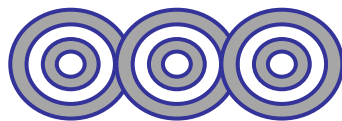


# Chemical Separation and Chromatographic Methods



## Separations Based on Mass or Density

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# **Centrifugation**

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# Centrifugation

If there is a difference in the mass or density of the analyte and interferent, then a separation using centrifugation may be possible.

The sample, as a suspension or two immiscible liquids, is placed in a centrifuge tube and spun at a high angular velocity (high numbers of revolutions per minute, **rpm**).

Particles experiencing a greater centrifugal force have faster sedimentation rates and are preferentially pulled toward the bottom of the centrifuge tube.

For particles of equal density the separation is based on mass, with heavier particles having greater sedimentation rates. When the particles are of equal mass, those with the highest density have the greatest sedimentation rate.

Bench-top centrifuge capable of reaching speeds up to 14,000 rpm and centrifugal forces of  $20,800 \times g$ .

This particular centrifuge is refrigerated, allowing samples to be cooled to temperatures as low as  $-4\text{ }^{\circ}\text{C}$ .



The concept of centrifugal force is applied in rotating devices

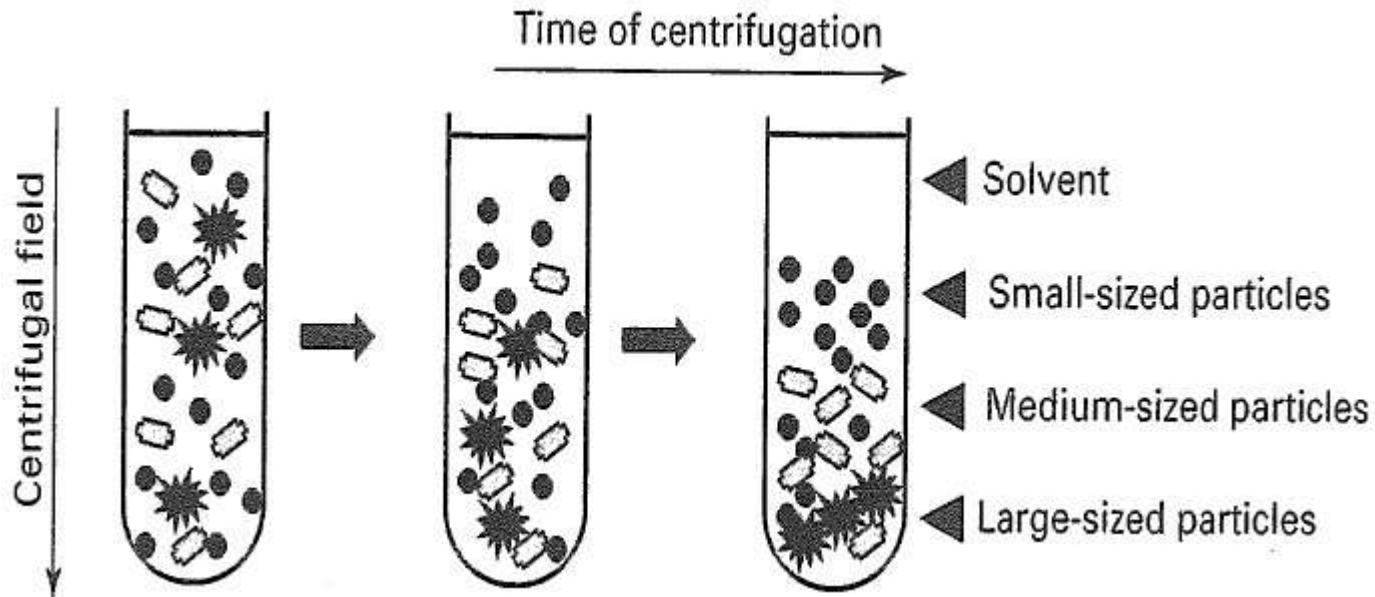
## Conditions for the separation of selected cellular components by centrifugation.

