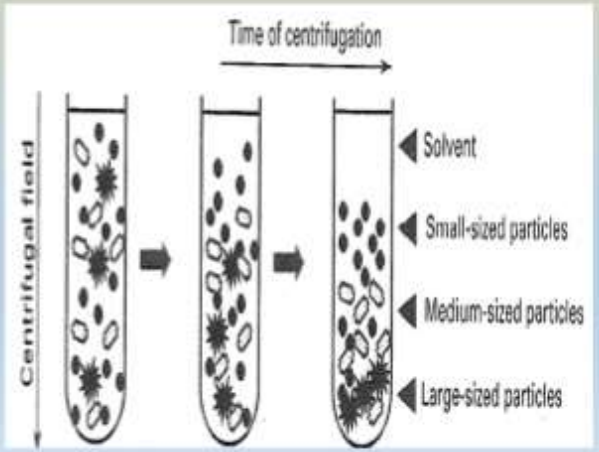
ULTRACENTRIFUGATION

Centrifuge

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



HISTORY

- Swedish Biochemist Theoder 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.

Ultracentrifugation Machine

ULTRACENTRIFUGATION

- It is an important tool in biochemical research. 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.

Example;

Red cells separated from plasma of blood, nuclei from mitochondria in cell homogenates, one protein from another in complex mixtures. And also isolation of macromolecules such as DNA, RNA, Lipids etc.

- Its rotational speed up to 150,000 rpm.
- It is creating a centrifugal force up to 900,000 x g.

TYPES

There are two types of ultracentrifugation:

1. **Analytical ultracentrifugation:- The aim of Analytical ultracentrifugation is use to study molecular interactions between macromolecules or to analyse the properties of sedimenting particles such as their apparent molecular weight.**

2. **Preparative ultracentrifugation:- The aim of Preparative ultracentrifugation to isolate and purify specific particles such as subcellular organelles.**

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 profile.**

Preparative ultracentrifugation

It is to isolate specific particles which can be reused

1. **Differential ultracentrifugation:-** Differential centrifugation is a common procedure in microbiology and cytology used to separate certain organelles from whole cells for further analysis of specific parts of cells.

2. **Density gradient ultracentrifugation:-** Based on density difference. There are two types of density gradient ultracentrifugation's under preparative ultracentrifugation such as.

   1.ZONAL or RATE & 2.ISOPYCNIC

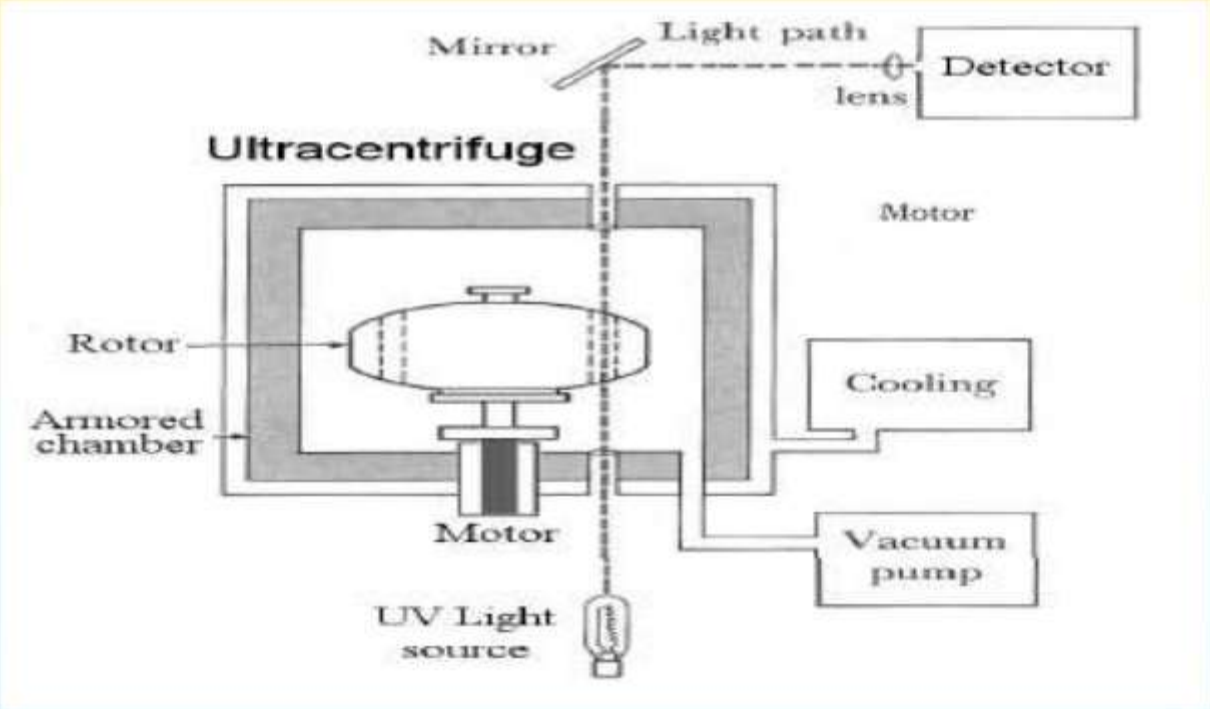
1} ZONAL or RATE Centrifugation:

- Mixture to be separated is layered on top of a gradient (increasing concentration down the tube).
- Provides gravitational stability as different species.
- Move down tube at different rates.

2} ISOPYCNIC Centrifugation:

- Isopycnic means “of the same density”.
- Molecules separated on equilibrium position.
- Each molecule floats or sinks to position where density.

Schematic presentation of a Ultracentrifuge:



Fig; A Beckman Ultracentrifugation.

Functions of analytical 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.

Functions of preparative ultracentrifugation:

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.

The Physics of UltraCentrifugation

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: $$\frac{dr}{dt} = \frac{M(1-\bar{v}\rho)}{N_A f}\omega^2 r$$

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 Svedberg units, 10^{-13}

  S (= second).

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


BECKMAN
BECKMAN
TL-100 Ultracentrifuge
Thank you