CENTRIFUGE

Centrifugation is a technique used for the separation of particles using a centrifugal field. The particles are suspended in liquid medium and placed in a centrifuge tube. The tube is then placed in a rotor and spun at a definitive speed. Rotation of the rotor about a central axis generates a centrifugal force upon the particles in the suspension.

The centrifuge involves principle of sedimentation, where the acceleration at centripetal force causes denser substances to separate out along the radial direction at the bottom of the tube. By the same concept lighter objects will tend to move to the top of the tube; in the rotating picture, move to the centre. 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. 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.

Two forces counteract the centrifugal force acting on the suspended particles:

- ✓ **Buoyant force:** This is the force with which the particles must displace the liquid media into which they sediment.
- ✓ **Frictional force:** This is the force generated by the particles as they migrate through the solution.

Particles move away from the axis of rotation in a centrifugal field only when the centrifugal force exceeds the counteracting buoyant and frictional forces resulting in sedimentation of the particles at a constant rate.

Particles which differ in density, size or shape sediment at different rates. The rate of sedimentation depends upon:

- ✓ The applied centrifugal field
- ✓ Density and radius of the particle.
- ✓ Density and viscosity of the suspending medium.

Angular velocity = ω radians / second;

since one revolution = 360^o = 2p radians,

One revolution/minute $= A = \text{rpm} = \frac{\omega \times 60}{2\pi}$

$$\omega = \frac{2\pi \times \text{rpm}}{60}$$

Therefore, Centrifugal field (CF) $= \omega^2 \times r = \frac{[2\pi \times (\text{rpm})]^2}{[60]^2} \times r$

$$= \frac{4\pi^2 \times (\text{rpm})^2}{3600} \times r$$

(r = radial distance of the particle from the axis of rotation)

As the centrifugal field acting on the particle is much greater than the Earth's gravitational field, CF is generally expressed relative to the Earth's gravitational field as multiples of g, the acceleration due to gravity (g= 980 cm/s^2)

Relative Centrifugal Field (RCF) $= \frac{\omega^2 \times r}{g}$

$$\text{RCF} = \frac{4\pi^2 \times (\text{rpm})^2}{3600 \times 980} \times r = 1.11 \times 10^{-5} \times (\text{rpm})^2 \times r$$

This expression relates relative centrifugal field (RCF) to the speed of the centrifuge (rpm) and and the radius of the rotor (r). For example, if a rotor with an average radius of 7 cm revolves at a speed of 20,000 rpm, a centrifugal field of 31,300 g is created.

The sedimentation rate of velocity (v) of a particle can be expressed in terms of its sedimentation rate per unit centrifugal field. This is termed as sedimentation coefficient (s). The sedimentation rate is proportional to w^2 r, the centrifugal field,

$$v = s\omega^2 r$$

Therefore,

$$s = \frac{v}{\omega^2 r}$$

Sedimentation velocity depends upon the mass of the particle, its density, shape and also on the density and viscosity of the medium in which the particle is suspended.

A **Svedberg** unit (**S**/**Sv**) is a non-SI unit for sedimentation rate. The sedimentation rate is the rate at which particles of a given size and shape travel to the bottom of the tube under centrifugal force. The Svedberg is technically it is measure of time, and is defined as exactly 10^{-13}seconds (100 fs). The Svedberg unit (S) offers a measure of particle size based on its rate of travel in a tube subjected to high g-force. Svedberg units are successful in classifying

ribosomes as 50S and 80S in eukaryotes. A substance with a sedimentation coefficient of 26S ($26x10^{-13}$s) will travel at 26 microns per second ($26x10^{-6}$ m/s) under the influence of an acceleration of a million gravities (10^7 m/s^2). (Svedberg unit Source: Wikipedia)

So, in summary, Centrifugation is the process of using centrifugal force to separate the lighter portion of solution, mixture or suspension from the heavier portions. In laboratory centrifuge is used to:

- ✓ Remove cellular debris from blood to separate cell free plasma or serum
- ✓ Concentrate cellular elements and other components for microscopic analysis or chemical analysis.
- ✓ Separate protein bound or antibody bound ligand from free ligand in immunological assay.
- ✓ Extract solutes from aqueous or organic solvents.
- ✓ Separate lipid components like chylomicrons from other components of plasma.

