Analytical

ULTRACENTRIFUGATION

Centrifugation

- Centrifugation is a process which involves the use of the centrifugal force for the sedimentation of heterogeneous mixtures with a centrifuge.
- Applied in industrial chemistry, biochemistry, cellular and molecular biology, environmental technology.
- Used for separation procedures such as precipitation of macromolecules, isolation of cell and subcellular organelles and concentration of sludge.

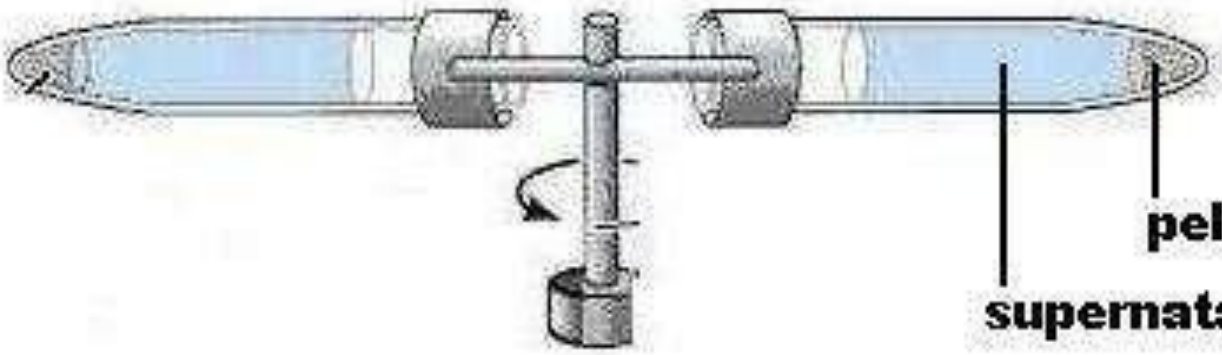
Pellet: precipitated form of particles

Supernatant: remaining solution or medium

Principle of Centrifugation

- Sedimentation of solute particles makes heavier particles sink and lighter particles to float in the solvent or medium.
- The soltution is subjected to high angular velocity measured in rpm, the effect of gravity and thereby the rate of sedimentation greatly increases due to application of an external force called centrifugal force.

A centrifuge spins the suspensions around a central axis, producing centrifugal force, which causes the suspended particles to collect as a pellet in the tube bottom.



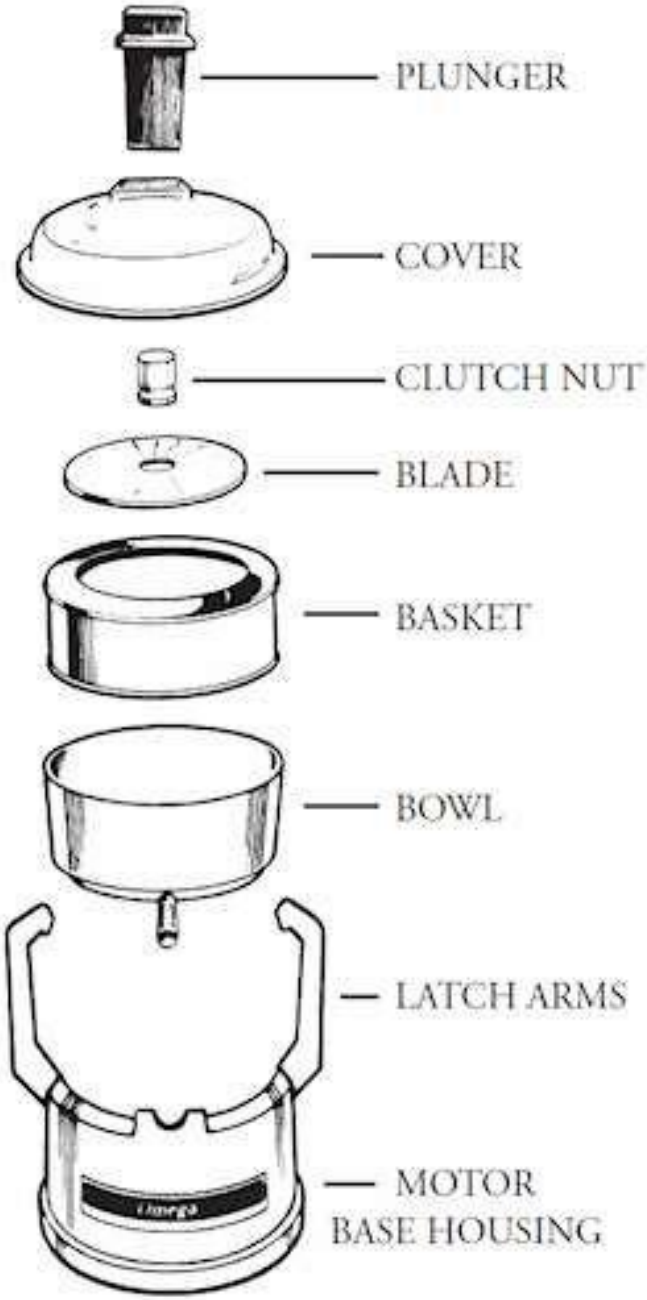
fluid containing suspended particulate matter

