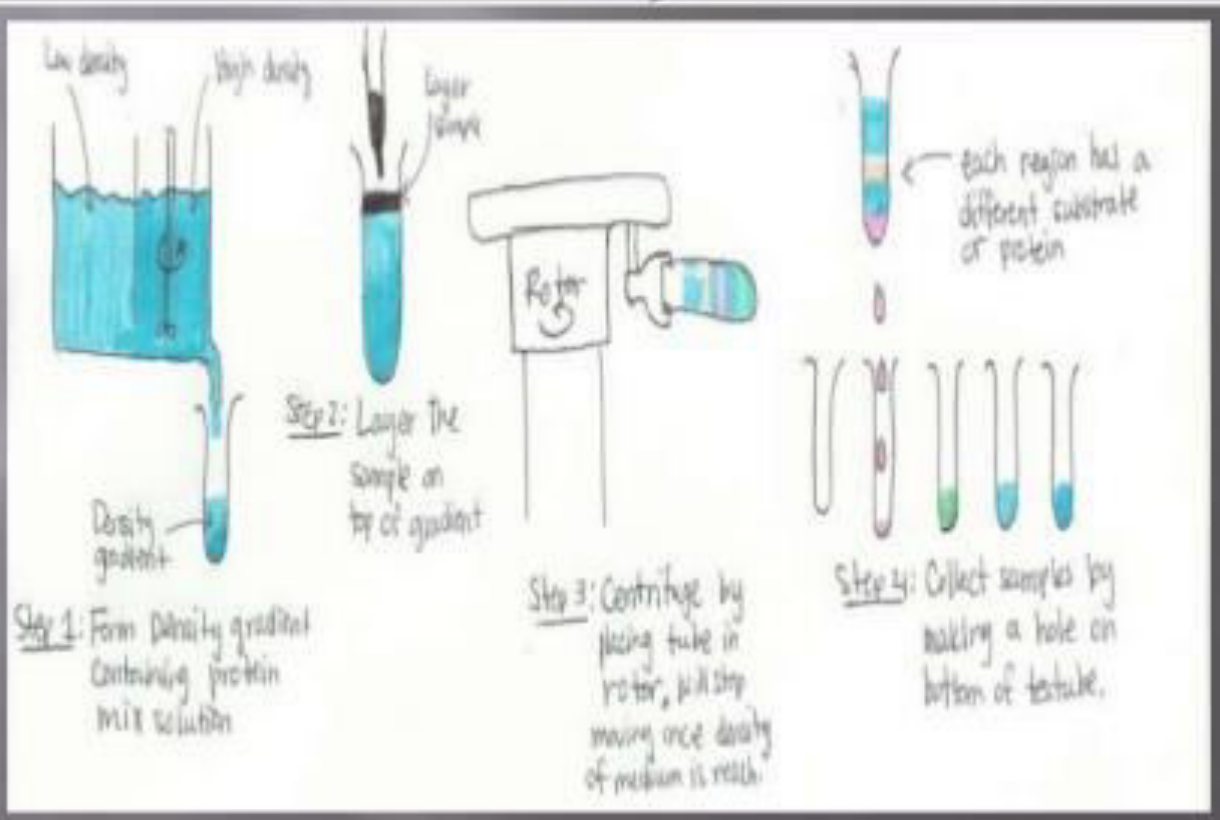
A sucrose gradient is shown here, but denser gradients can be formed with cesium chloride that are particularly useful for separating the nucleic acids (DNA and RNA).

The final bands can be collected from the base of the tube, as shown above.

ZONAL CENTRIFUGATION

- ZONAL CENTRIFUGATION
- a)zonal centrifugation is also known as band or gradient centrifugation
- b)it relies on the concept of sedimentation coefficient (ie movement of sediment through liquid medium)
- c)in this technique a density gradient is created in a test tube with sucrose and high density at the bottom.
- d)the sample of protein is placed on the top of the gradient and then centrifuged.
- e)the proteins sediment according to their sedimentation coefficient and the fractions are collected by creating a hole at the bottom of tube.


