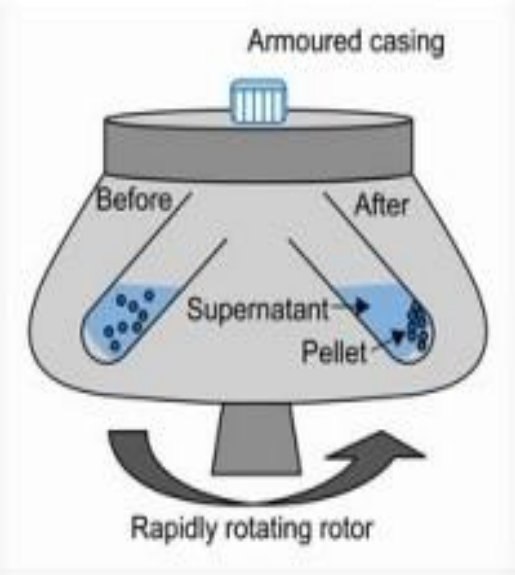
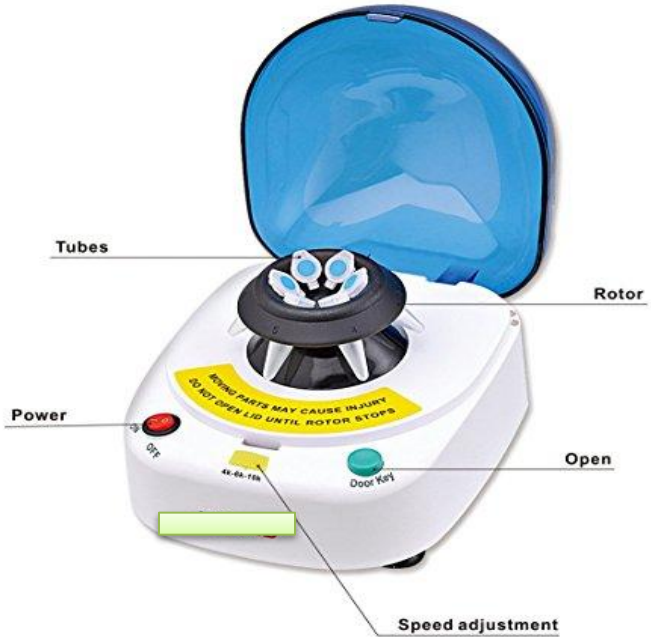
CENTRIFUGATION

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 the particles stay steady.

Description: the centrifuge has a stable body made of steel or aluminum. It consist of an electrical motor to which is attached a head, accessories for carrying samples, the head turns on a spindle which is an extraction of the motor shaft. There is a safety shield around the rotating head; the safety is maintained by the micro-switch which cut the current when the cover is not closed





1.1 Centrifuge Components

A centrifuge comprises of
• Electric motor
• Drive shaft
• Rotor to hold tubes

Sedimentation of suspended and some dissolved particles occurs due to centrifugal force.