Centrifuge Machines

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

Parts of a Centrifuge

- Core part is a rotor
- Fixed no. or arms radiating from it a certain angle
- Hollow tubes attached for sample placement
- Ball bearings
- Aluminium/Titanium graded drum
- Motor and brake assembly
- Controlling nobs



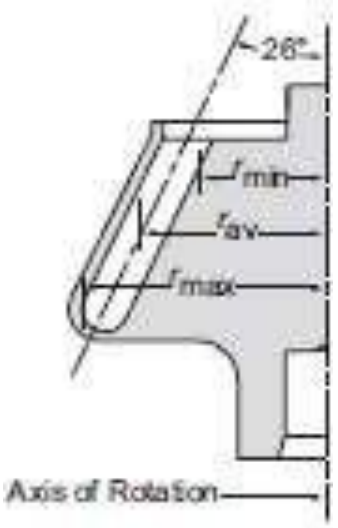
Rotor

- Five types of rotors are available for centrifugation:
  1. Fixed-angle rotor,
  2. Swinging-bucket rotor,
  3. Vertical rotor and
  4. Near-vertical rotor.
  5. Continuous-flow rotor

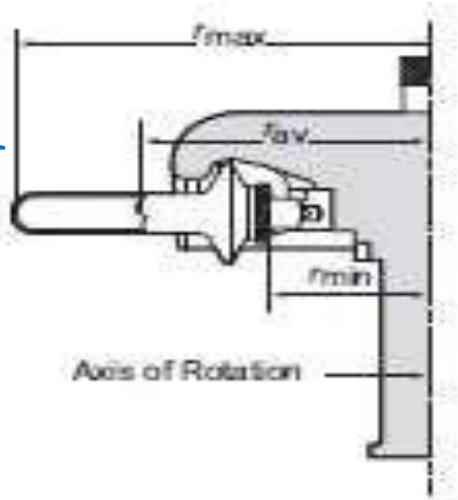
1. FIXED ANGLE ROTOR

- Fixed-angle rotors are general-purpose rotors that are especially useful for pelleting subcellular particles and in short column banding of viruses and subcellular organelles.

- Tubes are held at an angle (usually 20 to 45 degrees) to the axis of rotation in numbered tube cavities.

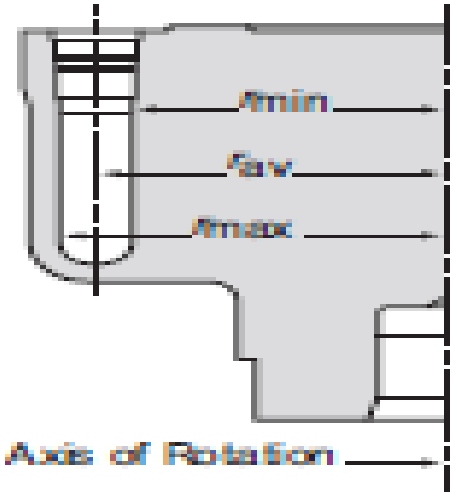


2SWINGINGBUCKETROTOR



- Swinging-bucket rotor are used for pelleting, isopycnic studies and rate zonal studies.
- Tubes are attached to the rotor body by hinge pins or a crossbar. The buckets swing out to a horizontal position.