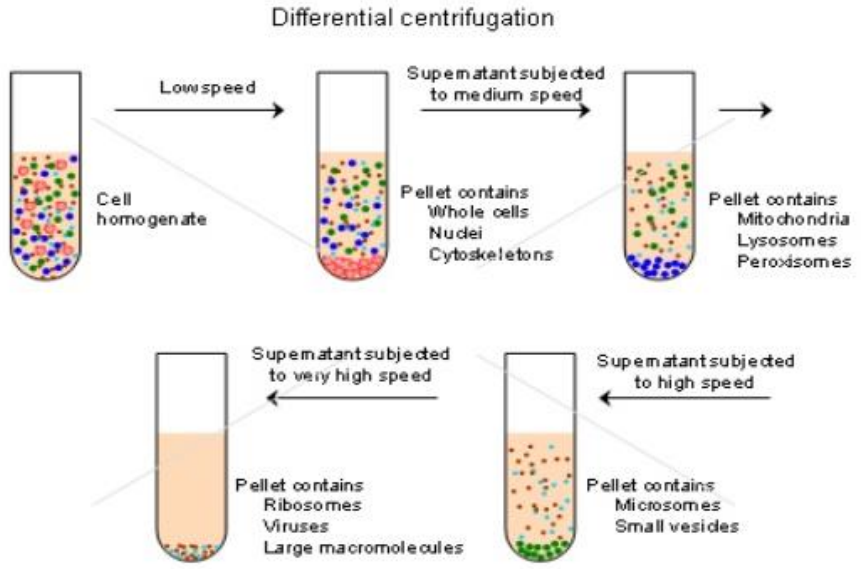
TYPES OF CENTRIFUGES AND THEIR USES:

DENSITY GRADIENT CENTRIFUGATION:

Density gradient centrifugation is used to separate macromolecules that differ only slightly in size or density. Two techniques are commonly used. In zonal centrifugation, the sample being separated (e. g., a cell extract or cells) is placed on top of the centrifugation solution as a thin layer. During centrifugation, the particles move through the solution due to their greater density. The rate of movement basically depends on their molecular mass. Centrifugation stops before the particles reach the bottom of the tube. Drilling a hole into the centrifugation tube and allowing the contents to drip out makes it possible to collect the different particles in separate fractions. During centrifugation, the solution tube is stabilized in the tube by a density gradient. This consists of solutions of carbohydrates or colloidal silica gel, the concentration of which increases from the surface of the tube to the bottom. Density gradients prevent the formation of convection currents, which would impair the separation of the particles. Isopycnic centrifugation, which takes much longer, starts with a CsCl solution in which the sample material (e. g., DNA, RNA, or viruses) is homogeneously distributed. A density gradient only forms during centrifugation, as a result of sedimentation and diffusion processes. Each particle moves to the region corresponding to its own buoyant density. Centrifugation stops once equilibrium has been reached. The samples are obtained by fractionation, and their concentration is measured using the appropriate methods.

MOVING BOUNDARY/ZONE CENTRIFUGATION:

In moving boundary (or differential centrifugation), the entire tube is filled with sample and centrifuged. Through centrifugation, one obtains a separation of two particles but any particle in the mixture may end up in the supernatant or in the pellet or it may be distributed in both fractions, depending upon it size, shape, density, and conditions of centrifugation.



