Centrifugation

- Centrifugation is the most popular technique used in biological sciences for separation of particles / microorganisms by centrifuge.

- A centrifuge is a device for separation of microorganisms from the **suspended fluid** using centrifugal force (g-force).

- Particles separate according to their size, shape, density, viscosity of the medium and rotor speed.

- Centrifugation can only be used when the **dispersed material** is denser than the **medium**.

- In a solution, higher density particles sediment in influence of gravitational field.

- Movement of particle under the gravitational force is called **sedimentation**.

- When centrifugal force applied by the centrifuge, particles move faster (> g).

- For example, when sand particles added in the water filled bucket it travels slower but it sediment faster when bucket is swung around in a circle.

- The biological materials show a drastic increase sedimentation when they undergo under acceleration in centrifugal force.

- Relative centrifugal force (RCF) is expressed as a multiple of the acceleration (G) due to gravity (g).

Basic Principle of Sedimentation

- When a biological sample moves in centrifuge, it experiences an outward **centrifugal force.**

- Rate of sedimentation of biological sample is depend on the applied centrifugal field.

- The applied centrifugal force is determined by the **radial distance** of the particle from the axis of rotation.

- If the angular velocity of particle ω and the radial distance of a particle r , applied centrifugal field is G would be

- $$\mathbf{G = \omega^2 r \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)}$$
- If the mass of the particle m, **centrifugal force F,**

$$F = mG = m\,\omega^2 r$$

Angular velocity $\omega = 2\pi s$

Where s = frequency

- Frequency s can defined as numbers of revolutions (cycles) per second. We can express the angular velocity in per minute then,

$$\omega = 2\pi \text{ (rev per min)}/ 60 \quad \ldots\ldots\ldots\ldots (2)$$

Put value of ω from equation (2) to equation (1)

$$G = 4\pi^2 (\text{rev per min})^2 / 3600 \text{ ----------(3)}$$

- The centrifugal field is generally expressed in multiples of the gravitational field g ($981 cm/s^2$).

❖ **Relative centrifugal force (RCF) is the ratio of the centrifugal acceleration (G) and gravitational acceleration (g).**

$$\mathbf{RCF = G/g}$$

Putting value of G from previous eq.(3),

$$\mathbf{RCF = 4\pi^2 r (rev\ per\ min)^2 / 3600*981}$$

$$\mathbf{RCF = 1.12 \times 10^{-5} (r.p.m.)^2 \times r}$$

RCF unit is dimensionless

So the relative centrifugal force (RCF) applied to the particle in centrifugation can be calculated.