Rate-zonal centrifugation versus equilibrium density gradient centrifugation
Sample
Stabilizing sucrose gradient
Centrifugation
Slow-sedimenting component
Fast-sedimenting component
Fractionation
Sample
Steep sucrose gradient
Low buoyant density component
High buoyant density component

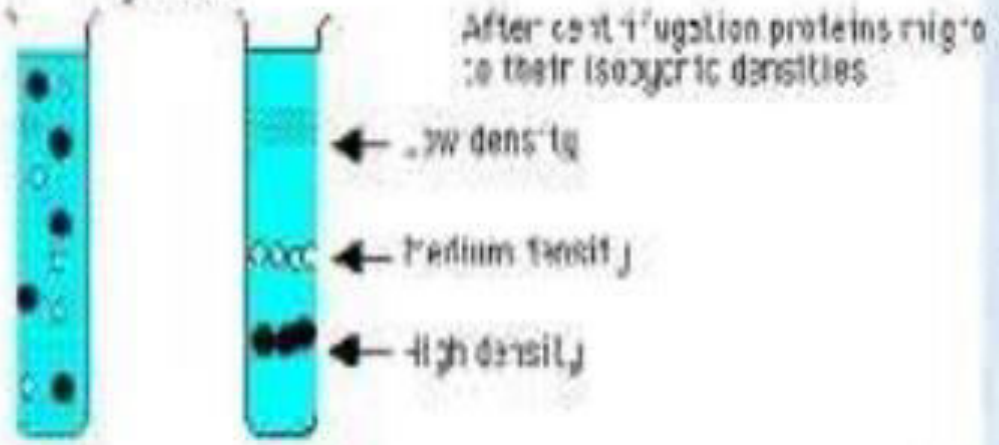

Low density
High density
Density gradient
Step 1: Form Density gradient Containing protein mix solution
Layer
Step 2: Layer the sample on top of gradient
Rotor
Step 3: Centrifuge by placing tube in rotor, with stop moving once density of medium is reach
each region has a different substrate or protein
Step 4: Collect samples by making a hole on bottom of testtube.

ISOPYCNIC CENTRIFUGATION

- ISOPYCNIC CENTRIFUGATION
- a)It is also called as density gradient centrifugation.
- b)the solution of biological sample and cesium salt is uniformly distributed in a centrifuge tube and rotated in an ultra centrifuge.
- c)under the influence of centrifugal force the cesium salts redistributes to form a density gradient from top to bottom.
- d)the sample molecules move to the region where their density equals to the density of gradient.

ISOPYCNIC-ZONAL CENTRIFUGATION

Figure 4. Isopycnic separation with a self-generating gradient



The sample is evenly distributed throughout the centrifuge tube before centrifugation.

