Centrifugation

The centrifuge **uses centrifugal force** (**the force generated when an object rotates around a single point**), **for** separating solids suspended in a liquid by **sedimentation** or liquids of diverse density. The rotational movements allow **forces** much greater than gravity to be generated in controlled periods of time. Centrifuges are generally used in the laboratory in processes such as the separation of solid components from biological liquids through **sedimentation** and in **particular of blood components**: **red cells, white cells, platelets among others** and for conducting multiple tests and treatments. **So, 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.

Figure 1: shows centrifuge

1. Parts of a Centrifuge

Centrifuge consist of the following parts as shown in figure 2:

1. on and off control, operation time control (timer), rotation speed control (in some centrifuges), temperature control (in refrigerated centrifuges), vibration control (safety mechanism) and brake system.

2. Refrigeration system (in refrigerated centrifuges).

3. Vacuum system (in ultracentrifuges, not shown in the Figure 2).

4. Base.

5. Cover.

6. Casing.

7. Electric motor.

8. Rotor to hold tubes**. There are four types of rotor are:-**

- **<u>Fixed angle</u>.**
- **<u>Swinging buckets</u>.**
- **<u>Vertical tube.</u>**
- **<u>Almost vertical tube.</u>**

9. Drive shaft

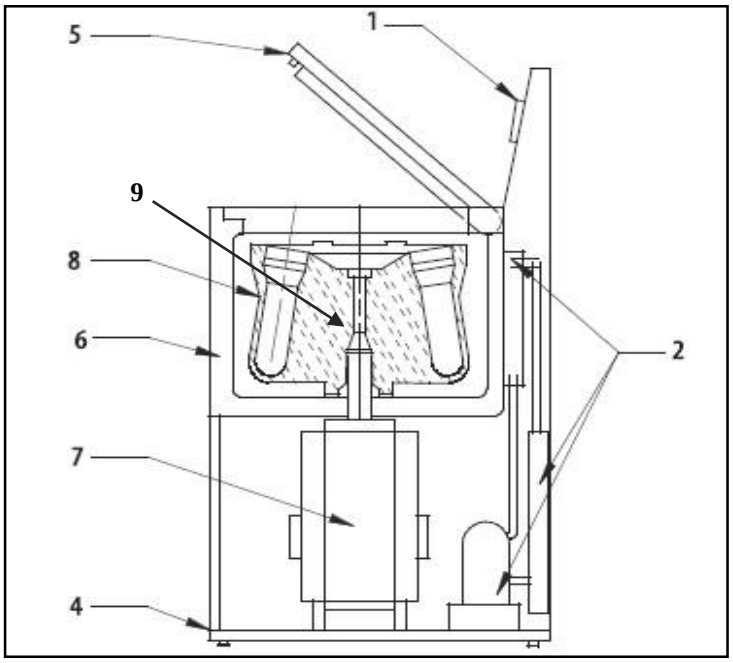
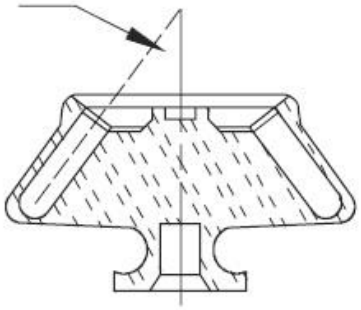
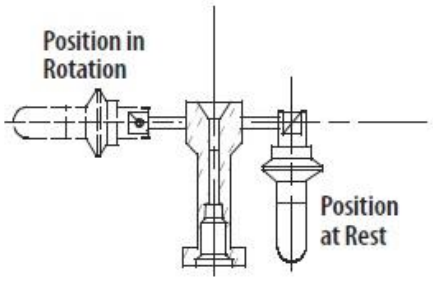
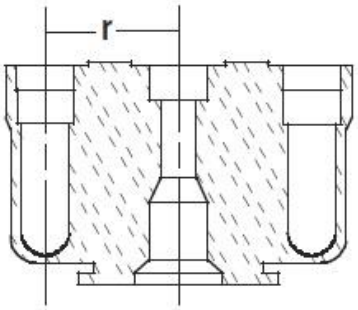
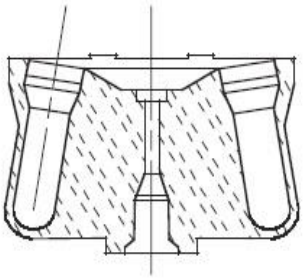


Figure 2: shows centrifuge components

2. Types of Rotor

Type of rotor	Characteristic	Cross- section
Fixed angle	Sedimenting particles have only short distance to travel before pelleting. Shorter run time. The most widely used rotor type	
Swinging buckets	Longer distance of travel may allow better separation in density gradient centrifugation. Easier to withdraw supernatant without disturbing pellet.	
Vertical tube	Keeps tubes parallel to the rotational axis. Thus, separate bands are formed across the tube's diameter, not its length	
Almost vertical tube	For gradient centrifugation when some sample components do not participate in the gradient.	

