Laboratory Centrifuge

Laboratory centrifuge is a device for separating particles from a solution according to **their size, shape, density by subjecting high gravitational force.**

In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it float to the top. If there is no difference in density the particles stay steady.

To take advantage of 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 used to separate particles or macromolecules such as :

-Cells

-Sub-cellular components (mitochondria, ribosome, membranes)

-Proteins

-Nucleic acids (DNA, RNA).

- salts

Principles of Centrifugation

When a suspension is rotated at a certain speed or **revolutions per minute (RPM),** centrifugal force causes the particles to move away from the axis of rotation. The force on the particles compared to gravity is called **Relative Centrifugal Force (RCF)**. For example, an RCF of 500 x g indicates that the centrifugal force applied is 500 times greater than Earth gravitational force.

It is much better to use g as a unit for centrifugation steps, RPM (revolutions per minute) is not a useful unit, because the force varies with the radius of centrifuge machine (the bigger the radius, the more acceleration is applied to your samples for the same RPM).

$g = (1.118 \times 10^{-5}) R S2$

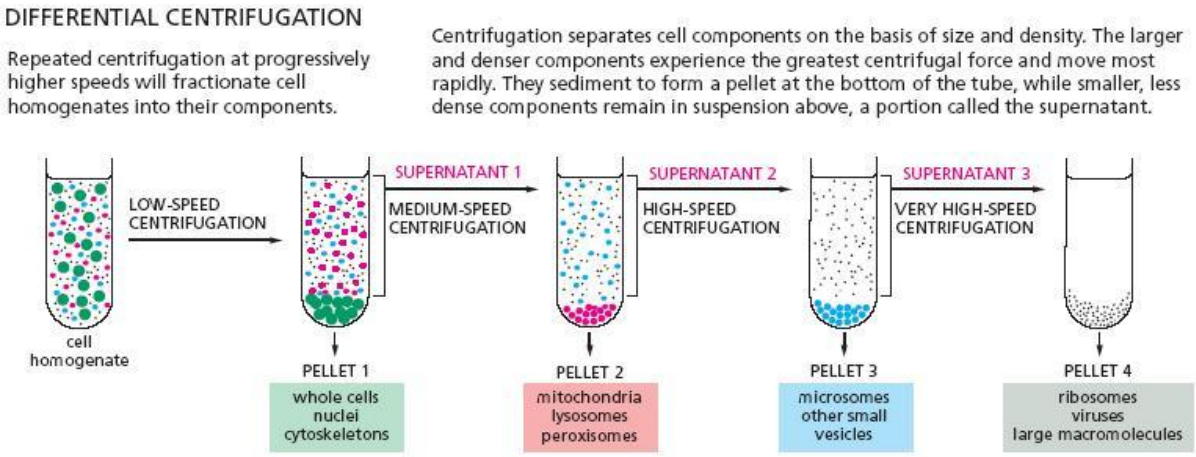
Where g: is the relative centrifugal force

R: is the radius of the rotor in centimeters

S is the speed of the centrifuge in revolutions per minute. For some centrifuges, there is a button "rpm/ rcf" for automatic conversion of the rpm-value in g.

Types of Centrifugal Separations :-

1.Differential centrifugation: Separation is based on the **size of the particles**. This type of separation is commonly used in obtaining partially-pure preparation of subcellular organelles and macromolecules. During centrifugation, a cell homogenate suspended in a uniform medium (same density), then larger particles will sediment faster than smaller ones. A cell homogenate can be centrifuged at a series of higher gravitational-forces and times to generate pellets of partially-purified organelles.



2. Density gradient centrifugation. Density gradient centrifugation is the preferred method to purify subcellular organelles and macromolecules. Density gradients can be generated by placing layer after layer of gradient media such as sucrose in a tube with the heaviest layer at the bottom and the lightest at the top. The cell fraction to be separated is placed on top of the layer and centrifuged. Density gradient separation can be classified into two categories.

A. Rate zonal (size) separation

Rate-zonal separation takes advantage of **particle size and mass** instead of particle density for sedimentation. Examples of common applications include separation of cellular organelles such as separation of **proteins**. Antibody classes all have very similar densities, but different masses. Thus, separation based on mass will separate the different classes, whereas separation based on density will not be able to separate these antibody classes.