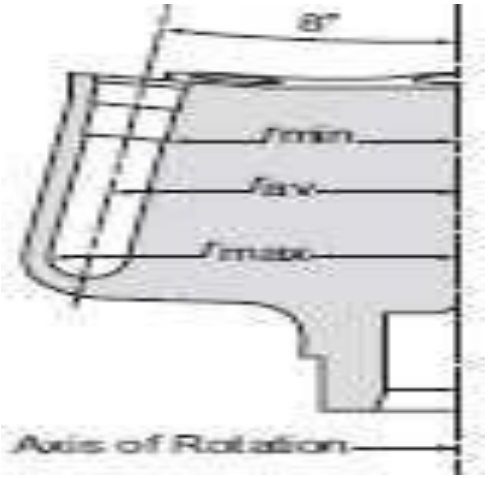
3.VERTICAL ROTOR



- Vertical rotors hold tubes parallel to the axis of rotation; therefore, bands separate across the diameter of the tube rather than down the length of the tube.

- Vertical rotors are useful for isopycnic and, in some cases, rate zonal separations when run time reduction is important.
- Not suitable for pelleting

4.NEAR VERTICAL ROTOR



- Near-vertical rotors are designed for gradient centrifugation when there are components in a sample mixture that do not participate in the gradient.
- Tubes are held at an angle (typically 7 to 10 degrees) to the axis of rotation in numbered tube cavities.
- Used in isolation of molecules like plasmid DNA, RNA, Lipoprotein.

5. Continuous Flow Rotor

- Used industrially and for few lab separations like recovery of bacteria from litres of culture solution.
- The sample is ejected through the centre and separated at a specific speed
- Sample is extracted by injecting high density liquid from outside wall while the rotor is running

Centrifugation

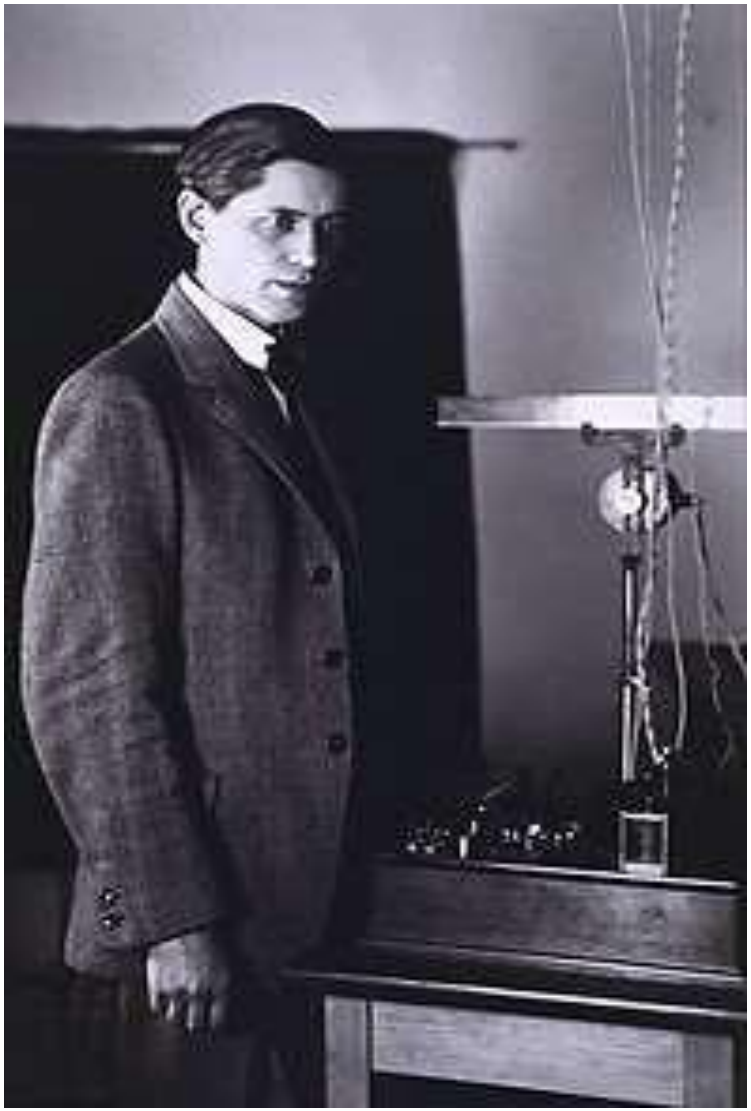
- Preparative centrifugation
  - Differential centrifugation
  - Density gradient centrifugation
    - Rate zonal centrifugation
    - Isopycnic centrifugation
- Analytical ultracentrifugation

