CENTRIFUGE

One of the most common equipments used to separate materials into subfractions in a biochemistry lab is the centrifuge. A centrifuge is a device that spins liquid samples at high speeds and thus creates a strong centripetal force causing the denser materials to travel towards the bottom of the centrifuge tube more rapidly than they would under the force of normal gravity. In other words, centrifuge is a device for separating particles from a solution based on their size, shape, density, viscosity of the medium and rotor speed. In biology, particles usually refer to the cells, subcellular organelles, viruses, biomolecules such as proteins and nucleic acids, etc.

BASIC PRINCIPLES OF SÉDIMENTATION

The rate of sedimentation is dependent upon the applied centrifugal force, directed radially outwards.

Relative Centrifugal Force (RCF)

When an object moves in a circle at a steady angular velocity, it experiences a force, \( F \), directed outwards. \( F \) is determined by the square of the angular velocity of the rotor (\( \omega \), in radians per second) and the radial distance of the particle from the axis of rotation (\( r \), in cm).

\[
\text{Centrifugal force} = (\text{angular velocity})^2 \times \text{radius}
\]

\[
F = \omega^2 r
\] (1)
The common way of expressing rotor speed is in terms of revolutions per minute (rev min\(^{-1}\) or rpm). Since one revolution of the rotor is equal to \(2\pi\) radians, its angular velocity can be expressed as,

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

(2)

Substituting the value of \(\omega\) in equation 1,

\[
F = \frac{4\pi^2 (\text{rev min}^{-1})^2 r}{3600} \cdot \omega^2 
\]

However, \(F\) is expressed as a multiple of the earth's gravitational field (\(g = 981\, \text{cm s}^{-2}\)).

The ratio of the weight of the particle in the centrifugal field to the weight of the same particle when acted by gravity alone is known as the relative centrifugal field (RCF) which is commonly referred to as the 'number times \(g\)' Hence,

\[\text{RCF} = \frac{4\pi^2 (\text{rev min}^{-1})^2 r}{3600 \times 981} \]

\[\text{RCF} = (1.118 \times 10^{-5})(\text{rev min}^{-1})^2 r\]

From the above relationship, it is clear that RCF depends upon the rpm and the radius of rotation, \(r\). If \(r\) is a constant for a given rotor, then variations in rpm alone determine the variations in RCF.

**Sedimentation Rate**

In the centrifugation process, the particles will sediment progressively with time towards the bottom of the sample tube. To simplify mathematical terminology we assume all biological materials to be spherical particles. The sedimentation rate of a given particle depends upon a number of factors such as density of the particle \(\rho_p\), radius of the particle \(r\), the density of the medium \(\rho_m\) and the viscosity \(\eta\) of the suspending medium. A mathematical expression relating to all these factors for sedimentations of a rigid spherical particle is given below:
where,
\[ \nu = \text{rate of sedimentation}, \]
\[ g = \text{gravitational field and} \]
\[ \frac{2}{9} = \text{shape factor constant for a spherical particle}. \]

From the above equation, it is clear that the sedimentation rate of a given particle is proportional to its size, the difference in density between the particle and the medium and to the applied centrifugal field. Sedimentation rate becomes zero when the density of the particle and the medium are equal. It increases when the force field increases and decreases when the viscosity of the medium increases. However, since the equation involves the square of the particle radius, it is apparent that the size of the particle has the greatest influence upon its sedimentation rate. Some other factors that affect the sedimentation rate are i) characteristics of the centrifuge and ii) concentration of the suspension.

Svedberg Unit or Sedimentation Coefficient

The sedimentation rate or velocity (\( \nu \)) of a particle can be expressed in terms of its sedimentation rate per unit of centrifugal field. It is commonly referred to as its sedimentation coefficient, \( S \). Since sedimentation rate studies are performed using a wide variety of solvent-solute systems or at different temperatures, the value is affected by temperature, solution viscosity and density. Therefore, these values are corrected to the sedimentation constant theoretically obtainable in water at 20°C. The equation for standard coefficient is

\[
S_{20w} = \frac{S_{\text{obs}} (1 - \bar{v} \rho_{20w})}{(1 - \bar{v} \rho_T)} \times \frac{\eta_T}{\eta_{20}} \times \frac{\eta}{\eta_{20}}
\]

where,
\[ S_{20w} = \text{the standard sedimentation coefficient}, \]
\[ S_{\text{obs}} = \text{the experimentally measured sedimentation coefficient}, \]
\[ \frac{\eta_T}{\eta_{20}} = \text{the relative viscosity of water at temperature } T \text{ compared with that at } 20^\circ C. \]
\[ \frac{\rho_T}{\rho_{20}} = \text{the density of water at } 20^\circ C. \]
\[ \rho_T = \text{the density of the solvent at temperature } T(\circ C) \text{ and } \]
\[ v = \text{the partial specific volume of the solute.} \]