- **Schematic presentation of a laboratory centrifuge:**

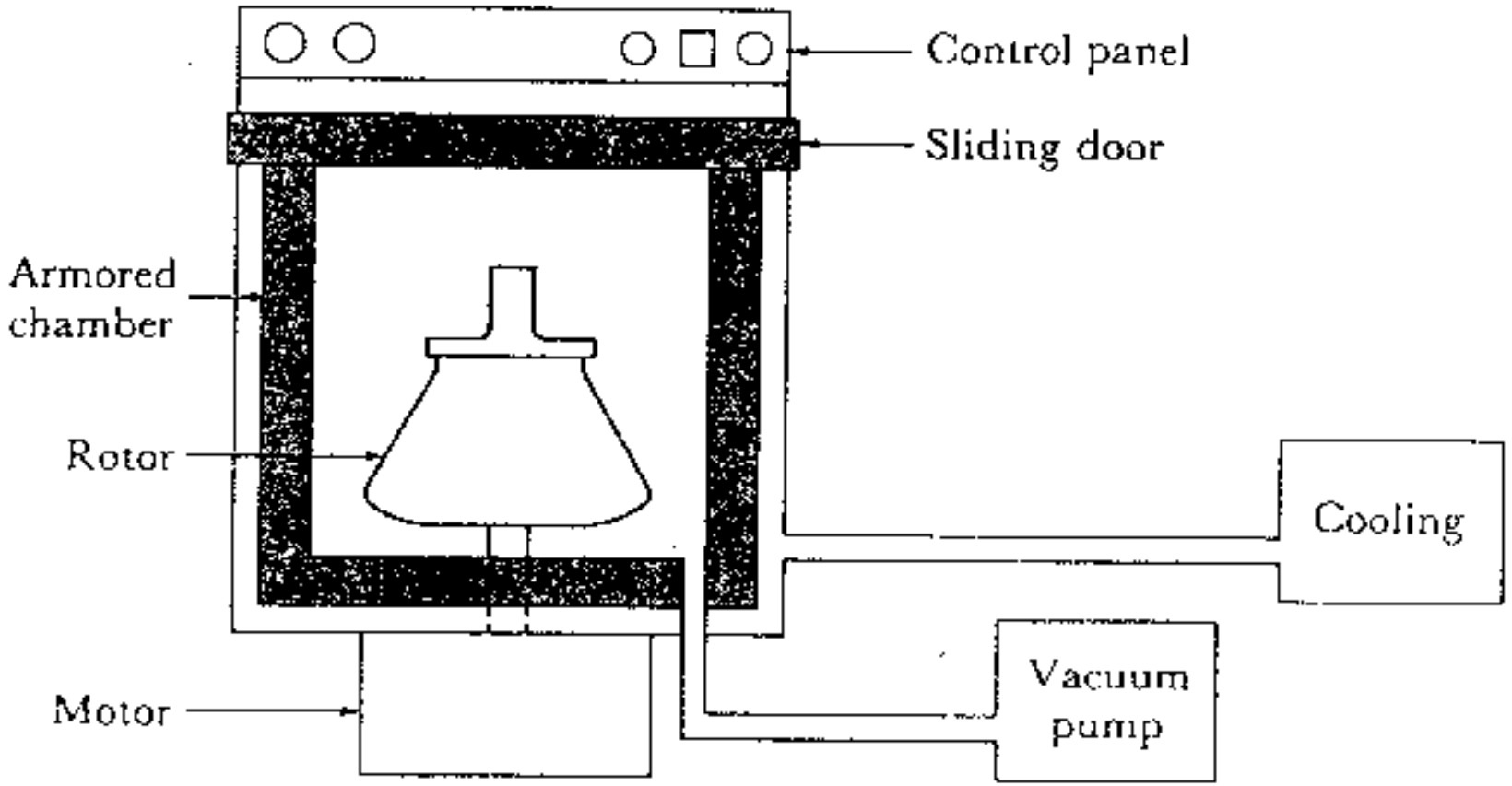
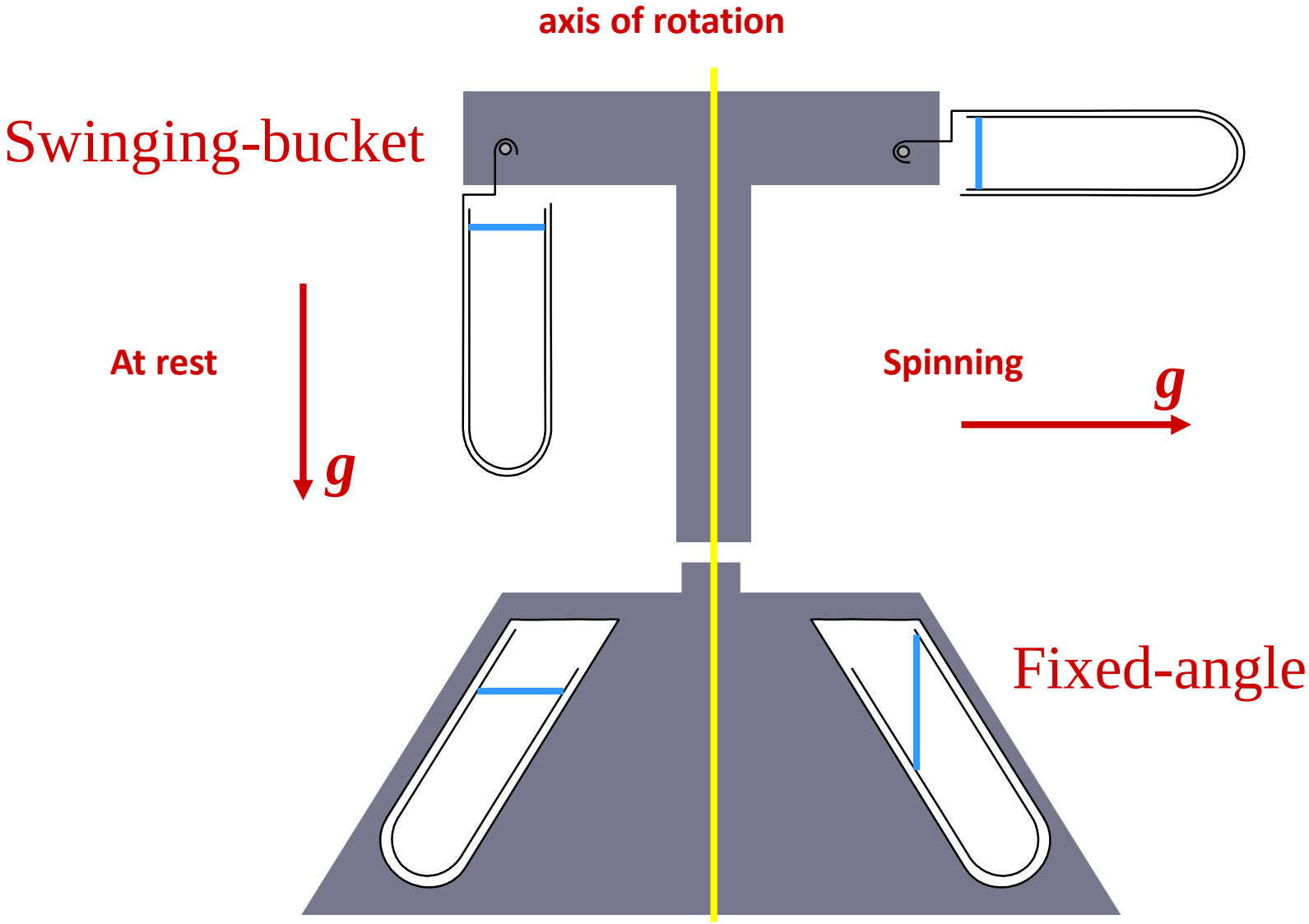