Components	Centrifugal Force ( $\times g$ )	Time (min)
eukaryotic cell	1000	5
cell membranes, nuclei	4000	10
mitochondria, bacterial cells	15,000	20
lysosomes, bacterial membranes	30,000	30
ribosomes	100,000	180

Centrifugation is of particular importance as a separation technique in **biochemistry**. Cellular components can be separated by centrifugation.

For example, lysosomes can be separated from other cellular components by repeated differential centrifugation, in which the sample is divided into a solid residue and a solution called the supernatant. After destroying the cell membranes, the solution is centrifuged at  $15,000 \times g$  (a centrifugal field strength that is 15,000 times that of the Earth's gravitational field) for 20 min, leaving a residue of cell membranes and mitochondria. The supernatant is isolated by decanting from the residue and is centrifuged at  $30,000 \times g$  for 30 min, leaving a residue of lysosomes.

# Differential Centrifugation



Based on differences in sedimentation rate of the sample particles of different size, shape and density.

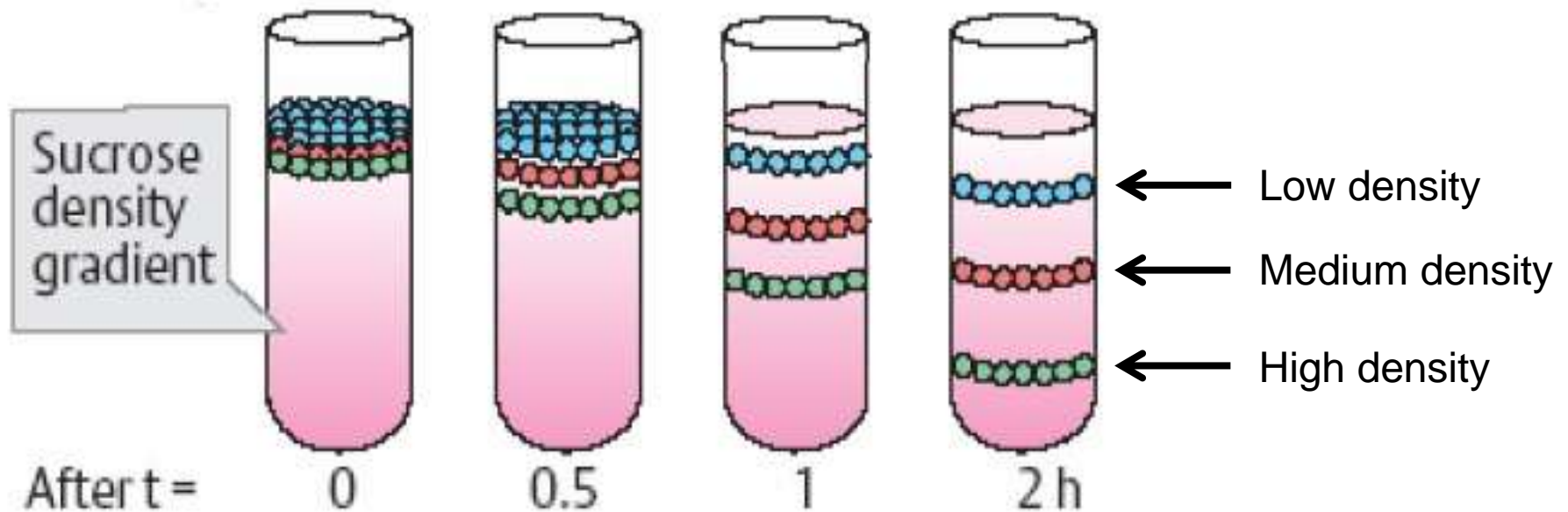
An alternative approach to differential centrifugation is **equilibrium-density-gradient centrifugation**. The sample is either placed in a solution with a preformed density gradient or in a solution that, when centrifuged, forms a density gradient.