The basic unit of sedimentation coefficient is $1 \times 10^{-13}$ sec. This is also termed as one Svedberg unit (S), in recognition of T. Svedberg's pioneering work. Generally, the larger the molecule or particle, the larger is its Svedberg unit and hence the faster is its sedimentation rate. Svedberg units for viruses are 40 to 1000S, for lysosomes 40000S and for mitochondria $20 \times 10^3$S to $70 \times 10^3$S.

**TYPES OF CENTRIFUGES**

Centrifuges may be classified into four major groups—small bench centrifuges, large capacity refrigerated centrifuges, high-speed centrifuges, and ultracentrifuges.

**Small Bench Centrifuges**

*Hand centrifuge*  
Hand centrifuge is manually operated consisting of two centrifuge tube holders (Figure 5.1). Hand centrifuges are used to sediment the larger particles for simple experiments.

![Figure 5.1 Hand centrifuge](image)
Clinical centrifuges. These are very simple and small and hence can be placed atop a desk (Figure 5.2). They are normally used to isolate red blood cells, yeast cells or bulky precipitates of chemical reactions. Their maximum speed is usually 3000 rev min⁻¹ with maximum relative centrifugal force of 7000 g. They do not usually have a temperature regulatory system. They are useful for the separation of large volumes of crude preparation. The rotors are mounted vertically on a rigid shaft. Therefore, centrifuge tubes must be placed diametrically opposite to each other after balancing their weights accurately.

![Clinical centrifuge](image)

Figure 5.2 Clinical centrifuge

Large Capacity Refrigerated Centrifuges

These have a maximum speed of 6000 rev min⁻¹ and produce a maximum relative centrifugal field approaching 6500 g. They have refrigerated rotor chambers and vary only in their maximum carrying capacity, all being capable of utilizing a variety of interchangeable swinging-bucket and fixed-angle rotors. Large total capacity centrifuges are also available which in addition to accommodating smaller tubes, are also capable of holding bottles (Figure 6.3). These instruments are most
often used to collect substances that sediment rapidly, for example erythrocytes, coarse or bulky precipitates, yeast cells, nuclei and chloroplasts.

Figure 5.3 Large capacity refrigerated centrifuge

High-speed Refrigerated Centrifuges

These centrifuges are available with maximum rotor speeds in the region of 25000 rev min\(^{-1}\), generating a relatively centrifugal field of about 60000 \(g\). They generally have a range of interchangeable fixed-angle and swinging-bucket rotors. They are equipped with refrigeration equipment to remove the heat generated due to friction between the air and the spinning rotor. The temperature can easily be maintained in the range \(-10^\circ\text{C} \text{ to } 40^\circ\text{C}\) (Figure 5.4). These instruments are most often used to collect microorganisms, cellular debris, larger cellular organelles and proteins precipitated by ammonium sulphate. They cannot generate sufficient centrifugal force to effectively sediment viruses or smaller organelles such as ribosomes.
Continuous flow centrifuges The continuous flow centrifuge is a relatively simple high-speed centrifuge. The rotor is long and tubular, through which particles suspended in medium flow continuously. As the medium enters the rotating rotors, particles are deposited against its wall and excess medium overflows through an outlet port. The major application of this type of centrifuge is in the harvesting of bacteria or yeast cells from large volumes of culture medium.

Ultracentrifuges

Ultracentrifuges are of two types—preparative and analytical ultracentrifuge.