Analytical Ultracentrifugation (AUC)

- Which through rapid spinning imposes high centrifugal forces on suspended particles, or even molecules in solution, and causes separations of such matter on the basis of differences in weight.
- Can run at about 120,000-150,000 rpm
- RCF as high as 625,000 X g
- Rotor chamber are evacuated to reduce friction
- Volume of single tube accomodated-0.2-5.5 ml
- Separate particles as less as 10 microns
- Equipped with self-balance system and micro-processor control

AUC Centrifuge

HISTORY



- Swedish Biochemist **Theodore Svedberg** invented the Ultracentrifuge in 1923.

- And he won the Novel Prize in chemistry in 1926 for his research on colloids and protein using the ultracentrifuge.

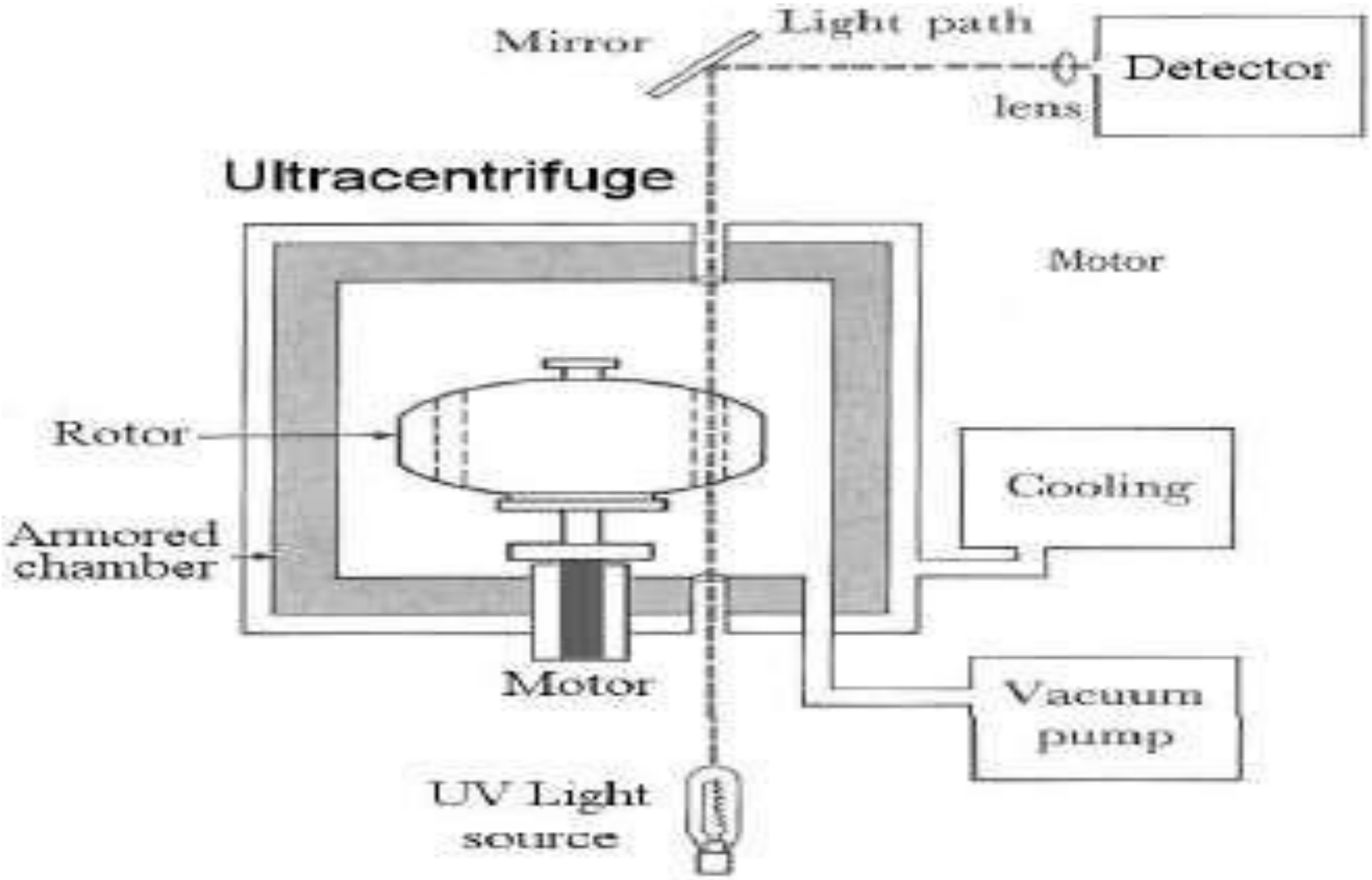
Analytical ultracentrifugation

Two kinds of experiments are commonly performed on these instruments:

1. Sedimentation velocity experiments:- Aim of SVEs to interpret the entire time-course of sedimentation, and report on the shape and molar mass of the dissolved macromolecules, as well as their size distribution.

2. Sedimentation equilibrium experiments:- SEEs are concerned only with the final steady-state of the experiment, where sedimentation is balanced by diffusion opposing the concentration gradients, resulting in a time-independent concentration

Schematic presentation of a Ultracentrifuge:



Fig; A Beckman Ultracentrifugation.

Functions of analytical and preparative ultracentrifugation:

Analytical

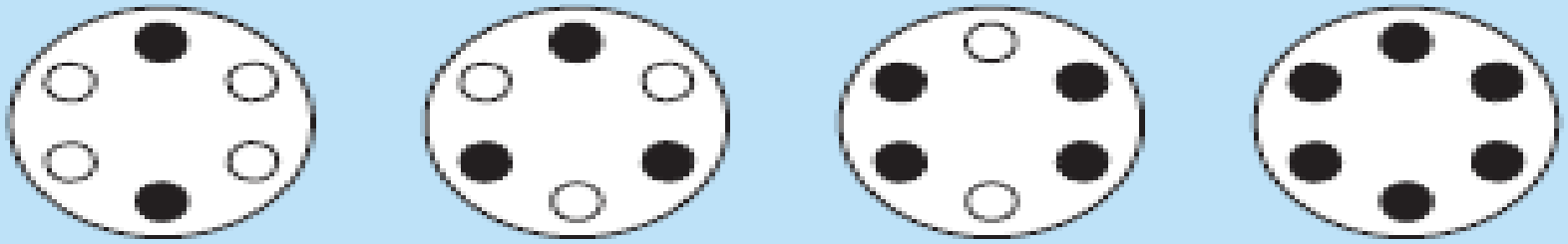
- Uses small sample size (less than 1 ml).
- Built in optical system to analyze progress of molecules during centrifugation.
- Uses relatively pure sample.
- Used to precisely determine sedimentation coefficient and MW of molecules.
- Beckman Model E is an example of centrifuge used for these purposes.

Preparative

- Larger sample size can be used.
- No optical read-out collect fractions and analyze them after the run.
- Less pure sample can be used.
- Can be used to estimate sedimentation coefficient and MW.
- Generally used to separate organelles and molecules. Most centrifugation work done using preparative ultracentrifuge.

Rotor Balance

- The mass of a properly loaded rotor will be evenly distributed on the ultracentrifuge drive hub, causing the rotor to turn smoothly with the drive.
- An improperly loaded rotor will be unbalanced; consistent running of unbalanced rotors will reduce ultracentrifuge drive life.
- To balance the rotor load, fill all opposing tubes to the same level with liquid of the same density.
- Weight of opposing tubes must be distributed equally.