The pellet is a mixture of all of the sediment components, and it is contaminated with whatever unsedimented particles were in the bottom of the tube initially. The only component which is purified is the slowest sedimenting one, but its yield is often very low. The two fractions are recovered by decanting the supernatant solution from the pellet. The supernatant can be recentrifuged at higher speed to obtain further purification, with the formation of a new pellet and supernatant.

RATE ZONAL CENTRIFUGATION:

Particles of the same size (M) but different shapes (e.g., linear versus globular) will separate the particle with the greater frictional coefficient (f) will move slower (rod shaped moves slower than globular). This technique is called velocity gradient centrifugation (a gradient of sucrose is used to linearize the motion of the particles).

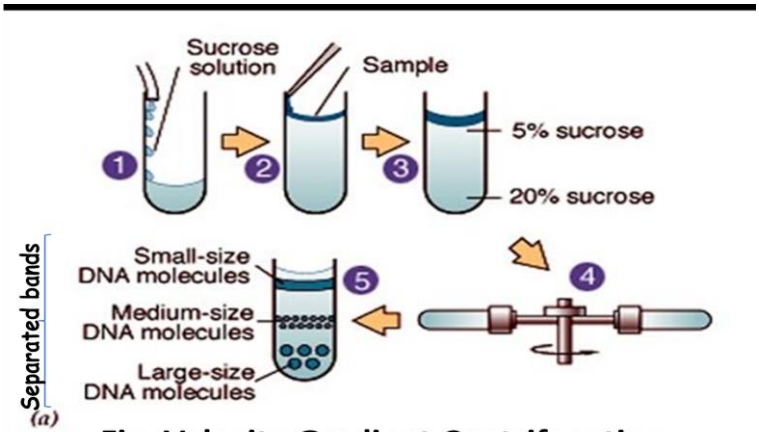
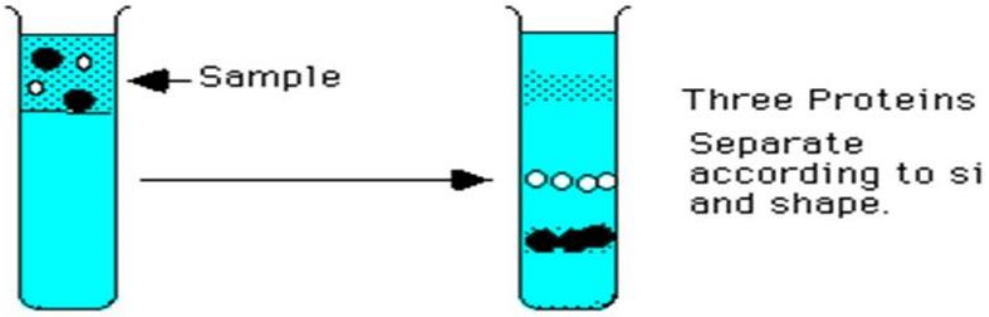


Fig: Velocity Gradient Centrifugation

In rate zonal centrifugation, the sample is applied in a thin zone at the top of the centrifuge tube on a density gradient. Under centrifugal force, the particles will begin sedimenting through the gradient in separate zones according to their size shape and density. The run must be terminated before any of the separated particles reach the bottom of the tube.

Figure 3b: Rate zonal centrifugation.



Particles can be separated by density. When the density in the solvent equals the density of the particle, the denominator of the equation equals zero and therefore velocity equals zero - the particle reaches its equilibrium density in the solvent this is called equilibrium density gradient centrifugation or isopycnic banding.

ISOPYCNIC CENTRIFUGATION:

In isopycnic technique, the density gradient column encompasses the whole range of densities of the sample particles. The sample is uniformly mixed with the gradient material. Each particle is sediment only to the position in the centrifuge tube at which the gradient density is equal to its own density, and there it will remain. The isopycnic technique, therefore, separate particles into separate zones solely on the basis of their density differences, independent of time. In many density gradient experiments, particles of both the rate zonal and the isopycnic principles may enter into the final separations. For example, the

gradient may be of such a density range that one component sediments to its density in the tube and remains there, while component sediments to the bottom of the tube. The self generating gradient technique often requires long hours of centrifugation.