Preparative ultracentrifuge Preparative ultracentrifuges are capable of spinning rotors to a maximum speed of 80,000 rev min⁻¹ and can produce a relative centrifugal field of up to 60,000 g. The rotor chamber is refrigerated, sealed and evacuated to minimize any
excessive rotor temperature being generated by frictional resistance between the air and the spinning rotor. The temperature monitoring system is more sophisticated than in simpler instruments, employing an infrared temperature sensor that can continuously monitor rotor temperature and control the refrigeration system. An over-speed control system is also incorporated into these instruments to prevent operation of the rotor above its maximum rated speed and there are electronic circuits to detect rotor imbalance. In order to minimize vibration caused by slight motor imbalance that may arise due to unequal loading of the centrifuge tubes, ultracentrifuges are fitted with a flexible drive shaft system. For safety reasons, rotor chambers of both high-speed and ultracentrifuges are always enclosed in heavy armour plating.

Airfuge An air-driven, tabletop preparative ultracentrifuge, called an airfuge, is available. This is capable of acceleration of a magnetically suspended 3.7-cm diameter rotor, accommodating $6 \times 175 \text{ mm}^3$ tubes on a friction-free cushion of air in a non-evacuated chamber. The rotor speed is $100,000 \ \text{rev min}^{-1}$ ($160,000 \ \text{g}$). The airfuge has found applications in biochemical and clinical research where there is only small volume of samples requiring high centrifugal forces. Examples include macromolecules/ligand binding-kinetic studies, steroid hormone receptor assays, separation of the major lipoprotein fraction from plasma, and deproteinization of physiological fluids for amino acid analysis.

Analytical ultracentrifuge Analytical ultracentrifuges are capable of operating at forces as great as $600,000 \pm 100 \ \text{g}$ and with temperature control within approximately $0.1^\circ \text{C}$. Analytical ultracentrifuge basically consists of a motor, a centrifuge rotor which is present in a protective and armoured chamber and an optical system for recording the distribution of the sample in the ultracentrifuge cell. The rotor is kept in an evacuated and cooled chamber and is suspended on a wire coming from the drive shaft of the motor. The tip of the rotor contains a thermistor for measuring temperature. The thermistor makes electrical contact with the control circuit by means of a pool of mercury, which the rotor tip touches. The rotor chamber contains an upper condensing lens and a lower
collimating lens. The lower lens allows the passage of the light so that the sample is illuminated by parallel light. The upper lens and the camera lens focus the light on the film (Figure 5.5). Several types of rotors are available. A rotor contains two cells, namely the analytical cell and the counterpoise cell.

![Diagram of analytical ultracentrifuge](image)

**Figure 5.5** Diagram of analytical ultracentrifuge

Three types of optical systems are available. They are

1. Ultraviolet light absorption system
2. Schlieren optical system
3. Rayleigh interference system

In the ultraviolet light absorption system, light of a suitable wavelength is passed through the moving analytical cell containing the solution under analysis. The intensity of the transmitted light is recorded on a photographic plate.

In Schlieren optical system, when light passes through a solution having different density zones it is refracted at the boundary between these zones. The Schlieren optical system plots the refractive index gradient against the distance along the analytical cell, which is useful for sedimentation velocity measurements. However, it is not sufficiently sensitive to detect small concentration differences.
Rayleigh interference system employs a double-sector cell. One sector contains the solvent and the other the solution. The optical system measures the difference in refractive index between the reference solvent and the solution by the displacement of interference fringes caused by splits placed behind the two liquid columns. Each fringe traces a curve of the refractive index gradient against the distance in the cell. Since the position of the fringes is determined by solute concentration, it is possible to measure the concentration of the solute at any point along the cell.

**Applications** The analytical centrifuge is used for the following:

1. To determine relative molecular mass of macromolecules such as proteins and DNA.
2. To investigate the purity of DNA preparations, viruses and proteins.
3. To detect conformational changes in macromolecules such as DNA and proteins.

**Types of Rotors**

Rotors used for low-speed centrifugation are made up of brass or steel because they experience a much lower degree of stress. Rotors made of alloys of aluminium or titanium are used in high-speed centrifugation. The different types of rotors used for centrifugation process are given in the following sections.

