Notes on centrifugation, and cell fractionation

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Centrifugation

Centrifugation is a process which involves the application of the centripetal force for the sedimentation of heterogeneous mixtures with a centrifuge. This process is used to separate two immiscible substances. More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis.

The rate of centrifugation is specified by the angular velocity measured in revolutions per minute (RPM), or acceleration expressed as *g*. The particles' settling velocity in centrifugation is a function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity.

Principle:

If a container is filled with a suspension of particles of varying size and density, the particles will gradually settle to the bottom of the container under the influence of gravity. The rate at which settling occurs can be greatly increased by increasing the gravitational effect on the particles. This is the rationale behind the use of centrifugation.

A tube containing a suspension of particles (e.g., a tissue homogenate) is placed in the rotor of a centrifuge and then is rotated at high speed.

The resulting acceleration greatly increases the force acting on the suspended particles, causing their more rapid sedimentation to the bottom of the tube along paths that are perpendicular to the axis of rotation.

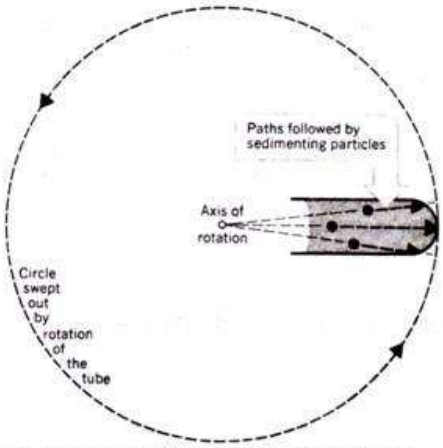


FIGURE 12–1 Paths followed by particles during centrifugation. The centrifuge tube is being viewed from a position that is above the plane in which the tube rotates.

The force acting on a particle during centrifugation is given by the equation: $F = m\omega^2 x$

Where m is the mass of the particle,

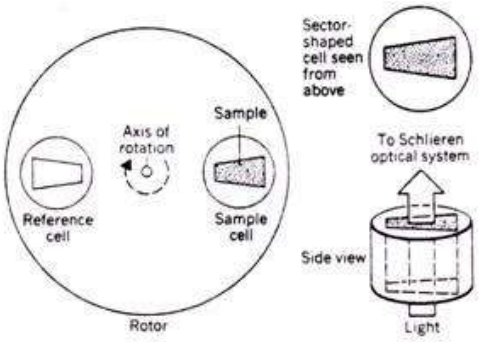
ω (omega) is the angular velocity of the spinning rotor in radians per second, and

x is the distance of the particle from the axis of rotation.

Usually, the value given for the force applied to particles during centrifugation is a relative one, that is, it is compared with the force that the earth's gravity would have on the same particles. It is called relative centrifugal force or RCF and is given by

$$\text{RCF} = \frac{F_{\text{centrifugation}}}{F_{\text{gravity}}} = \frac{m\omega^2 x}{mg} = \frac{\omega^2 x}{g}$$

Where g is acceleration due to gravity and equals 980 cm/sec/sec.



Ultracentrifugation:

Ultracentrifugation makes use of high centrifugal force for studying properties of biological particles. Ultracentrifuges can isolate much smaller particles, including ribosomes, proteins, and viruses. Ultracentrifuges can also be used in the study of membrane fractionation. This occurs because ultracentrifuges can reach maximum angular velocities in excess of 70,000 rpm.

Differential Centrifugation:

Differential centrifugation is one of the classic procedures for isolating subcellular particles and involves the stepwise removal of classes of particles by successive centrifugations at increasing RCF.

During centrifugation, particles sediment through the medium in which they are suspended at rates related to their size, shape, and density. Differences in the sedimentation coefficients of the various subcellular particles provide the means for their effective separation.