Types, uses, design and precautionary measures of Centrifuge

Introduction:

A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed.

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. 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. This technique plays crucial role in biochemistry or biotechnology as it is non-dispensable part of one or the other step in every method involved in biological study right from the separation of cell organelles to complex experiments involving separation of sub-cellular fractions.

Types of Centrifuges and their Uses:

There are four major types of centrifuges. They are:

Small Bench Centrifuges:

They are used to collect small amount of material that rapidly sediment like yeast cells, erythrocytes etc. They have maximum relative centrifugal field of 3000-7000 g.

Fig: Small Bench Centrifuge

Large Capacity Refrigerated Centrifuges:

They have refrigerated rotor chamber and have capacity to change rotor chambers for varying size. They can go up to maximum of 6500 g and use to sediment or collect the substances that sediment rapidly like erythrocytes, yeast cell, nuclei and chloroplast.

High Speed Refrigerated Centrifuges:

They can generate speed of about 60000g and are used to collect micro-organism, cellular debris, larger cellular organelles and proteins precipitated by ammonium sulphate.

Ultra Centrifuges:

a) Preparative ultracentrifuge:

It can produce relative centrifugal force of about 600000g and its chamber is refrigerated, sealed and evacuated. It is employed for separation of macromolecules/ligand binding kinetic studies, separation of various lipoprotein fractions from plasma and deprotonisation of physiological fluids for amino acid analysis.

b) Analytical ultracentrifuge:

It is capable of operating at 500000 g. Three kinds of optical systems are available in analytical ultracentrifuges: a light absorption system, and the alternative Schlieren system and Rayleigh interferometric system, both of which detect changes in the refractive index of the solution.

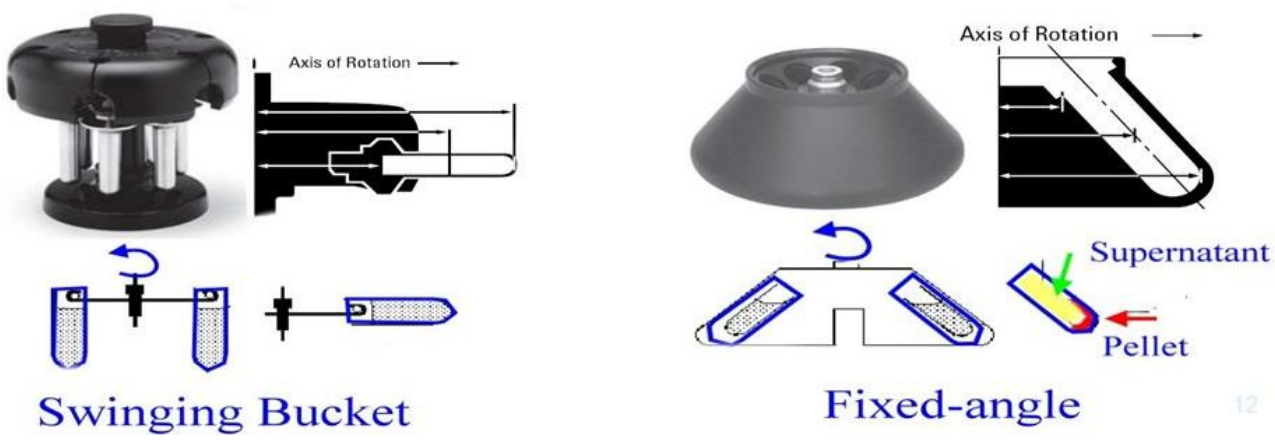
Design and Types of Rotors:

Swinging Bucket Rotors:

The swinging bucket rotor has buckets that start off in a vertical position but during acceleration of the rotor swing out to a horizontal position so that during centrifugation the tube and hence the solution in the tube, is aligned perpendicular to the axis of rotation and parallel to the applied centrifugal field, the tube returning to its original position during deceleration of the rotor.