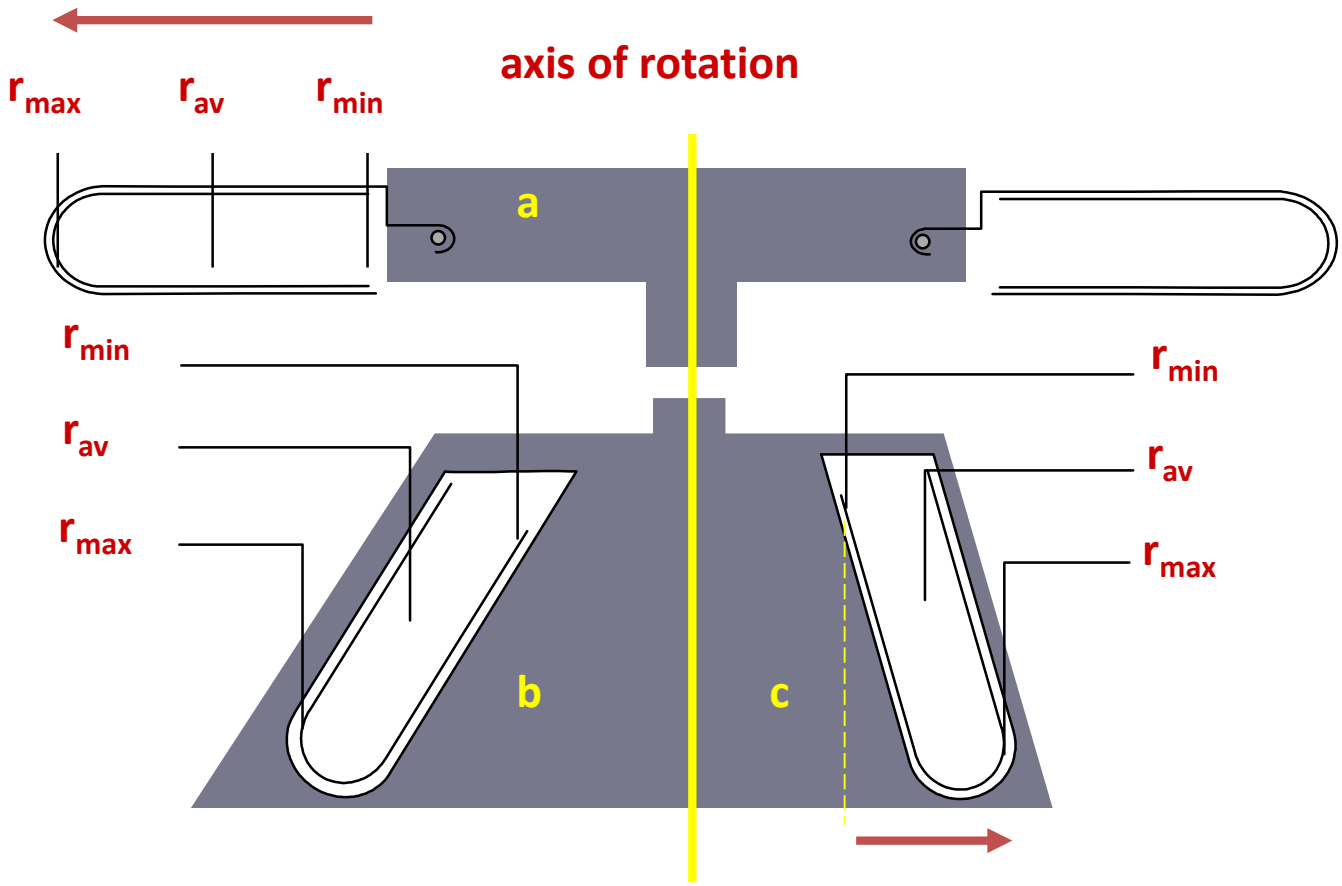
Figure 11-4

A Beckman preparative ultracentrifuge.

Centrifuge rotors



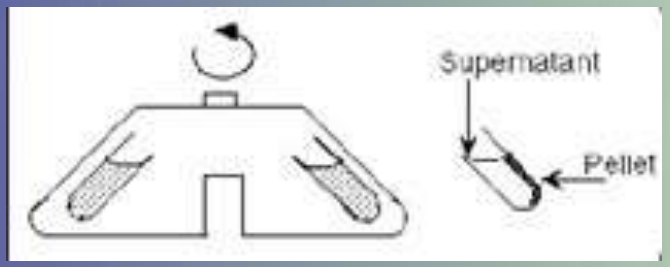
Geometry of rotors



Sedimentation path length →

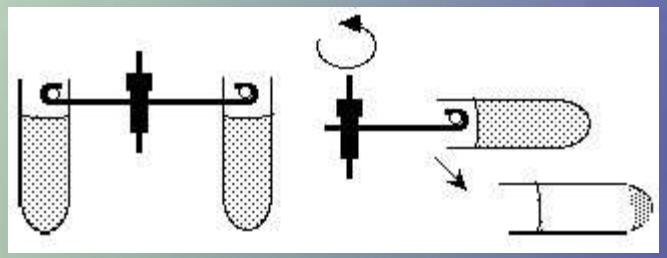
Centrifuge Rotors

- **Fixed Angle Rotor**



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

- **Swinging Bucket Rotor**



Longer distance of travel may allow better separation, such as in density gradient centrifugation. Easier to withdraw supernatant without disturbing pellet.