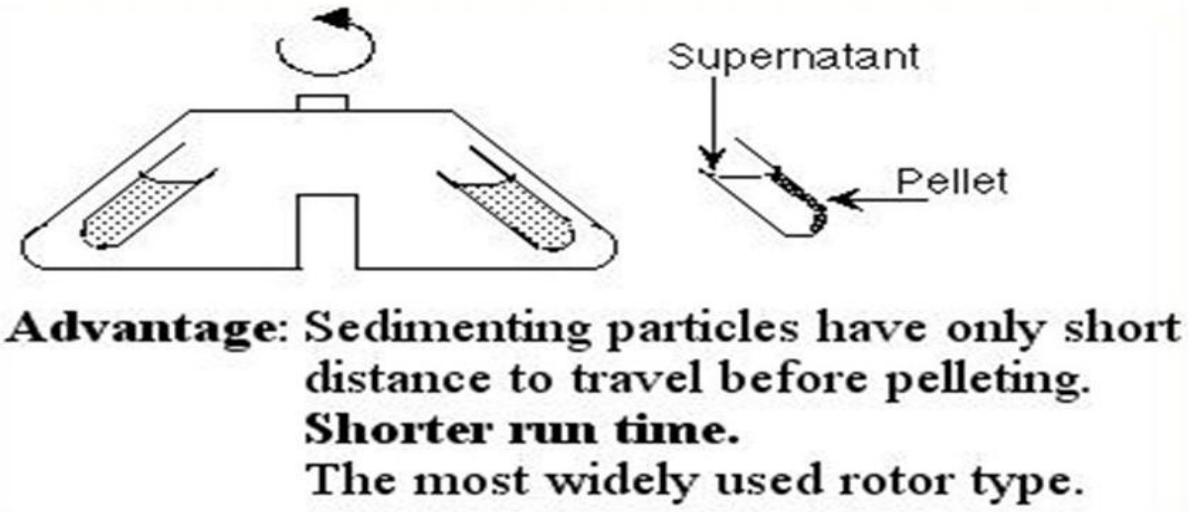
Two principal uses

1. Separate out solid matter as a PELLET from dissolved solutes as SUPERNATANT
2. Separate soluble macromolecules of different mass or density

Also sometimes used to provide centrifugal force to drive other processes, eg. ultrafiltration.

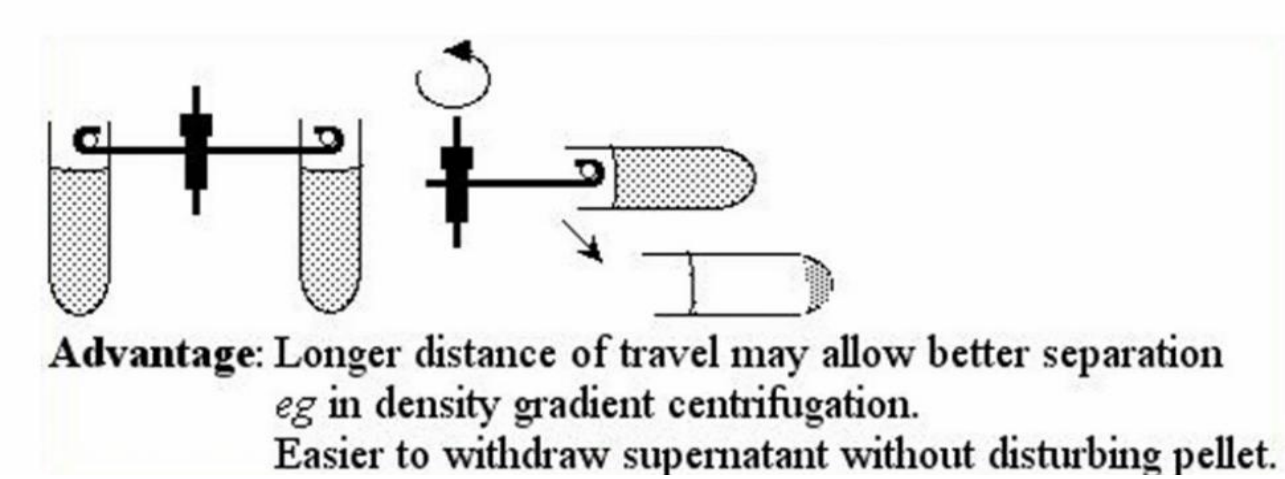
1.2 Centrifuge Rotors

A. Fixed Angle Rotor



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

B. Swinging Bucket Rotor



Advantage: Longer distance of travel may allow better separation
eg in density gradient centrifugation.
Easier to withdraw supernatant without disturbing pellet.