**Vertical Tube Rotors**

The vertical tube rotor is a fixed zero-angle rotor. In this, tubes are aligned vertically in the body of the rotor at all times. The design and operation of the vertical tube rotor is shown in Figure 5.6. In this type of rotor, the pellet is deposited along the entire length of the outer wall of the centrifuge tube. The major disadvantage in this rotor is that the pellet tends to fall back into the solution at the end of centrifugation.
Figure 5.6 Design and operation of the vertical tube rotor. (a) Vertical rotor (b) Cross-sectional diagram of a vertical tube rotor. (c) The centrifuge tube is filled with gradient; the sample is layered on top and is then placed in the rotor. (d) As the rotor accelerates, the sample and gradient begin to reorient. (e) The sample and medium reorientation is complete. (f) Sedimentation and separation of particles occur during centrifugation. (g) Reorientation of separated particles and gradient occur during the rotor deceleration. (h) Rotor is at rest: bands of separated particles and gradient are fully reoriented.

Fixed-Angle Rotors

In fixed-angle rotors, the tubes are located in holes in the rotor body set at a fixed angle between 14° and 40° to the vertical. Under the influence of the centrifugal field, particles move radially outwards
and have only a short distance to travel before colliding with the outer wall of the centrifuge tube. The design and operation of fixed-angle rotor is shown in Figure 5.7. They are used for the differential separation of particles whose sedimentation rates differ by a significant order of magnitude.

Figure 5.7 Design and operation of the fixed-angle rotor. (a) Fixed-angle rotor. (b) Cross-sectional diagram of a fixed-angle rotor. (c) The centrifuge tube, after being filled with gradient, is loaded with sample and then placed in the rotor. (d) During rotor acceleration, reorientation of the sample and gradient occur. (e) Sedimentation and separation of the particles occur during centrifugation. (f) Rotor is at rest; the gradient reorients and bands of separated particles appear.

Swinging-Bucket Rotors

This type of rotor has buckets. During acceleration of the rotor, they swing out from the vertical position to a horizontal position. They are then aligned perpendicular to the axis of rotation and parallel to the applied centrifugal field. The design and operation of
Swinging-bucket rotor is shown in Figure 5.8. An undesirable swirling effect that causes mixing of the tube contents are also produced during rotor acceleration and deceleration.

![Diagram of swinging-bucket rotor](image)

Figure 5.8 Design and operation of the swinging-bucket rotor. (a) Swinging-bucket rotor. (b) Cross-sectional diagram of a swinging-bucket rotor. (c) The centrifuge tube is initially loaded with gradient, the sample is then layered on top before the tube is placed in the bucket for attachment to the rotor. (d) During acceleration of the rotor, the rotor bucket reorients to lie perpendicular to the axis of rotation. (e) Sedimentation and separation of the particles occur during centrifugation. (f) At the end of centrifugation the rotor deaccelerates, the bucket coming to rest in its original vertical position.

Zonal Rotors

There are two types of zonal rotors, namely, the batch type and the continuous-flow type. The batch type zonal rotor is extensively used. It is designed to minimize the wall effects that are encountered
in swinging-bucket and fixed-angle rotors and to increase sample size. Low-speed batch rotors are designed to operate at near 5,000 rpm (5,000 g). The high-speed batch rotors are made of aluminium or titanium alloy and can operate at speeds up to 60,000 rpm (256,000 g). As shown in Figure 5.9, the density gradient is formed while the rotor is spinning; then the sample is layered and centrifuged until the isopycnic zonal layering of the particles is reached. At this moment, an injection of a denser sucrose solution pushes the layers toward the centre where they are collected in tubes of a fraction collector. Batch-type zonal rotors are used to remove contaminating proteins from a variety of preparations and for the separation and isolation of hormones, enzymes, ribosomal subunits, viruses and subcellular organelles from animal or plant tissue homogenates.

![Schematic section through a zonal rotor](image)

**Figure 5.9** Schematic section through a zonal rotor

Continuous flow zonal rotors are designed for high-speed separation of relatively small quantities of solid matter from large volumes of suspension. The rotors are similar in shape to batch type zonal rotors but differ in their design because of the continuous fluid flow in the rotor. They are useful for the harvesting of cells and isolating viruses in large scale.
Elutriator Rotors