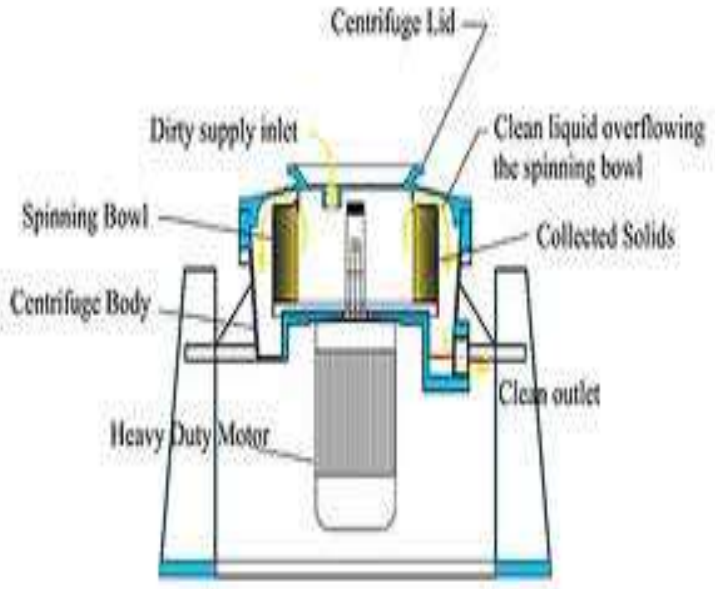
High-speed centrifuges

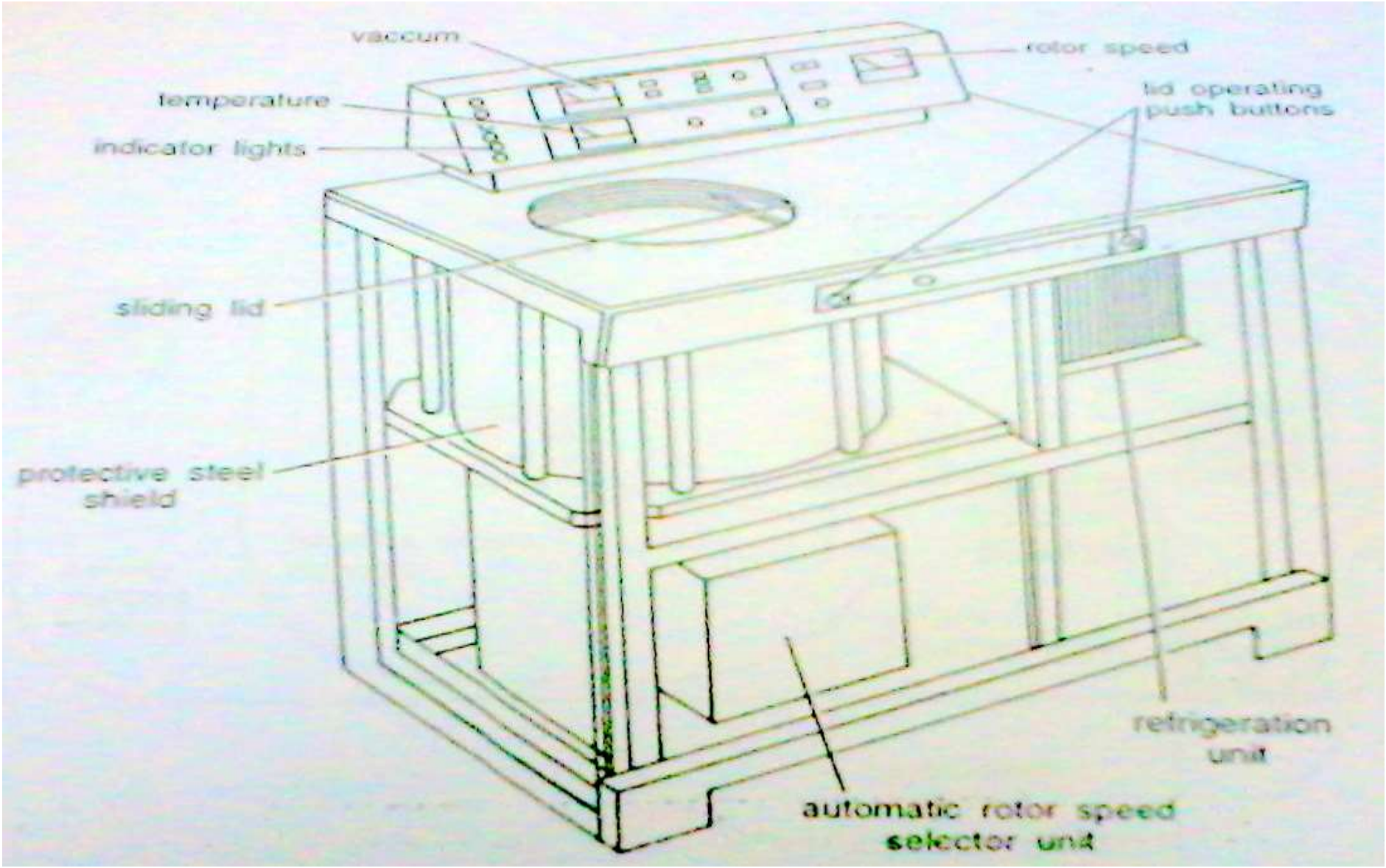
High-speed centrifuges

- High-speed or super speed centrifuges can handle larger sample volumes, from a few tens of millilitres to several litres.
- Additionally, larger centrifuges can also reach higher angular velocities (around 30000 rpm).
- The rotors may come with different adapters to hold various sizes of test tubes, bottles, or microliter plates.

Ultracentrifuges

- Ultracentrifuges can also be used in the study of membrane fractionation.
- This occurs because ultracentrifuges can reach maximum angular velocities in excess of 70000 rpm.
- Ultracentrifuges can separate molecules in batch or continuous flow systems.
- During the run, the particles or molecules will migrate through the test tube at different speeds depending on their physical properties and the properties of the solution.




vaccum
rotor speed
temperature
lid operating push buttons
indicator lights
sliding lid
protective steel shield
refrigeration unit
automatic rotor speed selecter unit

Types of Centrifuges

High Speed centrifuges	Ultracentrifuges
– 15,000 – 20,000 RPM	→ 65,000 RPM (100,000's x g)
