Centrifuge

Centrifugation

Centrifugation is a procedure that involves the use of centrifugal force for the sedimentation of heterogeneous mixture with a centrifuge, used in industry and in laboratory settings.

More dense components of the mixture move away from the axis of the centrifuge while less dense components of the mixture move towards the axis

Principle of centrifugation

A particle whether it is a precipitate a macromolecule or a cell organelle is subjected to a centrifugal force when it is rotated at a high rate of speed. The centrifugal force F is denoted by equation

$$F=m\omega^2 r$$

Where:

F= intensity of the centrifugal force

m= effective mass of the sedimenting particle

ω= angular velocity of rotation

r= distance of the migrating particles from the central axis of rotation

A more common measurement of F in terms of the earths gravitation force , g, is relative centrifugal force, RCF its defined by

$$\mathbf{RCF = (rpm)^2 (r)}$$

This equation relates RCF to revolutions per minute of the sample . Equation dictates that the RCF on a sample will vary with r, the distance of the sedimenting particles
from the axis of rotation . The RCF value is reported as "a number times gravity ,g ."

What are centrifuges ?

An apparatus that rotates at high speed and by centrifugal force separates substances of different densities

Depending upon " rotational speed" the centrifuges are classified into 3 main categories :

- Low speed centrifuges
- High speed centrifuges
- Ultracentrifuges

Laboratory tabletop centrifuge

A laboratory tabletop centrifuge. The rotating unit, called the rotor, has fixed holes drilled at an angle (to the vertical). Test tubes are placed in these slots and the motor is spun. As the centrifugal force is in the horizontal plane and the tubes are fixed at an angle, the particles have to travel only a little distance before they hit the wall and drop down to the bottom. These angle rotors are very popular in the lab for routine use.

Instrumentation for centrifugation

The basic centrifuge consists of two components one is Electric motor with drive shaft to spin the sample and a Rotor to hold tubes or other containers of the sample. A wide variety of centrifuges are available ranging from a low speed centrifuge used for routine pelleting of relatively heavy particles. There are three types of centrifuges

- **Low –speed centrifuges** .

Its also called as clinical centrifuge.

>> The common centrifuge has a maximum speed in the range of 4000 to 5000 rpm with RCF value approx. up to 3000Xg. These instruments usually operate at room temperature with no means of temperature control of the samples.

>> Two types of rotors **fixed angle** and **swinging bucket** may be used in this instruments.

Instrumentation for centrifugation

>> Low speed centrifuges are especially useful for the rapid sedimentation of coarse precipitates or red blood cells.

>> The sample is centrifuged until the particles are packed into a pellet at the bottom of the tube. The upper liquid portion, the supernatant, is then separated by decantation.

- **High Speed Centrifuges**:

>> High speed centrifuges used for more sophisticated biochemical applications.

Higher speeds and temperature control of the rotor chambers are essential.

>> Rotor chambers in most instruments are maintained at or near 4^0 C.
Three types of rotors are available for high speed centrifugation.

a. Fixed Angel rotor.
b. Swinging –bucket rotors
c. vertical rotor

>> High speed centrifuges are used to sediment
>Cell debris after cell homogenization.

High Speed centrifuges

- 15,000 – 20,000 RPM
- centrifugal field of 100,000 g
- large sample capacity depending on rotor
- normally refrigerated
- research applications.
- Differentiation separation of nucleus, mitochondrial, protein precipitate, large intact organelle, cellular debris, bulky protein aggregates.

Instrumentation for centrifugation

- **Ultracentrifuges**:

The most sophisticated of the centrifuges are the ultracentrifuges because of the high speeds attainable intense heat is generated in the rotor so the spin chamber must be refrigerated and placed under high vacuum to reduce friction.

>> it is a high speed centrifuge that has fixed head rotors .It is mainly used in separation of lipoproteins .since the separation is long process there is generation of heat and thus are provided with internal cooling system.

>> Ultracentrifuges can be used both for preparative work as well as for analytical. Preparative models its primarily used for separations and purification of samples for further analysis and analytical models which are designed for performing physical measurements on the sample during sedimentation.