3. Operation Principle

Centrifuges **represent** a practical application of **Newton's law of motion**. When a body of mass [m] turns around a central point [O], it is subjected to a centripetal force [N] directed towards the rotation axis with a magnitude $\mathbf{N = m\omega^2 R}$, where:-

m: - is the mass of the body.

R: - is the radius.

ω :- is Angular speed it's The turning rate of a body measured in radians per second. It is calculated using the following formula:

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

Where:

rpm = revolutions per minute.

π= constant with a value of 3.1416.

Centrifuges possess a rotating axis on which is mounted a rotor with sample receiving compartments. **Tangential speed** is defined by the following equation:

VT=ωR.

When the system spins at a speed of ω radians per second, the samples are subjected to the centrifugal force **Fp** of the same magnitude as **N**, but in an opposite direction.

Figure 3 shows features a diagram of the concept, of its actual application and of the obtained result. This Fp force acts on particles in the substance centrifuged, causing them to separate as a result of diff erences in density. Denser particles will settle at the bottom of the tube in shorter periods of time, while lighter ones require longer periods of time, settling onto those of greater density.

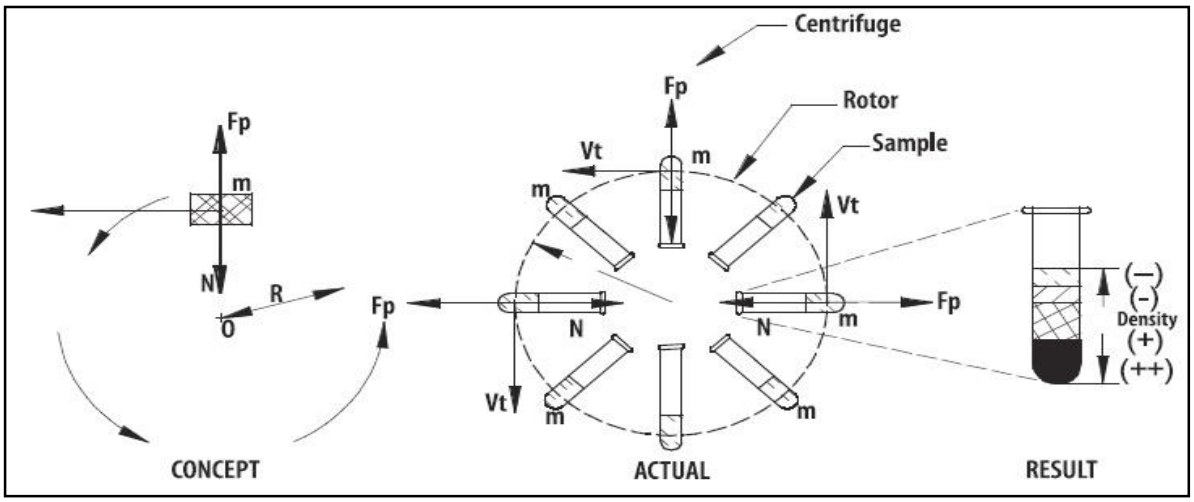


Figure 3: shows centrifugal force concept

The relationship between the **centrifugal acceleration** [$\mathbf{r\omega^2}$] to a given radius [r] and **the force of gravity** [**g**] is known as the **relative centrifugal field** or [**RCF**].

Where:-

$$\mathbf{RCF} = \frac{\mathbf{r\omega^2}}{\mathbf{g}}$$

Where:-

r: radius in mm.

ω: Angular speed in radians per second.

g: Standard gravity acceleration = 9.807 mm/s^2.

The **RCF** is the tool which **allows rotors of different specifications to be compared** when equivalent centrifugal effects are required.

4. 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)**

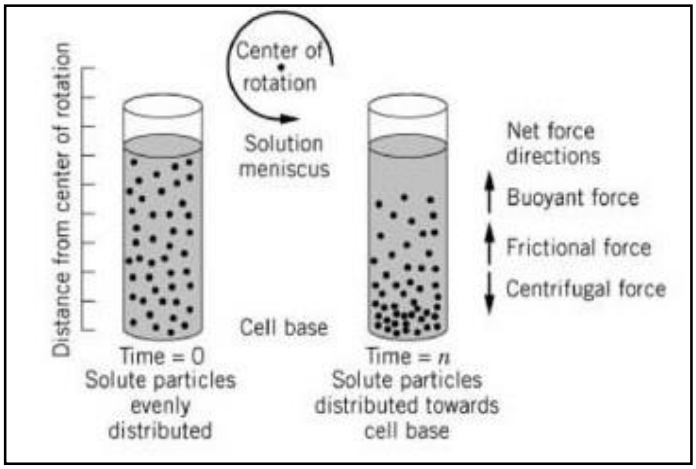
2. **Frictional Resistance**

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.