The elutriator rotor is a type of continuous flow rotor that contains recesses to hold a single conical-shaped separation chamber, the apex of which points away from the axis of rotation, and a bypass chamber on the opposite side of the rotor that serves as a counterbalance and to provide the fluid outlet. Particles suspended in a uniform low-density medium are pumped into the rotor chamber and the rotor is spinned at a preselected speed (usually between 1000 and 3000 rpm). Since the separation chamber is conical in shape, larger particles accumulate towards the centrifugal end of the chamber where the liquid flow velocity is high, while the smaller particles accumulate towards the centripetal end of the chamber where the liquid flow velocity is low (Figure 5.10). Either by a stepwise decrease in rotor speed or by a stepwise increase in liquid flow rate through the separation

![Diagram](attachment:image.png)

**Figure 5.10** (a) Cross-section through an elutriator rotor and (b) the separation of particles in the separation chamber of an elutriator rotor by centrifugal elutriation
chamber, collection of the separated uniformly sized particles can be made centripetally in order of successively increasing diameter by elutriation from the chamber.

With the technique of centrifugal elutriation, the elutriator rotor has been used successfully to separate various cell types from mammalian testis and different types of monocytes and lymphocytes from human blood, to purify Kupffer and endothelial cells from sinusoidal liver cells and fat-storing cells from rat liver, and for the bulk separation of rat brain cells and the fractionation of yeast cell populations.

TYPES OF CENTRIFUGATION

Centrifugation is classified based on the (i) purpose of centrifugation (ii) speed at which centrifuge is operated and (iii) method of application of the samples. Based on the purpose, centrifugation is classified into two most common types—analytical and preparative centrifugation. Analytical centrifugation involves measuring the physical properties of the sedimenting particles such as sedimentation coefficient or molecular weight. The other form of centrifugation is called preparative and the objective is to isolate specific particles, which can be reused.

Analytical Centrifugation

Optical methods are used in analytical ultracentrifugation. Molecules are observed by optical system during centrifugation, to allow observation of macromolecules in solution as they move in the gravitational field. The samples are centrifuged in cells (tubes with quartz windows) having windows that lie parallel to the plane of rotation of the rotor head. As the rotor turns, the images of the cell (proteins) are projected by an optical system onto a film or a computer. The concentration of the solution at various points in the cell is determined by absorption of a light of appropriate wavelength (Beer’s law is followed). This can be accomplished either by measuring the degree of blackening of a photographic film or by the pen deflection of the recorder of the scanning system and fed
into a computer.

The analytical ultra centrifuge has found many applications in biology, especially in the field of protein chemistry and nucleic acid chemistry. This technique is used to determine the sedimentation coefficient, relative molecular mass and also to test the purity of macromolecules.

The relative molecular mass of macromolecules is determined either by sedimentation velocity method or sedimentation equilibrium method.

**Sedimentation velocity method** In sedimentation velocity method the sedimentation coefficient of the molecule is initially determined either by boundary sedimentation or zonal sedimentation. The equation used for calculating molecular weight by sedimentation equilibrium method is

\[ M = \frac{RTS}{D(1 - \bar{v}\rho)} \]

where,

- \( M \) = relative molecular weight of the molecule,
- \( D \) = diffusion coefficient of the molecule,
- \( \bar{v} \) = partial specific volume of the molecule,
- \( \rho \) = density of the solvent at 20°C,
- \( R \) = molar gas constant,
- \( T \) = absolute temperature and
- \( S \) = sedimentation coefficient.

The measured values of \( S \) and \( D \) are corrected to standard conditions of zero concentration of solute in water at 20°C. However the determination is complicated by the difficulties encountered in the accurate determination of the diffusion coefficient of the particles and in correction in differences in viscosity and temperature. The determination of the relative molecular weight of a macromolecule using sedimentation velocity analysis is therefore less accurate and more time-consuming than determination by sedimentation equilibrium method.
Sedimentation equilibrium method  Sedimentation equilibrium method is more versatile and accurate. This method can be used to determine relative molecular mass values ranging from a few hundred to several million. This versatility is due to the large range of centrifugal fields available for ultracentrifugation. In this method, the ultracentrifuge is operated until a balance is established between sedimentation and diffusion of material in the opposite direction. Molecular weight can be calculated using the equation

$$M = \frac{2RT \ln(C_2/C_1)}{\omega^2(1 - \bar{\nu}\rho)(x_2^2 - x_1^2)}$$

where,

- $C_1$ and $C_2$ are the concentrations of solute at distances $x_1$ and $x_2$ respectively from the centre of rotation,
- $R$ = molar gas constant,
- $T$ = absolute temperature,
- $\omega^2$ = square of the angular velocity of the rotor,
- $\bar{\nu}$ = partial specific volume of the molecule and
- $\rho$ = density of the solvent.