Ultracentrifuges

- 65,000 RPM (100,000's x g)
- limited lifetime
- Expensive
- require special rotors
- care in use – balance critical!
- research applications
- The high speeds used in such devices generate considerable amounts of heat
- Therefore cooling arrangements and vaccuum are required in ultracentrifuges

Types of centrifugation

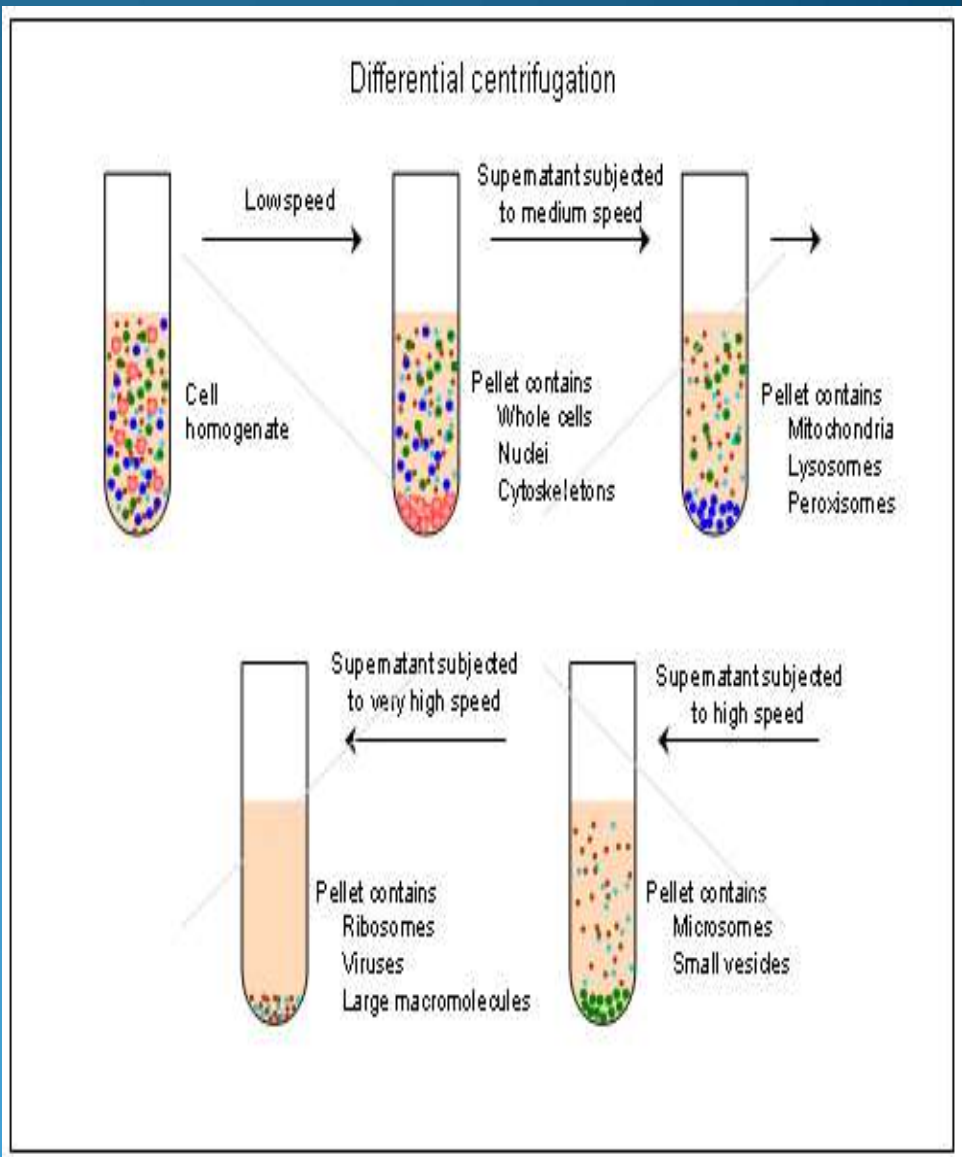
1. Differential pelleting

- Most common – separate according to size
- Material initially uniformly distributed in the solution
- During spin, particles moves with varying velocities down tube
- After spin, pellet contains larger to smaller particles (usually mixture)
- Supernatant = liquid + most slowly sedimenting component
- Pellet can be washed and respun
- If material not cleanly pelleted (smeared ; fixed angel rotor),

Then : - acceleration too rapid

Or

-sample too concentrated



2. Density – gradient centrifugation

- Separates particles on basis of relative velocities and density
- Tube filled with inert liquid of varying density preformed gradient within tube
- Sample layered onto top of liquid in tube (sample capacity greatly reduced)
- Gradient unchanged by centrifugation
- With centrifugation, faster-sedimenting particles in sample moves a head of slower ones i.e. samples separates as zones in the gradient .
- Swinging-bucket rotors preferred

Examples of gradient materials :

- Simple sugars – sucrose, sorbitol, glycerol
- Polysaccharides – ficoll , dextran, glycogen
- Proteins – bovine serum albumin
- Inorganic salts – CsCl