Fixed Angle Rotors:

In fixed angles the tubes are located in holes in the rotor body set at a fixed angle between 14° and 40° to the vertical. Under the influence of centrifugal field, particles move radially outward and have only a short distance to travel before colliding with, and precipitating on, the outer wall of the centrifuge tube. A region of high concentration is formed that has a density greater than surrounding medium, with the result that the precipitate sinks and collects as a small compact pellet at the outermost point of the tube.



Vertical Tube Rotors:

They are considered as zero angles fixed angle rotors in which the tubes are aligned vertically in the body of the rotors at all times.

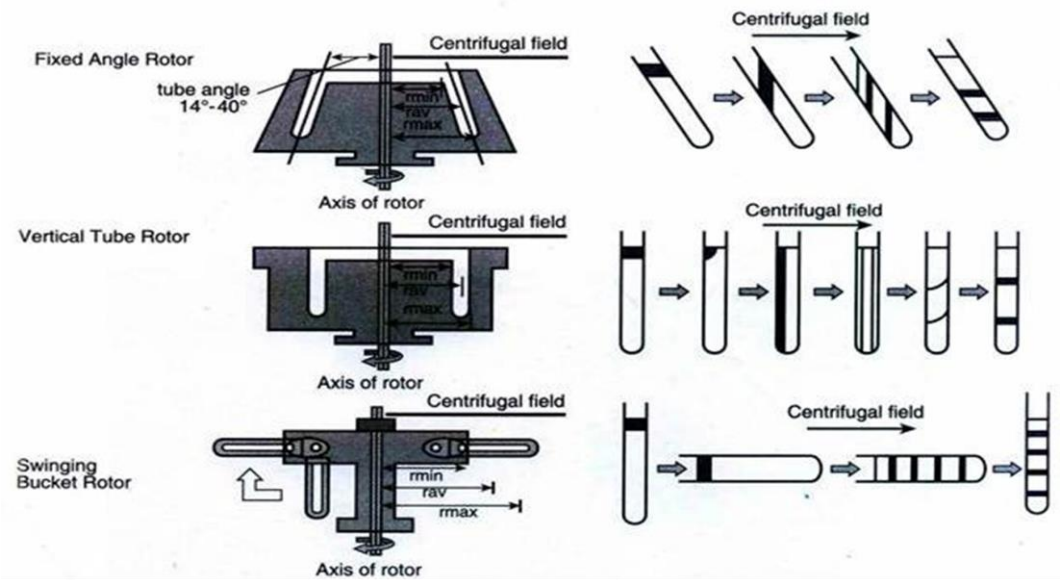


Fig: Types of centrifugation rotors

Zonal Rotors:

The zonal rotors may be of the batch or continuous flow type. The former being more extensively used than the latter, and are designed to minimize the wall effect that is encountered in swinging- bucket and fixed angle rotors, and to increase sample size.

Elutriator Rotors:

The elutriator is a kind of continuous flow rotor that contains recesses to hold a single conical shaped separation chamber, the apex of which points away from the axis of rotation, and a bypass chamber on the opposite side of the rotor that serves as a counter balance and to provide the fluid outlet.

Care of Rotors:

The protective anodized coating on aluminium rotor is very thin and does not provide a high degree of protection against corrosion; thus rotors should always be handled with care to prevent scratching. Rotors should always be thoroughly washed preferably with de-ionised water and since moisture is a potential source of corrosion, they should be allowed to dry upside down in a warm atmosphere; they should then be stored in a clean, dry environment. Rotors' outer surface only can be given a protective coat of lanolin or silicone polish.