This technique does not require diffusion coefficient and hence the method is more convenient and widely used for the determination of molecular weight of proteins.

Preparative Centrifugation

There are many types of preparative centrifugation such as differential and density gradient centrifugation.

Differential centrifugation (cell fractionation)  The process of separation of cell organelles is known as subcellular fractionation. To isolate a specific organelle, initially, the organs (liver, brain, or kidney) are homogenized in a suitable homogenizing medium at 4°C. The resulting suspension, containing many intact organelles, is known as a homogenate.

Fractionation of the contents of a homogenate is done by a classical biochemical technique called differential centrifugation. This
Figure 5.11 Differential sedimentation of a particulate suspension in a centrifugal field. (a) Particles are uniformly distributed throughout the centrifuge tube. (b) to (e) Sedimentation of particles during centrifugation is dependent upon their size, shape and density.

The method is based upon the differences in the sedimentation rate of particles of different sizes and density (Figure 5.11). This method uses a series of four different centrifugation steps at successively greater speeds. Each step yields a pellet and a supernatant. The supernatant from each step is subjected to centrifugation in the next step. This procedure provides four pellets, namely, nuclear, mitochondrial, lysosomal, and microsomal fractions (Figure 5.12). At the end of each step, the pellet is washed several times by resuspending in the homogenization medium followed by recentrifugation under the same conditions. This procedure minimizes contamination of other subcellular organelles and gives a fairly pure preparation of pellet fraction.

The purity of organelles obtained by differential centrifugation is measured by estimating some marker activity. A marker is one that is almost exclusively present in one particular organelle. A marker may be an enzyme molecule or a biochemical compound. Table 5.1 gives a list of various fractions, their functions and markers.
Table 5.1  Cellular fractions and their functions

<table>
<thead>
<tr>
<th>Organelle</th>
<th>Function</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma membrane</td>
<td>Regulates entry and exit of compounds</td>
<td>5' Nucleotidase</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Site of DNA-directed RNA synthesis</td>
<td>DNA</td>
</tr>
<tr>
<td>Mitochondrion</td>
<td>Citric acid cycle, ammonia release for urea formation</td>
<td>Glutamate dehydrogenase</td>
</tr>
<tr>
<td>Lysosome</td>
<td>Site of many hydrolases</td>
<td>Acid phosphatase</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>Oxidation of many xenobiotics</td>
<td>Glucose 6-phosphatase</td>
</tr>
<tr>
<td>Cytosol</td>
<td>Enzymes of glycolysis, fatty acid synthesis</td>
<td>Lactate dehydrogenase</td>
</tr>
</tbody>
</table>

The microsomal fraction contains mostly a mixture of smooth endoplasmic reticulum and free ribosomes. The contents of the final supernatant correspond approximately to those of cytosol.

10% w/v liver homogenate in 0.25M sucrose (50 ml)

Centrifuged 1000 g
$(r_w, 8 \text{ cm}) \times 10 \text{ min}$

Pellet (Nuclear)  
Supernatant

Centrifuged 3300 g
$(r_w, 8 \text{ cm}) \times 10 \text{ min}$

Pellet (Mitochondrial)  
Supernatant

Centrifuged 16300 g
$(r_w, 8 \text{ cm}) \times 20 \text{ min}$

Pellet (Lysosomal)  
Supernatant

Centrifuged 100,000 g
$(r_w, 6 \text{ cm}) \times 60 \text{ min}$

Pellet (Microsomal)  
Cytosol

Figure 5.12  Cellular fractionation
Density gradient centrifugation  Differential centrifugation uses a homogeneous medium for separation whereas density gradient centrifugation uses a medium that has gradients. The commonly used gradient materials and their applications are given in Table 5.2.