The material to be fractionated is subjected first to low-speed centrifugation to sediment the largest (or densest) particles present. Following this, the unsedimented material (called the supernatant) is transferred to another tube and centrifuged at a higher speed (and usually also for a longer period of time) to sediment particles of somewhat smaller size (or lower density).

Cell Fractionation using differential centrifugation:

- The removed liver tissue is homogenized in cold buffer and centrifuged 10 minutes at 700 g. This is usually sufficient to sediment all the cell nuclei to the bottom of the centrifuge tube, thereby providing the nuclear fraction.

- The overlying supernatant (called the nuclear supernatant) is removed and transferred to another tube for a second centrifugation at 20,000 g for 15 minutes. These sediments nearly all the mitochondria (i.e., the mitochondrial fraction).
- Again, the supernatant (i.e., mitochondrial supernatant) is removed and is subjected to a third centrifugation at 105,000 g for 60 minutes. This causes the sedimentation of a fraction called microsomes, which includes ribosomes and small fragments of intracellular membranes.

In this way the liver tissue is separated into four major fractions.

Uses:

1. **Laboratory separations**

Centrifuges are used in chemistry, biology, biochemistry and clinical medicine for isolating and separating suspensions and immiscible liquids.

2. **Isotope separation**

The Zippe-type centrifuge, separate isotopes, and these kinds of centrifuges are in use in nuclear power and nuclear weapon programs. Gas centrifuges are used in uranium enrichment.

3. **Aeronautics and astronautics**

Human centrifuges are exceptionally large centrifuges that test the reactions and tolerance of pilots and astronauts to acceleration above those experienced in the Earth's gravity.

4. **Geotechnical centrifuge**

Geotechnical centrifuge modeling is used for physical testing of models involving soils.

5. **Synthesis of materials**

High gravity conditions generated by centrifuge is applied in the chemical industry, casting, and material synthesis.

6. **Commercial applications**

- Standalone centrifuges- for drying (hand-washed) clothes – usually with a water outlet.
- Washing machines
- Large industrial centrifuges are commonly used in water and wastewater treatment to dry sludges.
- Large industrial centrifuges are also used in the oil industry to remove solids from the drilling fluid.
- Centrifuges are used to separate cream (remove fat) from milk.

Use:

- Remove cellular debris from blood to separate cell free plasma or serum
- Concentrate cellular elements and other components for microscopic analysis or chemical analysis.
- Separate protein bound or antibody bound ligand from free ligand in immunological assay.
- Extract solutes from aqueous or organic solvents.
- Separate lipid components like chylomicrons from other components of plasma.

Cell Fractionation

Cell fractionation is the process of producing pure fractions of cell components. The process involves two basic steps: disruption of the tissue and lysis of the cells, followed by centrifugation.

Method:

A. Tissue Disruption and Lysis-

The first step in cell fractionation is tissue disruption and cell lysis. Tissues can be broken up and cells lysed in a number of ways such as

1) Homogenization- Homogenization involves the use of a mechanical homogenizer, like a blender or a mortor and pestle, to break the tissue and lyse the cells.

2) Sonication- Sonication involves the use of ultrasound to disrupt the cells. Sonication is often used when prokarytic cells are to be lysed.

3) Osmotic lysis- Red blood cells are a perfect example of a cell that can easily be lysed through osmotic stress.

B. Centrifugation.

The second step in the cell fractionation process is **centrifugation**. Most of the cellular components in a cell lysate will settle to the bottom of a tube. To accelerate this process, the lysate can be subjected to centrifugation. In centrifugation, the lysate is rotated at a certain speed (expressed as rotations per minute (RPM)). This rotation imposes a force on the particles perpendicular to the axis of rotation. The force is called a **relative centrifugal force (RCF)**, expressed as a multiple of the force of Earth's gravitational force (x g). For example, an RCF of 1000 x g is a force 1000 times greater than Earth's gravitational force. When a particle is subjected to centrifugal force, it will migrate away from the axis of rotation at a rate dependent on the particle's size and density.