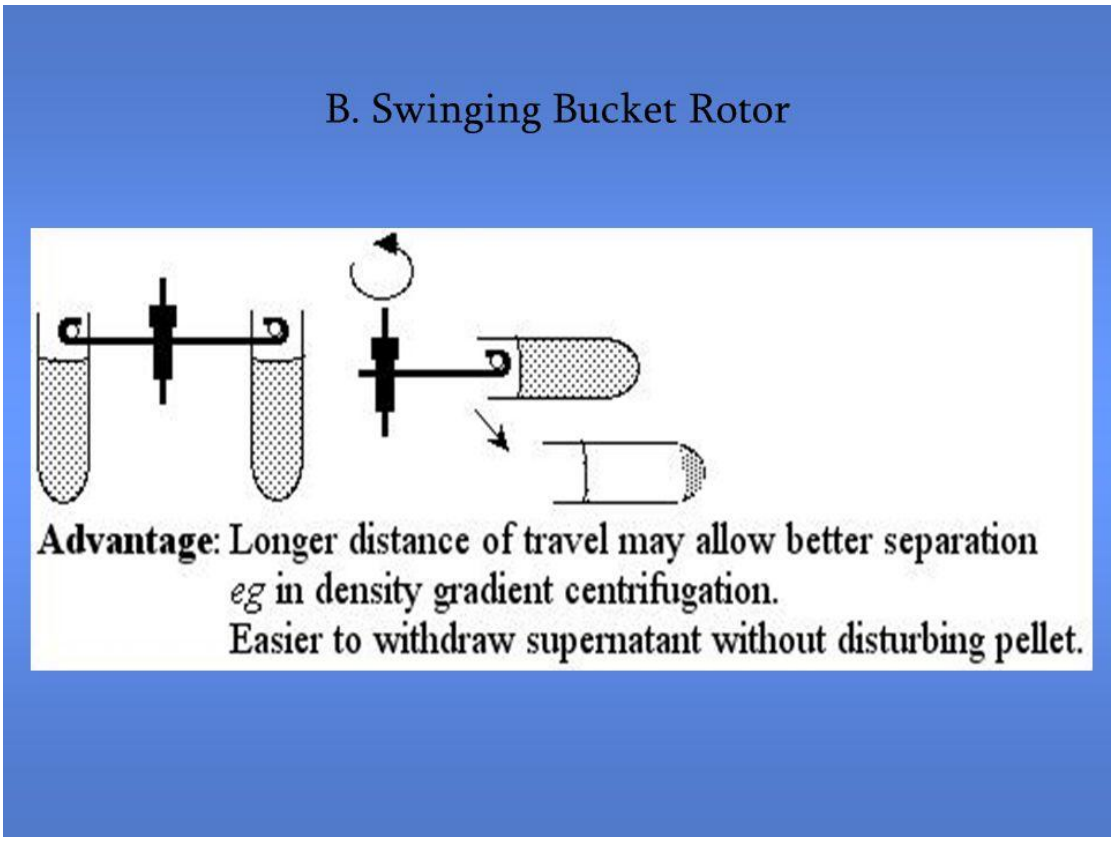
B. Isopycnic (density) separation.

In this type of separation, a particle of a particular density will sink during centrifugation until a position is reached where the density of the

surrounding solution is exactly the same as the density of the particle. Once this is reached, the time length of centrifugation does not have any influence on the movement of the particle. A common example for this method is separation of nucleic acids in a CsCl gradient.

