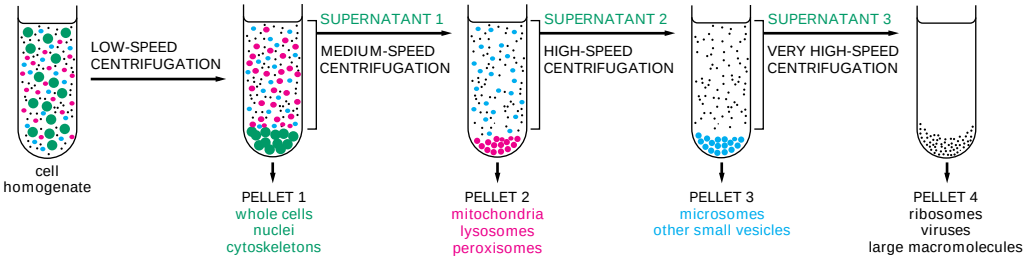
Cell Breakage and Fractionation - Part 2

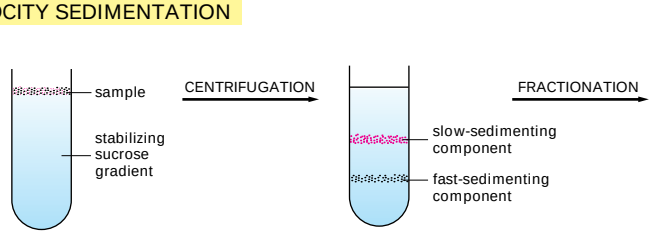
DIFFERENTIAL CENTRIFUGATION

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

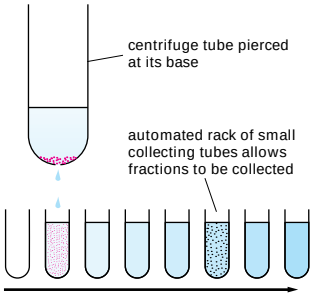
Centrifugation separates cell components on the basic of size and density. The larger and denser components experience the greatest centrifugal force and move most rapidly. They sediment to form a pellet at the bottom of the tube, while smaller, less dense components remain in suspension above, called the supernatant.



VELOCITY SEDIMENTATION



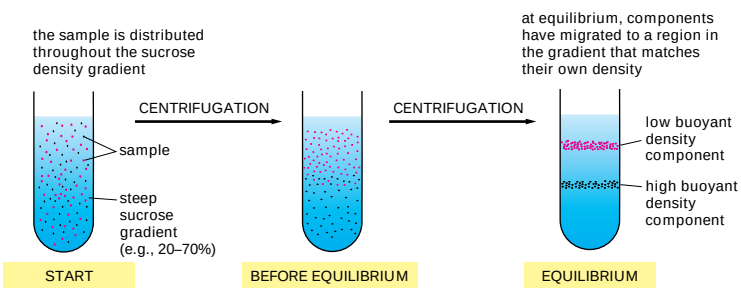
Subcellular components sediment at different speeds according to their size when carefully layered over a dilute salt solution. In order to stabilize the sedimenting components against convective mixing in the tube, the solution contains a continuous shallow gradient of sucrose that increases in concentration toward the bottom of the tube. This is typically 5–20% sucrose. When sedimented through such a dilute sucrose gradient, different cell components separate into distinct bands that can, after an appropriate time, be collected individually.



After an appropriate centrifugation time the bands may be collected, most simply by puncturing the plastic centrifuge tube and collecting drops from the bottom, as shown here.

EQUILIBRIUM SEDIMENTATION

The ultracentrifuge can also be used to separate cellular components on the basis of their buoyant density, independently of their size or shape. The sample is usually either layered on top of, or dispersed within, a steep density gradient that contains a very high concentration of sucrose or cesium chloride. Each subcellular component will move up or down when centrifuged until it reaches a position where its density matches its surroundings and then will move no further. A series of distinct bands will eventually be produced, with those nearest the bottom of the tube containing the components of highest buoyant density. The method is also called density gradient centrifugation.



A sucrose gradient is shown here, but denser gradients can be formed with cesium chloride that are particularly useful for separating the nucleic acids (DNA and RNA).

The final bands can be collected from the base of the tube, as shown above.