  Density Gradient Centrifugation

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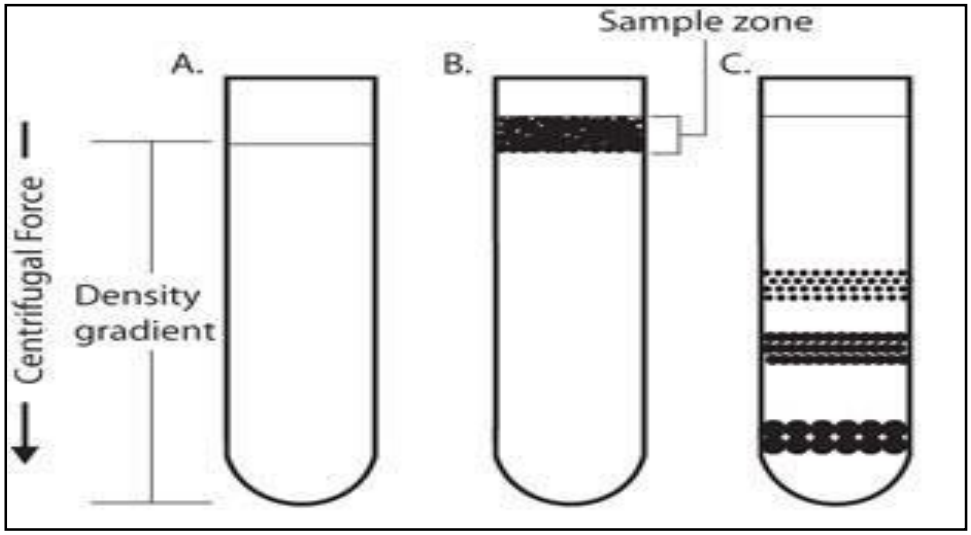
5. Density Gradient Centrifugation

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.



Great 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 Centrifugation or (Rate zonal centrifugation)** (Sucrose density gradient centrifugation).
- **Isopycnic Centrifugation** (Caesium chloride density gradient centrifugation).

5.1 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 **αM(1-vp)**

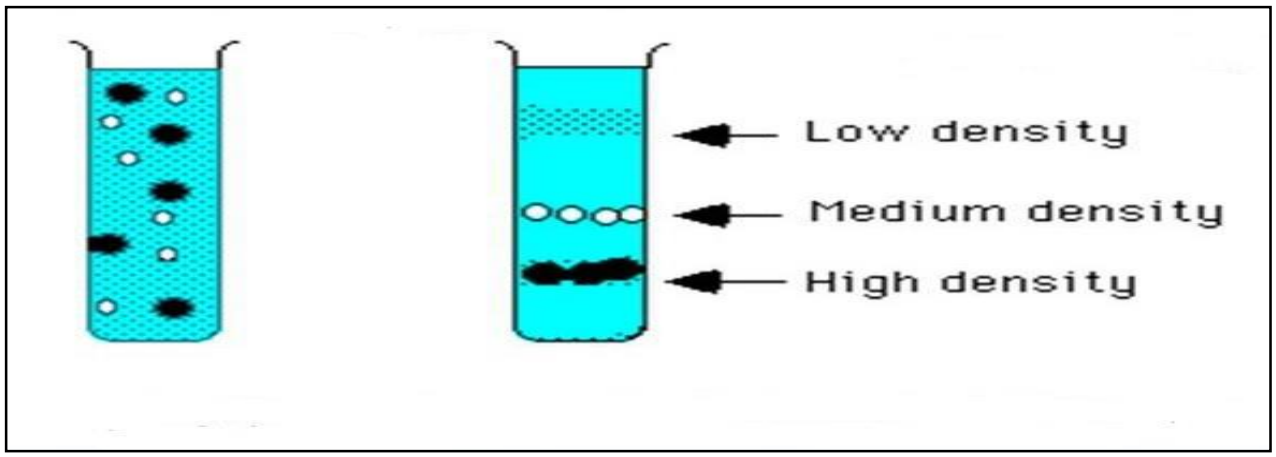
S: is also increased for more compact structures of equal particle mass (**frictional coefficient is less**).

5.2 Isopycnic 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