<table>
<thead>
<tr>
<th>Gradient materials</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caesium chloride</td>
<td>Banding of DNA, nucleoproteins, viruses, plasmid isolation</td>
</tr>
<tr>
<td>Caesium sulphate</td>
<td>Banding of DNA and RNA, purification of proteoglycans</td>
</tr>
<tr>
<td>Sodium bromide</td>
<td>Fractionation of lipoproteins</td>
</tr>
<tr>
<td>Sodium iodide</td>
<td>Banding of DNA and RNA</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Banding of membrane fragments, protein separation</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Separation of subcellular particles, proteins, viruses and membranes</td>
</tr>
<tr>
<td>Ficoll</td>
<td>Separation of whole cells, subcellular particles and viruses</td>
</tr>
<tr>
<td>Dextran</td>
<td>Separation of whole cells, banding of microsomes</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>Separation of whole cells</td>
</tr>
</tbody>
</table>

In density gradient centrifugation, separation depends upon the buoyant densities of the particle. This method gives a much better separation than differential centrifugation. There are two types of density gradient centrifugation, namely, the rate-zonal technique and the isopycnic technique.

Rate-zonal centrifugation technique  Rate-zonal centrifugation is a type of density gradient centrifugation in which particle separation is based upon

1. Differences in the size, shape and density of the particle,
2. The density and viscosity of the medium and
3. The applied centrifugal field.
The gradient used in rate-zonal centrifugation technique has the maximum density at the bottom but its density is lesser than the most dense sedimenting particle to be separated. The density gradient is shallow. It is produced by layering different samples of very narrow densities in decreasing order. Thus, a gradient is produced whose density continuously increases from the top towards the bottom of the sample tube. Centrifugation is then performed at a comparatively low speed for a short time. The sample particles travel through the steep gradient and form discrete zones depending upon their sedimenting rate (Figure 5.13). The centrifugation must be terminated before any of the zones reaches the bottom of the centrifuge tube. This method is extremely useful for the separation of proteins possessing nearly identical densities but differing slightly in their size. It is used for the separation of RNA-DNA hybrids, ribosomal subunits and subcellular organelles.

![Diagram](image)

**Figure 5.13** Rate zonal centrifugation

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**Isopycnic centrifugation** Isopycnic centrifugation depends only upon the buoyant density of the particle. It does not depend upon the shape or size of the particle and is independent of time. In isopycnic centrifugation, the maximum density of the gradient always exceeds the density of the densest particle. A continuous density gradient is always used. During centrifugation,
sedimentation of the particle occurs until the buoyant density of the particle and the density of the gradient are equal. At this point of isodensity, no further sedimentation occurs, irrespective of how long centrifugation continues. This technique is used to separate particles of similar size but of differing density (Figure 5.14). Subcellular organelles such as Golgi apparatus, mitochondria and peroxisomes can be effectively separated by this method.

![Centrifugal field](image)

**Figure 5.14** Isopycnic centrifugation using the equilibrium isodensity method. (a) Particles distributed homogeneously throughout the tube prior to centrifugation. (b) During centrifugation the gradient is allowed to establish itself, sample particles redistribute and band in a series of zones at their respective isopycnic positions.

**Centrifugal elutriation** In this technique the separation and purification of a large variety of cells from different tissues and species can be achieved by a gentle washing action, using an elutriator rotor. The technique is based upon differences in the equilibrium set-up in the separation chamber of the rotor, between the opposing centrifugal liquid flow and applied centrifugal field being used to separate particles mainly on the basis of differences in their size. The technique does not employ a density gradient and has the advantage that any medium totally compatible with the particles can be used, for example buffered salt solutions or culture medium; because pelleting of the particles does not occur, fractionation of delicate cells or particles, between 5 and 50 μm diameter, can be achieved with minimum damage so that cells
tain their viability. Separations can be achieved very quickly, giving high cell concentrations and a very good recovery yield.

A second system of classification of centrifugation is the rate speed at which the centrifuge is operated. Ultracentrifugation is carried out at a speed faster than 20,000 rpm. Superspeed centrifu-gation is at speeds between 10,000 and 20,000 rpm. Low-speed centrifugation is at a speed below 10,000 rpm.