Swinging-bucket rotors, however, should never be completely immersed in water as the bucket hanging system is difficult to dry. Titanium rotors are essentially resistant to corrosion. To prevent possible damage to the drive shaft of the centrifuge due to vibration caused by rotor imbalance, sample loads should be balanced within the limits specified by the manufacturer. Swinging-bucket rotors should not be run with any bucket or caps removed or individual rotor buckets interchanged as they form integral part of the balance of the rotor.

During acceleration and deceleration of the rotor, cyclic stretching and relaxing of metal can cause metal fatigue, leading to eventual failure of rotor. To avoid overstressing the rotor and to ensure its continued safe operation, an accurate record should be kept of its total usage, i.e, number of runs (at any speed) and time of each run so that rotor can either be de-rated after a certain number of runs or replaced after a set period of time as specified by manufacturer.

Sample Containers:

Centrifuge tubes and bottles are available in different range of sizes, thickness and rigidity from different variety of materials including glass, cellulose, esters, polyallomer, polycarbonate, polyethylene, polypropylene, kynar, nylon and stainless steel. The type of container used will depend upon nature and volume of sample to be centrifuged along with centrifugal forces to be withstood.

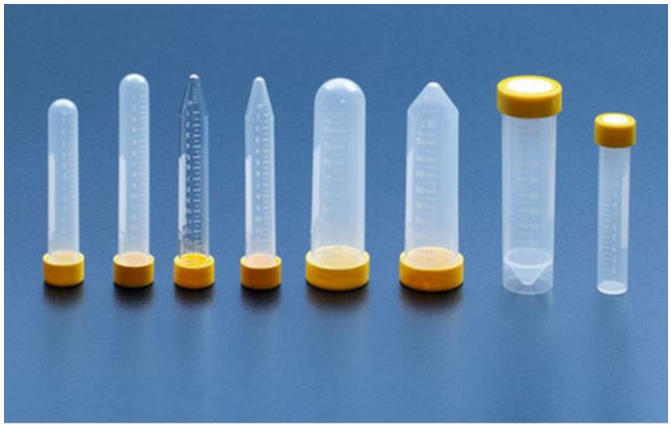


Fig: Different types of centrifugation tubes

Glass centrifuge tubes are suitable only for centrifugation at low speeds as they disintegrate at higher centrifugal fields. Thin walled tubes may be used in swinging bucket rotors because the tube is protected by the surrounding bucket; however, thick walled tubes are required with fixed angle and vertical tube rotors. The centrifuge tubes should be filled to accurate level and need to cap the tube or bottle depends upon the speed and type of the container used.

Table: Tube type and rotor compatibility

Rotor type			
Tube type	**Fixed angle**	**Swinging-bucket**	**Vertical**
Thin-walled open top	No	Yes	------
Thick-walled open top	Yes	Yes	No
Thick-walled sealed	Yes	Some tube types	Yes
Oak ridge	Yes	No	No

Precautionary Measures with Centrifuges:

Centrifuges are extremely dangerous instruments if not properly maintained and correctly used. It is, therefore, always advisable that one must read and understand the operating manual for particular centrifuge.

Manufacturers of centrifuges should ensure effective lid locks. Access to the rotor chamber of centrifuges should always be avoided when spin is in progress. Centrifuges should have imbalance detectors, over speed detectors and devices and ability to contain any failure of rotor.

To prevent possible physical injury when rotors are filled and emptied, care must be taken to ensure that the moving rotor is not touched and that long hair and loose clothing (e.g., Ties) do not get caught in any rotating part. This is especially important with older centrifuges where lid can be opened before the rotor has stopped rotating.

It is important when is centrifuging hazardous materials like pathogenic microorganisms, infectious viruses, carcinogenic, corrosive or toxic chemicals, radioactive materials), especially in low speed non-refrigerated centrifuges in which rotor temperature is controlled by air-flow through the rotor bowl , samples should be kept in air-tight, leak-proof containers. This is to prevent aerosol formations arising from accidental spillage of sample which would contaminate the rotor, centrifuge and possibly the whole laboratory.