1.3 Principles of Centrifugation

- **Sedimenting force** on particle = Mass x centrifugal field

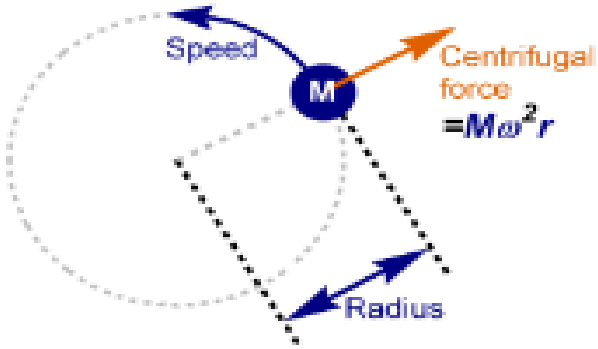
$$= Mw^2 r$$

Where

M= mass

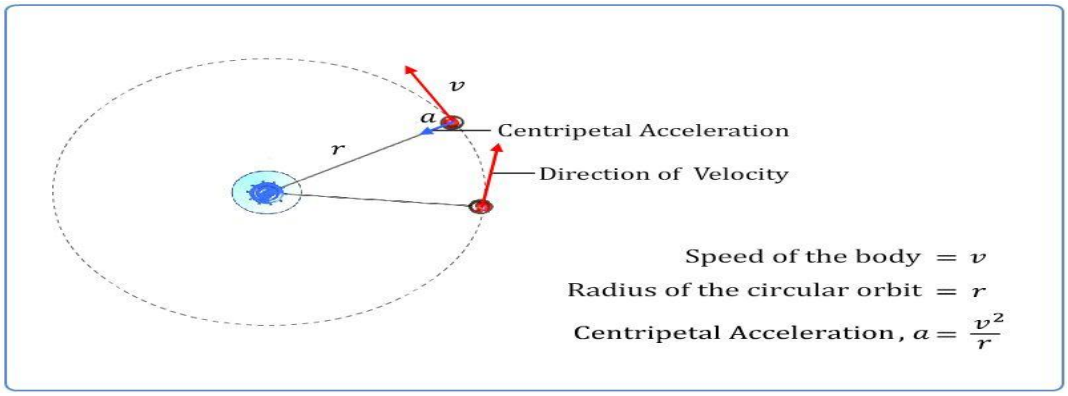
w = angular velocity of rotor (radians/sec)

r = radius (ie distance of particle from axis of rotation)



- **Relative Centrifugal Force (RCF)** = $1.119 \times 10^{-5} \times (rpm)^2 \times r$

The radial force generated by the spinning rotor is expressed relative to the earth's gravitational force (a) and therefore is known as the relative centrifugal force (RCF) or the "a force." The (a) force acting on particles is exponential to the speed of rotation (defined as revolutions per minute; rpm).



1.3.1 Interacting Forces in Centrifugation

Sedimenting force, mw^2r, is opposed by...

1- **Flotation Force** (Archimedes) = mw^2rvp

Where:

v = partial specific volume (volume displaced by l g of sedimenting particles)

ρ = density of solution

Net sedimenting force on particle, after allowing for flotation **= mw^2r (1- vp)**

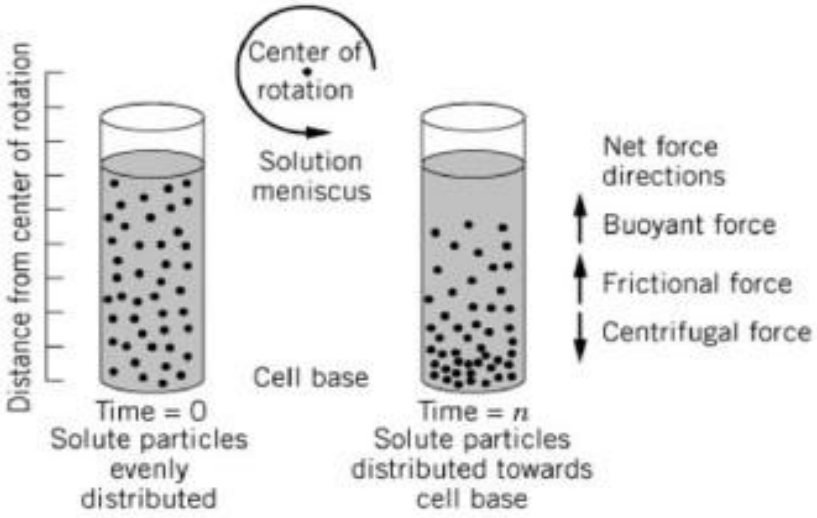
2- **Frictional Resistance**

Against particle moving through fluid. = f.v

Where:

f = frictional coefficient

v = particle velocity



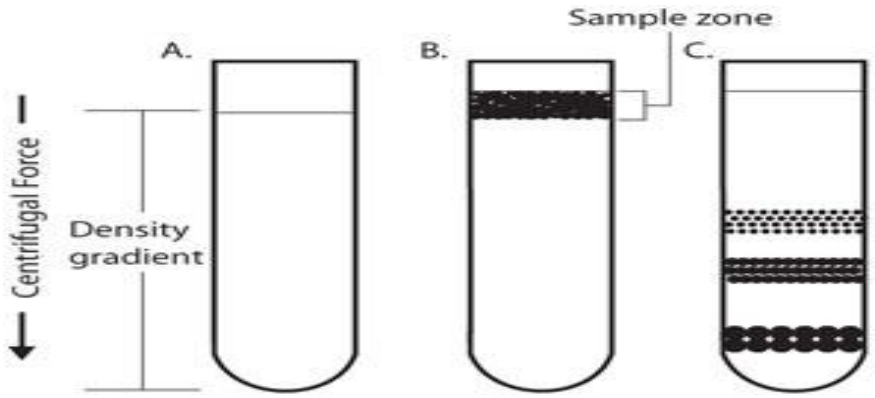
3- **Diffusion** - acting to counter uneven concentration distributions set up when dissolved molecules sediment.

BALANCE between the sedimenting force and counteracting forces leads to various formulae and equations used in

- **preparative centrifugation** to calculate the time required to sediment a particle to the bottom of the tube and in
- **Analytical ultracentrifugation** techniques used to determine sedimentation coefficients and molecular masses of dissolved macromolecules.

1.4 Density Gradient Centrifugation

In absence of a density gradient, separated bands of solute in the centrifuge are gravitationally unstable.



CAN'T OCCUR because layer of concentrated, dense solution overlaying less dense solvent would lead to mixing by convection and nullify the separation.

In absence of stabilizing density gradient, can form boundaries but not zones. **In analytical ultracentrifuge**, moving boundaries and concentration distributions observed by optical device.

Create DENSITY GRADIENT in tube

Use a non-interacting, low M. Wt solute in continuously increasing concentration from meniscus to bottom of tube.

Important technique for purifying **proteins** and **particularly nucleic acids**.

Two different types of density gradient centrifugation, for two different purposes are:

- **Zonal (or Rate Zonal) Centrifugation** (Sucrose density gradient centrifugation)
- **Isopycnic Centrifugation** (Cesium chloride density gradient centrifugation)

1.4.1 Zonal Centrifugation

Mixture to be separated is layered on top of a SUCROSE, or FICOLL, GRADIENT (increasing concentration down the tube)

- provides gravitational stability as different species move down tube at different rates forming separate bands.

Species are separated by differences in **sedimentation coefficient (s)**

$$= \frac{\text{Rate of movement down tube}}{\text{Centrifugal force S}}$$

Where

S is increased for particle of larger mass (because sedimenting force α M (1-vρ)