Start A Run

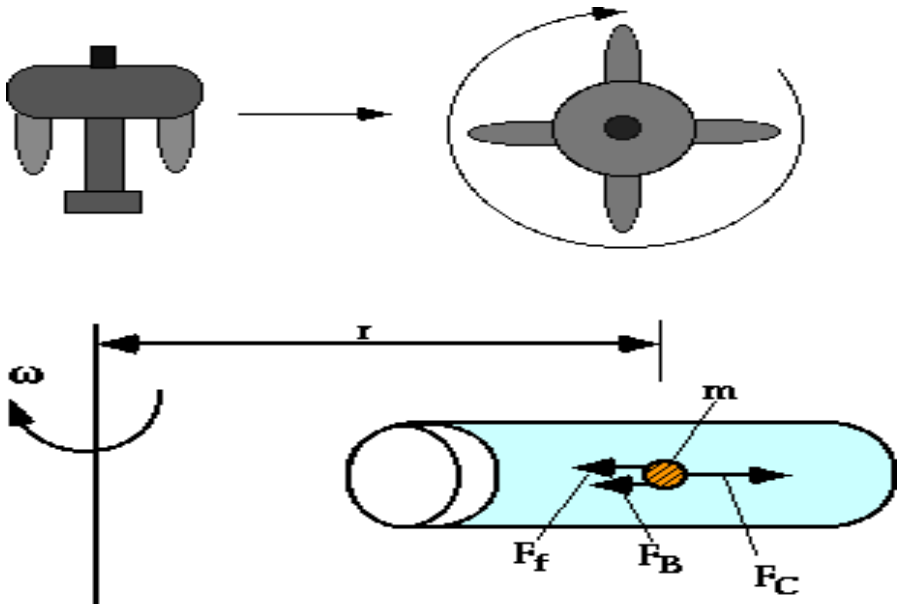
- Set the RPMs, time, and the temperature of the run by gently pressing the setup screen.
- Never exceed the manufacturer's stated maximum speed for any rotor.
- Press the run/start button when settings are correct.
- Wait until reaching desired speed.

After the Spin

- Once a run is complete, make sure the rotor has completely stopped before opening the centrifuge door. Never attempt to open the door of a centrifuge or slow the rotor by hand while the rotor is in motion.
- Please log in after every use which will provide info for maintenance and repair.
- Each centrifuge has a log book as the following.
- Return the clean (have to clean if spillage has occurred) rotors to their location.

DATE	ROTOR	Serial No.	RPM	Temp.	Duration	Operator	Tel. ext.

What happens to a particle in a centrifugal field



The particle (m) is acted on by three forces:

FC: the centrifugal force

FB: the buoyant force

Ff: the frictional force between the particle and the liquid

Equation that describes the motion of this particle as follows:

$$F = ma$$

where m is the mass of the particle and a is the acceleration.

The Physics of Ultra Centrifugation

1. Centrifugal force:- The tube containing the suspension of particles is rotated at a high speed, which exerts a centrifugal force directed from the center of the rotor towards the bottom of the tube.

Centrifugal Force: $F = M\omega^2 r$

Where,

M: mass of particle

r: radius of rotation (cm) (*ie* distance of particle from axis of rotation)

ω : Average angular velocity (radians/sec)

Centrifugal field :- **Depends on the radical distance of the particle from the rotation axis and the square of the angular velocity.**

$$G = r\omega^2$$

OR

$$G = \frac{4\pi^2 \left(\text{rev min}^{-1}\right)^2 r}{3600}$$

Angular Velocity:- **Detect to revolution per minute (r.p.m)**

$$\omega = \frac{2\pi \text{ rev min}^{-1}}{60}$$

2.Sedimentation rate:- This force acts on the suspended particles pushing them towards the bottom of the tube at a rate determined by the velocity of the spinning rotor.

Rate of Sedimentation $\frac{dr}{dt} = \frac{M(1-\bar{\nu}\rho)}{N_A f}\omega^2 r$

Where,

r = radius at which the organelle is located

t = time

M = molecular weight

v = partial specific volume of the molecule; inverse of the density

ρ = density of the solvent

f = translational frictional coefficient

ω = angular velocity

NA = Avagadro's number

3.Sedimentation coefficient:- Centrifugation separates particles in a suspension based on differences in size, shape and density that together define their sedimentation coefficient.

Sedimentation Coefficient:

$$S = \frac{dr}{dt} (1/ \omega^2 r)$$

- This is know as the Svedberg equation and is usually expressed in S10^{-13}dberg units,

  S (= second).

- This equation indicates that 'S' is dependent upon the molecular weight, the density and the frictional coefficient.

Marker Enzymes

- An enzyme that is known to be localized exclusively in the target organelle
- Examples-Acid phosphatase in lysosomes; Succinate dehydrogenase in mitochondria
- Isolation of any organelle requires a reliable test for the presence of the organelle
- By monitoring where each enzyme activity is found during a cell fractionation protocol, one can monitor the fractionation of organelle protocol
- Marker enzymes also provide information on the biochemical purity of the fractionated organelles. The presence of unwanted marker enzyme activity in the preparation indicates the level of contamination by other organelles


BECKMAN
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TL-100 Ultracentrifuge
Thankyou