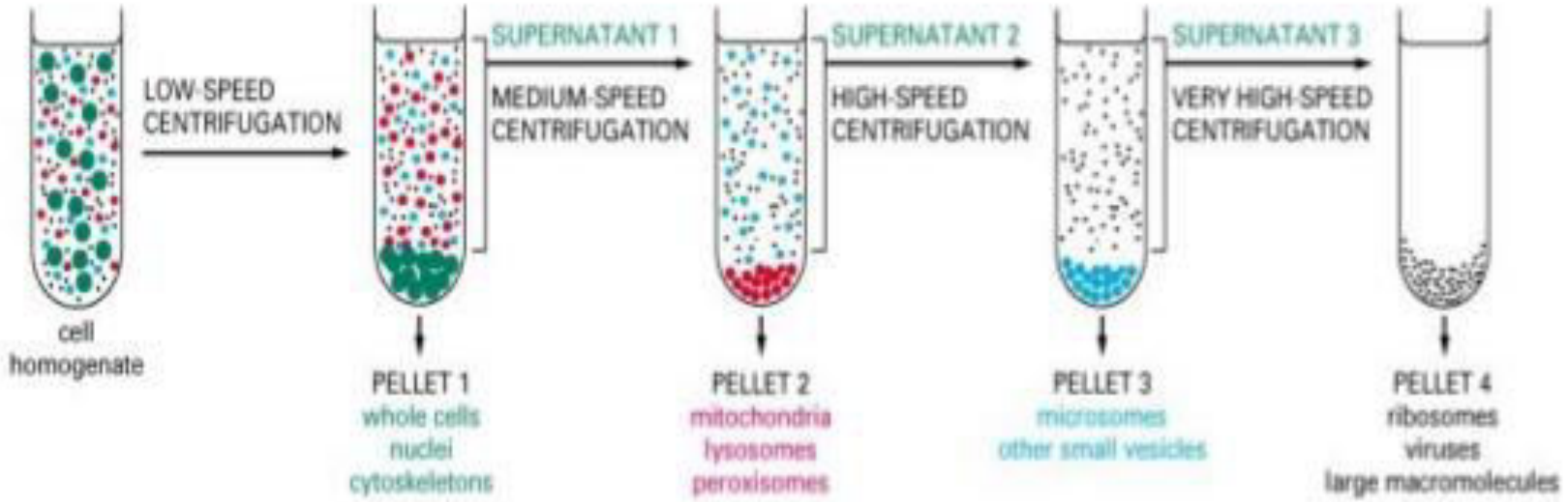
DIFFERENTIAL CENTRIFUGATION

- 1)DIFFERENTIAL CENTRIFUGATION
- a)Differential centrifugation is a technique commonly used by biochemists.
- b)tissue such as liver is homogenisfd at 32 degree in a sucrose solution that contains buffer
- c)the homogenate is then placed in a centrifuge and spun at constant centrifugal force at constant temperature.
- d)after sometime a sediment forms at the bottom of centrifuge called pellet and overlying solution called supernatant.
- e)the overlying solution is then placed in another centrifuge tube which is then rotated at higher speeds.

DIFFERENTIAL CENTRIFUGATION

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

Centrifugation separates cell components on the basic 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, called the supernatant.



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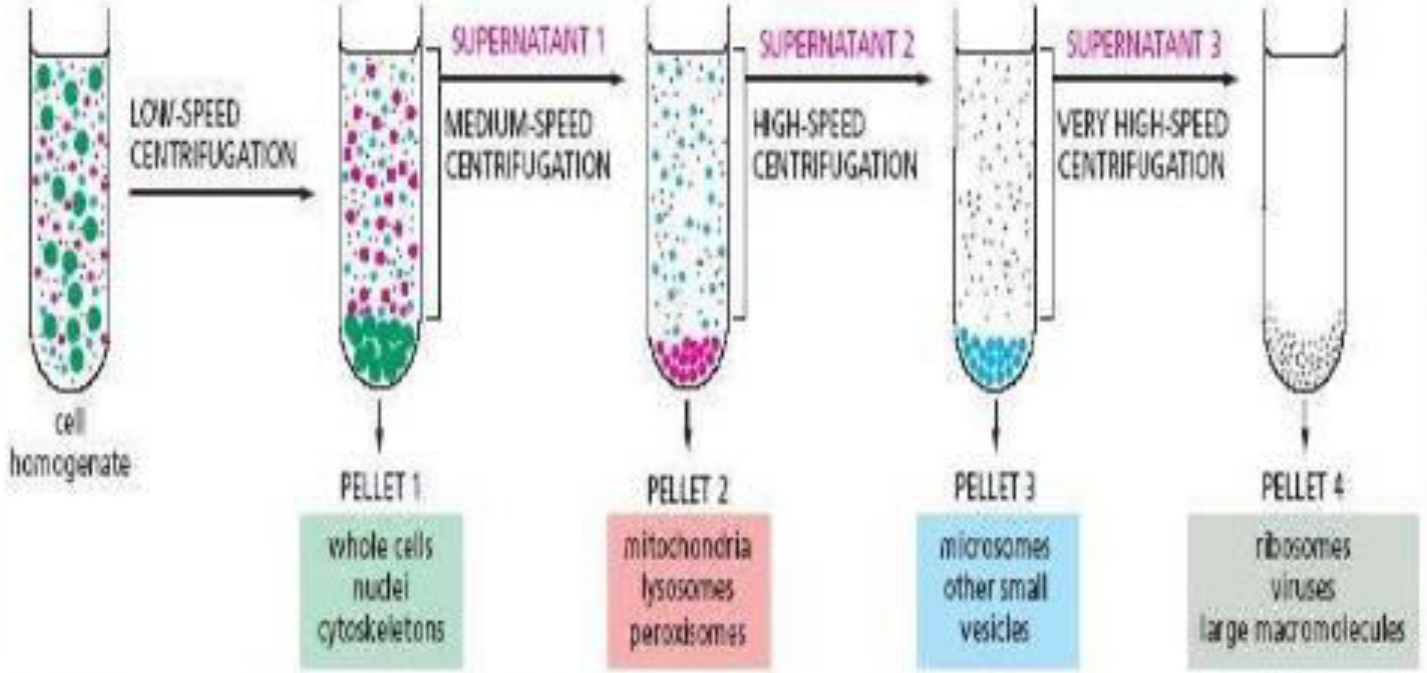