– large sample capacity depending on rotor	– limited lifetime
– normally refrigerated	– expensive
– research applications	– require special rotors
	– care in use – balance critical!
	– research applications

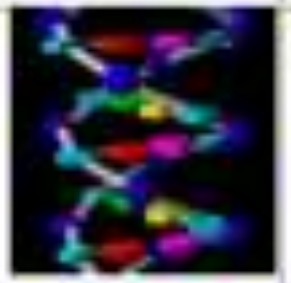
Microcentrifuges

- Microcentrifuges are used to process small volumes of biological molecules, cells, or nuclei.
- Microcentrifuge tubes generally hold 0.5 - 2 ml of liquid, and are spun at maximum angular speeds of 12000–13000 rpm.
- Microcentrifuges are small enough to fit on a table-top and have rotors that can quickly change speeds.
- They may or may not have a refrigeration function. This is the important process.

Small Bench Centrifuge

- Simplest centrifuges that are used to separate erythrocytes, Blood samples, coarse precipitates and cells are known an bench or laboratory centrifuges.
- They have a speed ranging from 4000 – 6000 RPM and a relative centrifugal force of 3000 – 7000 g.
- Small samples are sedimented now a days with microfuge that after a speed of 8000-13000 RPM and relative RCF of approximately 10000 g.
- They sediment small volume (250 mm^3 to 1.5 cm^3) of material in 1 or 2 min.