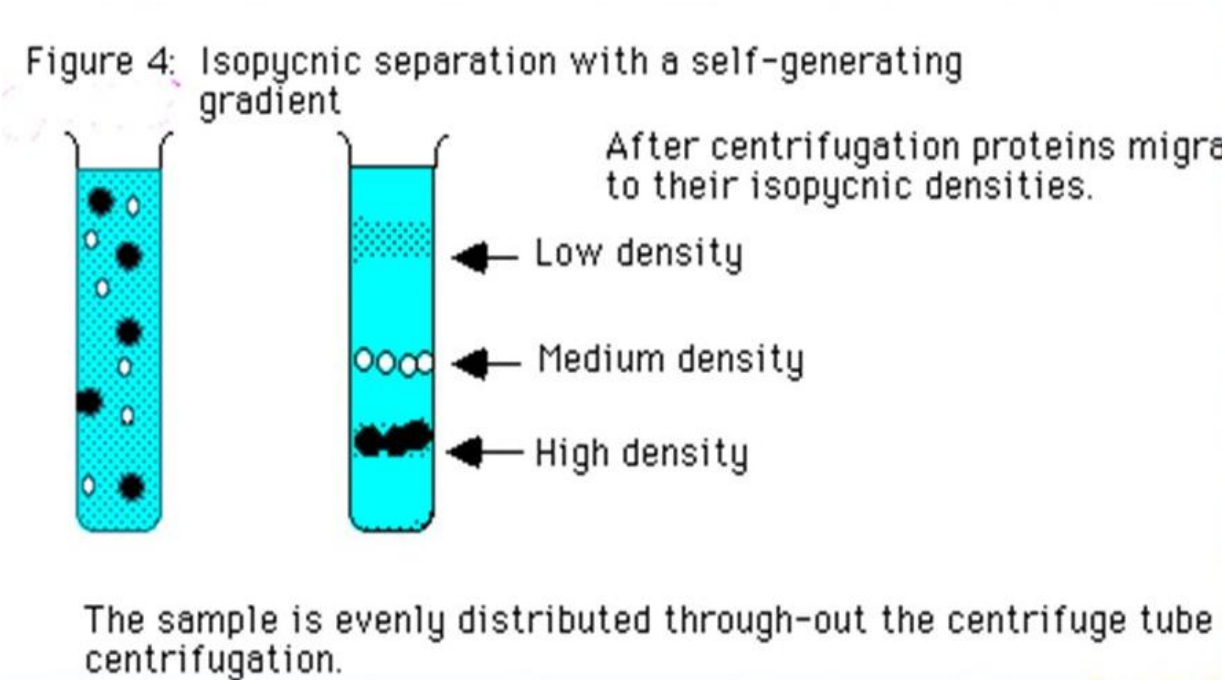
S is also increased **for more compact structures of equal** particle mass (frictional coefficient is less)

1.4.2 Isopyncic Centrifugation

Molecules separated on equilibrium position, not by rates of sedimentation. Each molecule floats or sinks to position where density equals density of CsC1 solution. Then no net sedimenting force on molecules.

Isopycnic = Equal density

And separation is on basis of different densities of the particles.



Very useful for purifying nucleic acid species of different density; also in separating proteoglycans extracted from cartilage.

Density gradients are used in many different operations:

- To separate particles of different densities (isopycnography, which is short for "equilibrium density gradient centrifugation)
- To separate particles of different sizes (sedimentation centrifugation)
- Column elution's that must smoothly go from one concentration to another

- Isolation of diamond dust (isopycnography)
- Isolation of bovine X-sperm from Y-sperm (dairy industry) (sedimentation without centrifugation)

There are several ways for making density gradients including those that use syringes, twin linked containers, and other devices. Here are two very simple additional ways to make linear density gradients:

1. This might be simple but it is one that takes many hours: fill a plastic centrifuge tube with - say - a 10% sucrose solution. Put it in the freezer. The first part that freezes will be almost pure water at the top, with only a little sucrose trapped in it, but as the overlying ice layer gets thicker, more and more sucrose is trapped within it. Thus when it is completely frozen, the top has little sucrose and the bottom has much. Upon subsequent thawing, the bottom melts first, and the melting proceeds upwards reinforcing the preparation of the gradient.

2. Here is one that takes about 60 seconds of time from start to finish!

The centrifuge works if a structure is denser or has a greater mass than its surroundings, because that causes it to move downward in the tube. If it has a lesser mass or is less dense, the structure separates to the top of the test tube. There are several speeds of centrifugation, and each have a specific purpose:

• Low-speed: large, dense bodies or leftover debris from the preparation of a substance

• Higher speed: intermediate sized objects such as chloroplasts and mitochondria

• Very high speed: microtubules, microfilaments, ribosomes, etc.

For extremely precise centrifugation, two different methods are used:

• Density gradient centrifugation

• Buoyant density centrifugation