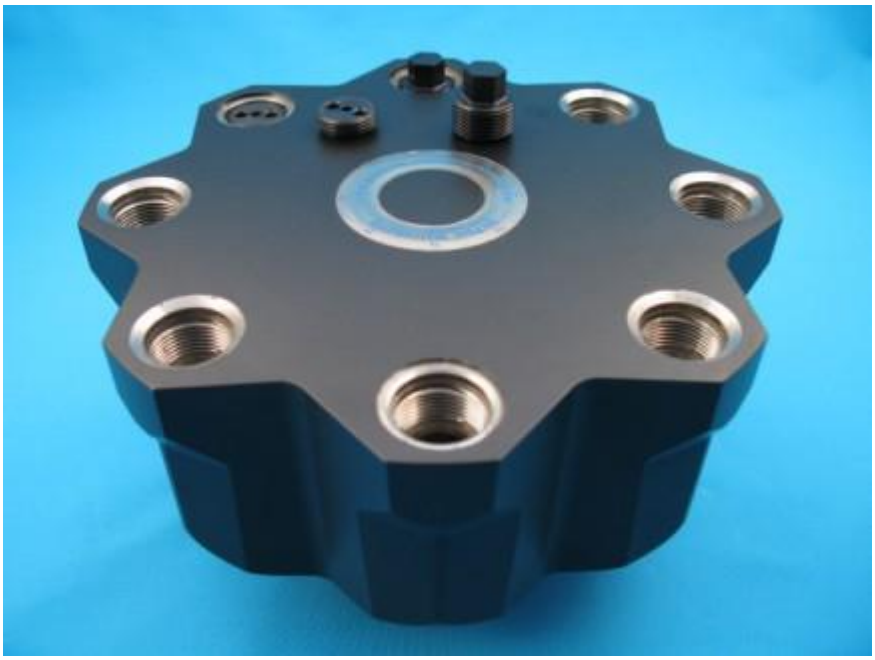
Centrifuge Models

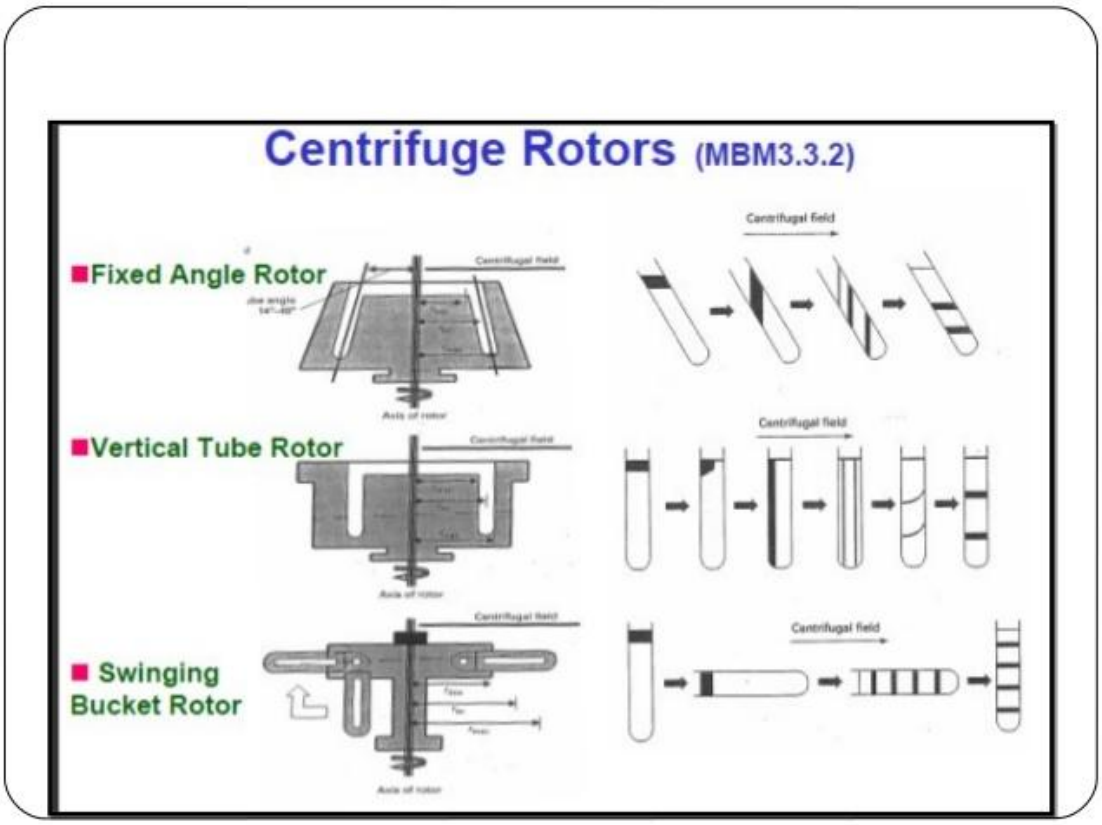
1. swinging-bucket: the sample tubes are loaded into individual buckets that hang vertically while the rotor is at rest. When the rotor begins to rotate the buckets swing out to a horizontal position . This rotor is particularly useful when samples are to be isolated in density gradients but relatively inefficient for pelleting.



2. fixed-angle: the sample tubes are held at fixed angle to the rotor cavity. This rotor type is most commonly used for pelleting. Examples include pelleting bacteria, yeast, and other mammalian cells.

3. Vertical rotor: sample tubes are held in vertical position during rotation. This type of rotor is not suitable for pelleting.




Centrifuge Rotors (MBM3.3.2)
Fixed Angle Rotor
Centrifugal field
Vertical Tube Rotor
Centrifugal field
Swinging Bucket Rotor
Centrifugal field

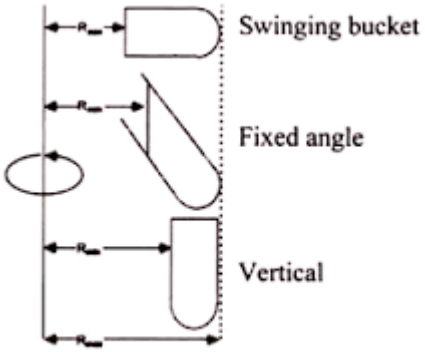


Figure 13.13: Different type of rotors