Density gradients can be established with solutions of **sucrose** or **CsCl**. To prepare a sucrose density gradient, for example, a solution with a smaller concentration of sucrose - and, thus, of lower density - is gently layered upon a solution with a higher concentration of sucrose. Repeating this process several times, fills the centrifuge tube with a multi-layer density gradient.

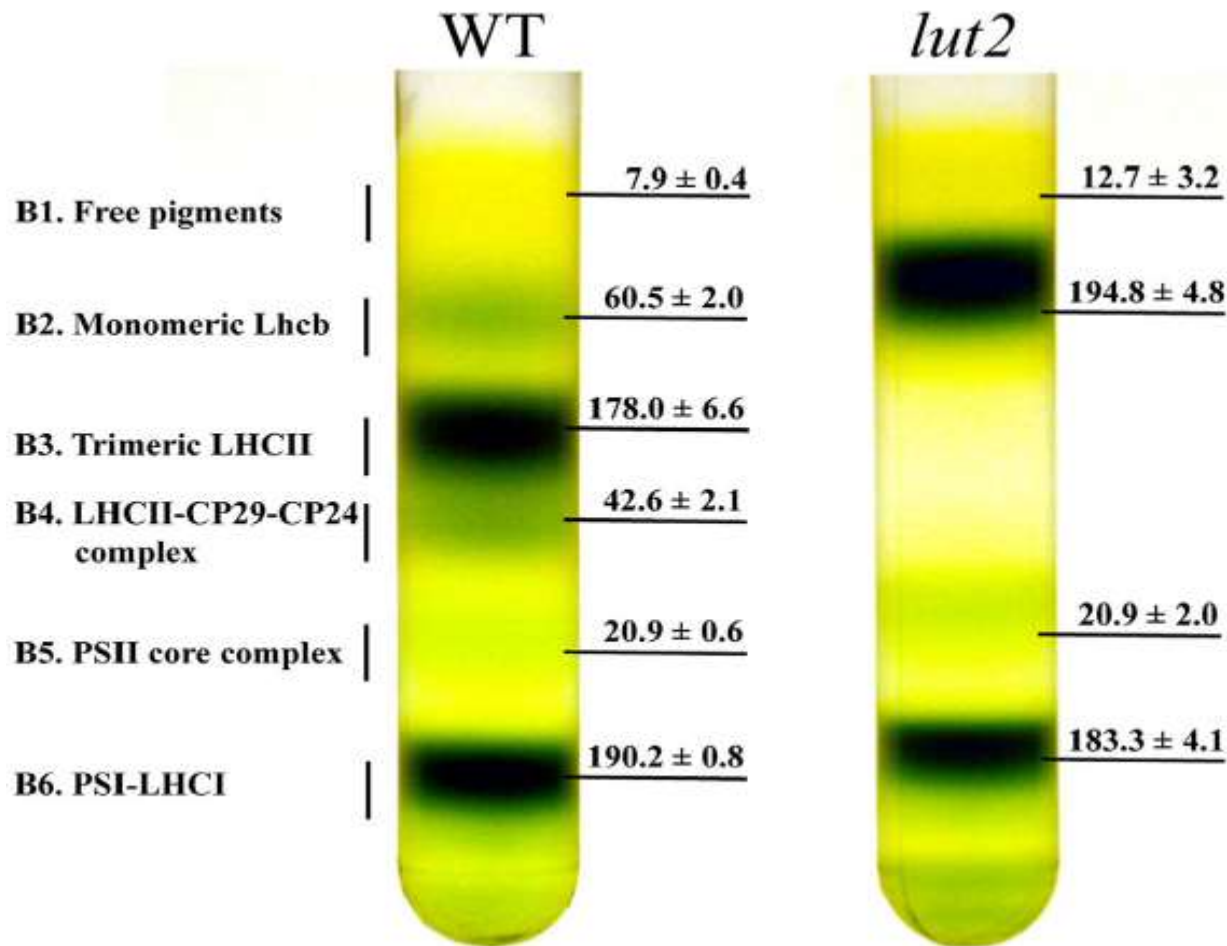
The sample is placed on top of the density gradient and centrifuged using a force greater than  $150,000 \times g$ . Because the solution's density increases toward the bottom of the centrifuge tube, the sedimentation rate for each component decreases as it moves toward the bottom of the centrifuge tube.