Small Bench top	Microcentrifuges ("microfuge", "Eppendorf")
– with or without refrigeration – slow speed (eg up to 4000 RPM) – common in clinical labs (blood/plasma/serum separation) – can take approx (up to) 100 tubes, depending on diameter	– take tubes of small vols (up to 2 mL) – very common in biochemistry/molecular biology/ biological labs – can generate forces up to ~15,000 x g – with or without refrigeration

	Sample	Typical Applications	Most Appropriate Rotor	Typical Separation Method
	**DNA and RNA**	Sequencing, gene therapy, cloning, gene expression	Vertical and Fixed angle	CsCl gradient, 400,000xg; Ethidium Bromide staining
	**Viruses**	Vaccines, gene therapy vector	Swinging bucket	Sucrose gradients, 100,000xg
	**Proteins**	Protein structure studies, Proteomics, HDL/LDL studies	Fixed angle	Rate-zonal separation, 600,000xg
	**Cells and organelles**	Cell function, membrane biology, mitochondrial DNA	Fixed angle	Differential pelleting. Low speeds for cells. High speeds - smaller organelles.

Gradient Media

There are several gradient materials used in centrifugation:-

Gradient media	Their uses
Sucrose and Ficoll	Helps in preserving the morphology and activity of subcellular fractions.
Cesium chloride	Useful in isopycnic density gradient centrifugation technique.
Potassium bromide	Useful in isopycnic density gradient centrifugation technique.
Percoll	Because of its low osmolarity, low viscosity and large particle size, is suitable for separating cells, bacteria, viruses and sub cellular organelles
Metrizamide	For the isolation of membrane fractions by floatation
Nycodenz	For the isolation of membrane fractions by floatation
Renografin	For cell fractionation

Centrifugal fractionation of cell organelles

Cell Organelle	Required centrifugal force
Nuclei	800 – 1000 g
Mitochondria	20,000 – 30,000 g
Chloroplasts	20,000 – 30,000 g
Lysosomes	20,000 – 30,000 g
Microbodies	20,000 – 30,000 g
Rough ER membranes (microsomes)	50,000 – 80,000 g
Plasma Membranes, Smooth ER membranes	80,000 – 1,00,000 g
Free Ribosome particles	1,50,000 – 3,00,000 g

Corrosion

- Many rotors are made from either titanium or aluminium alloy, chosen for their advantageous mechanical properties.
- While titanium alloys are quite corrosion-resistant, aluminium alloys are not.
- When **corrosion** occurs, the metal is weakened and less able to bear the stress from the centrifugal force exerted during operation.
- The combination of stress and corrosion causes the rotor to fail more quickly and at lower stress levels than an uncorroded rotor.

Precautions

A centrifuge user should strictly observe the following precautions :

1. Manufacturer's manual should be strictly followed.
2. Rotor should be stored in proper containers.
3. Attention should be given to imbalance detectors.
4. Rotor speed should not exceed the assigned speed.
5. Lid of the rotor chamber should remain locked during operation.
6. To avoid the rotor failure, manufactures instructions regarding rotor care and use should always be followed.

Applications in Biological Sciences

- To separate cellular and subcellular components
- Separating one cell type from another.
- Removing cells or other suspended particles from their surrounding milieu on either a batch or a continuous-flow basis.
- Isolating viruses and macromolecules, including DNA, RNA, proteins, and lipids or establishing physical parameters of these particles from their observed behaviour during centrifugation.
- To study the effects of centrifugal forces on cells, developing embryos, and protozoa.
- These techniques have allowed scientists to determine certain properties about cells, including surface tension, relative viscosity of the cytoplasm, and the spatial and functional interrelationship of cell organelles when redistributed in intact cells.

Conclusion

- The centrifugation is a modern & easy technique of separation and sedimentation on the basis of shape, size and density of macromolecules and other particles.
- In the centrifugation there are different types of forces are applied like as centrifugal force, gravitational force and centripedal force etc. and also different types of rotors are to be used that is; Swinging Bucket Rotor and fixed angle rotors at different RPM/RCF.