A third method of defining centrifugation is by the way the samples are applied to the centrifuge tube. In moving boundary (or differential centrifugation), the entire tube is filled with sample and centrifuged. Through centrifugation, one obtains a separation of particles but any particle in the mixture may end up in the supernatant or in the pellet or it may be distributed in both fractions, pending upon its size, shape, density, and conditions of centrifugation. The pellet is a mixture of all of the sedimented components, and it is contaminated with whatever unsedimented articles are in the bottom of the tube initially. The only component, rich is purified, is the slowest sedimenting one, but its yield is then very low. The two fractions are recovered by decanting the supernatant solution from the pellet. The supernatant can be centrifuged at higher speed to obtain further purification, with the formation of a new pellet and supernatant.

In rate-zonal centrifugation, the sample is applied in a thin layer at the top of the centrifuge tube on a density gradient (figure 5.13). Under centrifugal force, the particles will begin sedimenting through the gradient in separate zones according to their size, shape and density. The run must be terminated before the separated particles reach the bottom of the tube.

In isopycnic technique, the density gradient column compasses the whole range of densities of the sample particles, each sample is uniformly mixed with the gradient material (figure 5.14). Each particle will sediment only to the position in a centrifuge tube at which the gradient density is equal to its own density, and will remain there. The isopycnic technique, therefore, separates particles into zones solely on the basis of their density.
SAFETY ASPECTS IN THE USE OF CENTRIFUGES

There are a number of safety precautions that must be adhered to when using any centrifuge and rotor.

1. Before running a centrifuge, check the classification details on the centrifuge to ensure that the rotor is safe to use in the centrifuge at hand.

2. Never use an alkali detergent on a rotor (most are highly alkaline—be sure to check before use).

3. Always clean and completely dry the rotor after every use. Any spilled materials, especially salts and corrosive solvents must be removed immediately with running water. Fixed angle rotors are stored upside down, to drain after thorough cleaning and rinsing. Swinging buckets have only the buckets cleaned and dried, and stored inverted and with the caps removed. Never immerse the rotor portion of a swinging bucket rotor. Inevitably the linkage pins will rust, as it is virtually impossible to remove all fluids from them.

4. Be especially careful not to scratch the surface of a rotor or bucket. Use plastic brushes only. Normal wire brushes will scratch the anodized surface of aluminum rotors, which will increase the likelihood of corrosion. The anodized layer is extremely thin and is the main defence against corrosion of an aluminum rotor.

5. Always use a proper centrifuge tube. Glass tubes are used in clinical centrifuges only. High Speed Corex tubes can be used up to 15,000 rpm (in SS34 rotor). All ultracentrifugation use employs nitrocellulose or polyallomer tubes. Nitrocellulose tubes will collapse in a strong centrifugal field if old.

6. Always fill the centrifuge tubes to the proper level. (Usually fill to within 1/2 inch of the top). The tubes are thin-walled and will collapse if improperly filled.

7. Always balance the rotor properly. Use a precision scale for most work. Always balance the tube with a medium that is identical to that being centrifuged, i.e., do not balance an alcohol solution with water, or a dense sucrose solution with
water. For swinging buckets; be sure the buckets are weighed with their caps in place, that the seals are intact and that the caps are secure. Be careful in the placement of tubes within a rotor to ensure proper balance. Check the manufacturer’s guidelines for complex rotors that hold multiple tubes.

8. Ensure that the rotor is properly seated within the centrifuge. For swinging buckets, ensure that they are hanging properly. For preparative rotors, be sure the rotor cover is in place and properly screwed down, where appropriate. Never use a rotor without its lid.

9. Check that the centrifuge chamber is clean, defrosted and that all membranes or measuring devices are intact and functional (Beckman speed and temperature controls) and that the lid is securely closed.

10. Adjust acceleration rates, deceleration rates, temperature and rpm controls as appropriate. Set brake on or off as appropriate and check vacuum level where appropriate.

11. Start the centrifuge and set the timer. Do not attempt to open the centrifuge until the rotor has come to a complete stop.

12. Before opening the centrifuge, record the appropriate information in the centrifuge log.

If properly balanced and used, the rotor should accelerate smoothly and with a constant change in the pitch of the motor sound. In the case of any vibrations or unusual sounds the operator should immediately cease operation. Never leave the centrifuge until you are certain that it has reached its operating speed and is functioning properly. All rotors go through a minor vibration phase when they first start. There will be a minor flutter when the rotor reaches this vibration point; do not confuse this with a serious vibration caused by imbalance.