During centrifugation, each of the sample's components moves through the gradient until it reaches a position where its density matches the surrounding sucrose solution. When a component reaches a position where its density is equal to that of the solution, the centrifugal force drops to zero and sedimentation stops. Each component, therefore, is isolated as a separate band positioned where its density is equal to that of the local density within the gradient solution.

# Density Gradient Centrifugation







Example of a typical sucrose density gradient centrifugation for separating plant thylakoid membranes from wild type (WT) and *lut2* plants. The thylakoid membranes were extracted from the plant's leaves and separated by centrifuging in a 0.1–1 M sucrose gradient for 22 h at  $280,000 \times g$  at 4 °C. Six bands and their chlorophyll contents are shown.

For example, a mixture of **proteins**, **RNA**, and **DNA** can be separated in this way since their densities are different.

A density gradient from 1.65 g/cm<sup>3</sup> to 1.80 g/cm<sup>3</sup> is established using CsCl.

Proteins, with a density of less than 1.3 g/cm<sup>3</sup> experience no sedimentation, whereas RNA, with a density of greater than 1.8 g/cm<sup>3</sup> collects as a residue at the bottom of the centrifuge tube. The DNA, which has a density of approximately 1.7 g/cm<sup>3</sup> separates as a band near the middle of the centrifuge tube.

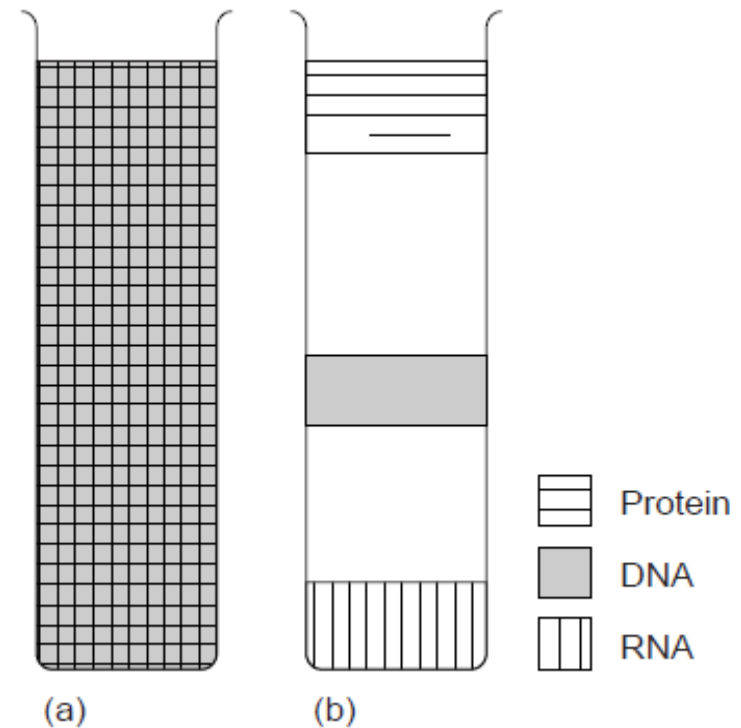


Illustration showing separation by equilibrium-density-gradient centrifugation.

The homogeneous mixture in (a) separates into three bands (b) after applying centrifugal force.

# Other Applications

- Water treatment (separation of solid substances, separation of oily suspensions).
- Remove fat from milk to produce skimmed milk
- Separating textile.
- Removing water from plant leaves (e.g. lettuce) after washing it in a salad spinner.
- Separating particles from an air-flow using cyclonic separation (removing particulates from an air, gas or liquid stream, without the use of filters, through vortex separation).
- It is used to separate urine components.
- It is used in labs and forensic labs to separate the components of blood (separation plasma from blood samples).