Ultracentrifugation

- Svedberg coined the term “ultracentrifuge". He was colloid chemist.
- He used the ultracentrifuge to determine the MW and subunit structure of hemoglobin , studies which changed the ideas concerning the structure of proteins.
- The first commercial ultracentrifuge was produced in 1940 by SPINCO.

Analytical ultracentrifuge

- In an analytical ultracentrifuge, a sample being spun can be monitored in real time through an optical detection system, using ultraviolet light absorption and/or interference optical refractive index sensitive system
- This allows the operator to observe the sample concentration versus the axis of rotation profile as a result of the applied centrifugal field.

Preparative ultracentrifuge

- Preparative ultracentrifuges are available with a wide variety of rotors suitable for a great range of experiments.
- Most rotors are designed to hold tubes that contain the samples. Swinging bucket rotors allow the tubes to hang on hinges so the tubes reorient to the horizontal as the rotor initially accelerates.
- Preparative rotors are used in biology for pelleting of fine particulate fractions, such as cellular organelles mitochondria, microsomes,ribosomes and viruses.

Continuous flow centrifuge

- Relatively simple
- High capacity
- Separating mixed liquids^

Refrigerated high-speed centrifuge

- Lower capacity
- Collect

microorganisms	O
cellular debris	O
cells	O
large cellular organelles	O
ammonium sulfate precipitates	O
immunoprecipitates	O
viruses	X
small organells	X

The ultracentrifuge

- Attain the speed of 75000rpm
- Isolate viruse
  DNA
  RNA
  protein

Composition

- Centrifuge consist of four parts:

1. Drive and speed control
2. Temperature control
3. Vacuum system
4. Rotors

Drive & Speed control

- Drive: water-cooled electric motor
- Speed control:
  1. selected by rheostat
  2. monitored with a tachometer

Overspeed system

- Prevent operation of a rotor above its maximum rated speed
- Consist of ^

1.a ring of alternating reflecting and nonreflecting surfaces attached to the bottom of the rotor.

2.a small but intense point source of light

3.a photocell

Temperature control

- highspeed centrifuge:

✓ placing a thermocouple in the rotor chamber

✓ monitoring only the rotor chamber temperature

- Ultracentrifuge:

✓ an infrared radiometric sensor placed beneath the rotor

✓ continuously monitors the rotor temperature

Vacuum system

- The speed of centrifuge < 15000 to 20000rp

  Not required

- The speed of centrifuge > 4000rpm

  Required

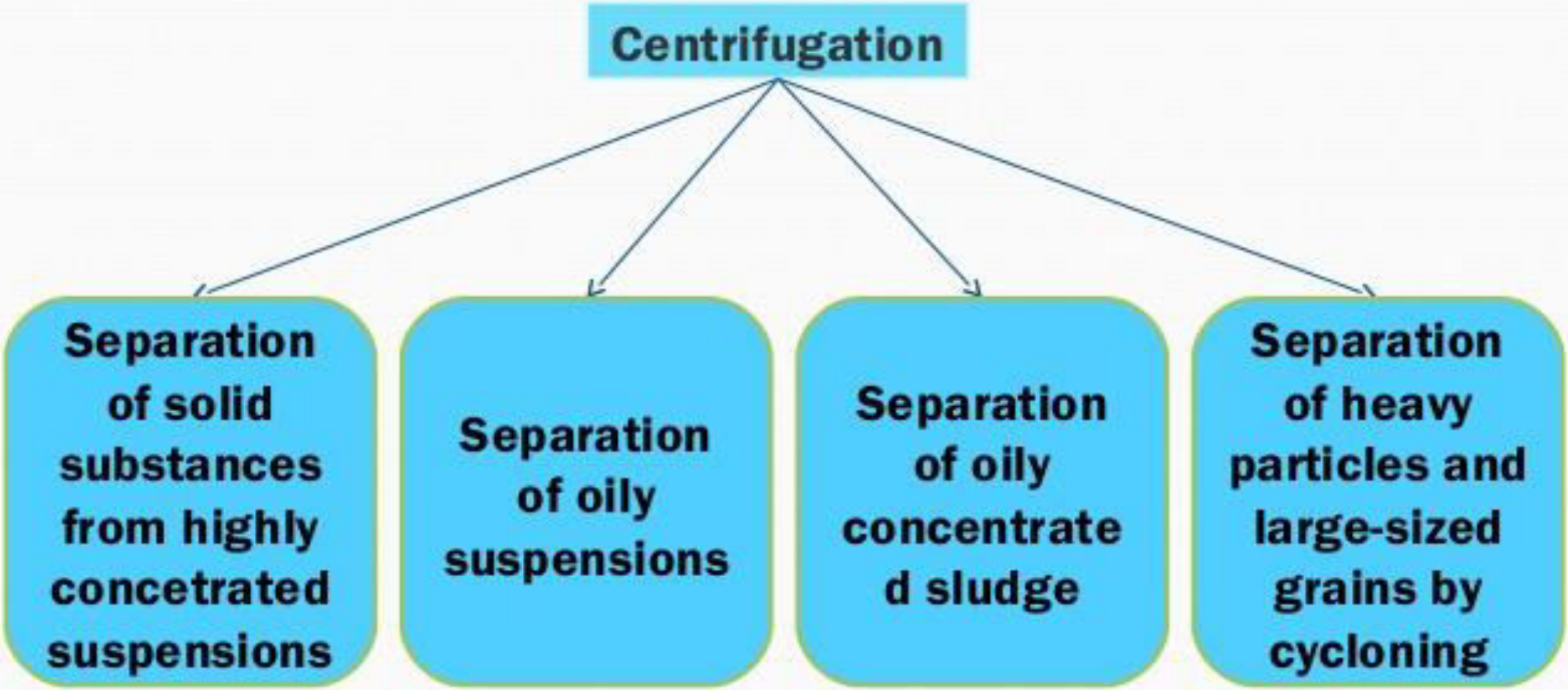
CARE OF CENTRIFUGE AND ROTORS

- 1)Carefully read the manual before using centrifuges
- 2)Select proper operating conditions
- 3)Check rotor for cleanliness and for damage
- 4)Select proper rotor of definate size
- 5)Be suer the rotor is clean and undamaged
- 6)keep accurate record of centrifuge and rotors
- 7)carefully clean rotors after centrifugation.

SIGNIFICANCE

- 1)THE CENTRIFUGATION IS A MODERN AND EASY TECHNIQUE OF SEPERATION
- 2)DUE TO CENTRIFUGATION IT IS EASY TO SEPARATE CELLULAR AND SUB CELLUAR COMPONENTS
- 3)IT IS USED TO STUDY THE EFFECTS OF CENTRIFUGAL FORCES ON CELLS

Application in Water Treatment



Commercial applications

- **Centrifuges with a batch weight of up to 2,200 kg per charge are used in the sugar industry to separate the sugar crystals from the mother liquor .**
- **Standalone centrifuges for drying (hand-washed) clothes – usually with a water outlet.**
- **Large industrial centrifuges are also used in the oil industry to remove solids from the drilling fluid.**

OTHER APPLICATIONS

- Separating chalk powder from water
- Removing fat from milk to produce skimmed milk
- Separating textiles
- Removing water from lettuce after washing it in a salad spinner
- Separating particles from an air-flow using cyclonic separation


ULTRACENTRIFUGE
BECKMAN
COULTER
Optima MAX


HIGH SPEED
CENTRIGUGE
2334
LID OPEN