

## Lab#6

## Centrifugation

The main use of centrifuge is to separate biologically important substances, and very few experiments can be done without at least one spin in a centrifuge.

A **centrifuge** is a device for separating particles from a solution. In the biological research lab, these particles are usually cells, organelles, or large molecules, such as DNA.

### Centrifugation

There are two main kinds of centrifugation procedures: **Preparative**, the isolation of specific particles; and **analytical**, the measuring of the physical properties of a sedimenting particle.

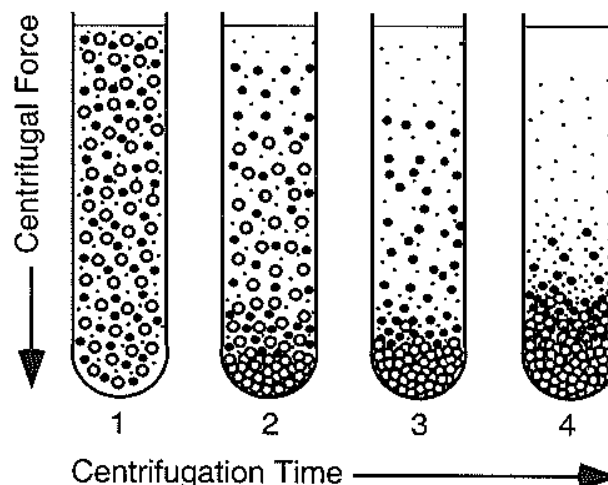
Most of centrifugation done in a molecular or cell biology lab is preparative centrifugation.

#### **Differential centrifugation (pelleting)**

**Principle:** Samples are spun at a given speed, resulting in a supernatant and a pellet fraction. The sample is isolated by sedimentation velocity that is proportional to the size of the particle and the difference between the density of the particle and the liquid.

**Disadvantage:** The pellet is a mixture of all the sedimented components, not all of which are desired.

**Examples:** Pelleting bacteria or cells from growth medium, collecting precipitated DNA.



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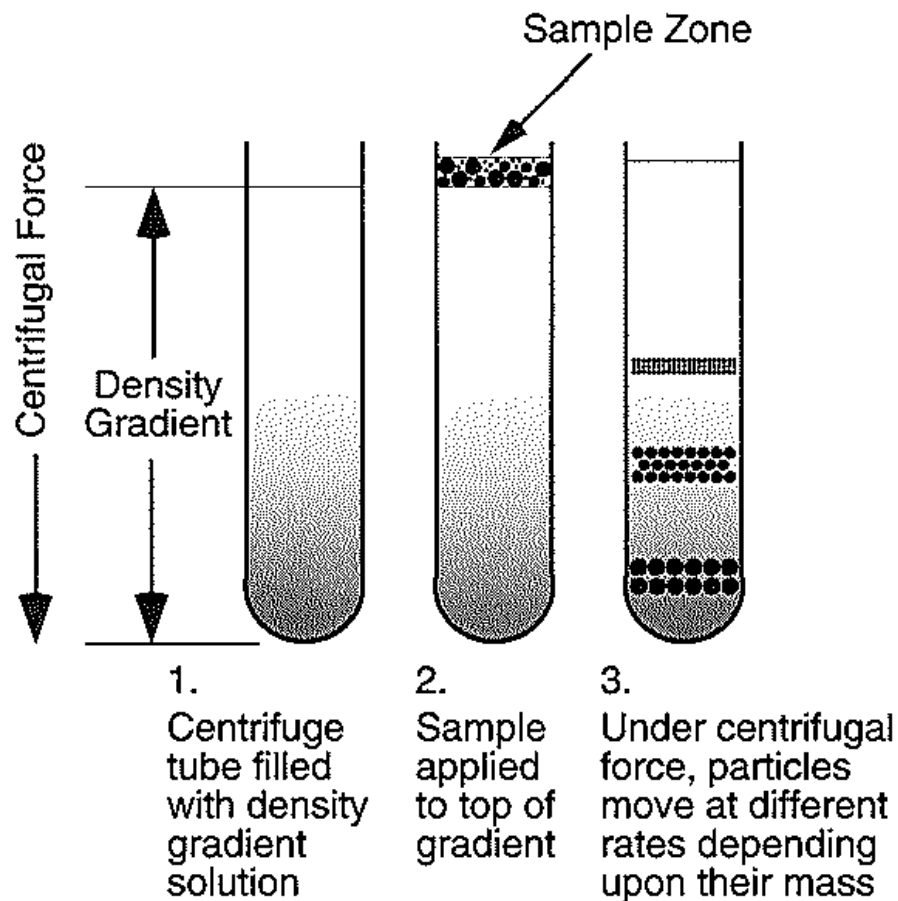
## Centrifugation

### Density gradient centrifugation

- **Rate-zonal centrifugation**

*Principle:* Separates particles having a similar buoyant density but differing in shape or particle size. Sample is layered on the top of a gradient of sucrose or other viscous medium. The particle density is higher than the liquid density, so the particle will ultimately pellet. Centrifugation must be stopped when the particles have been separated, but before all particles have reached the bottom of the tube.

*Examples:* Isolation of ribosomal subunits on sucrose gradient.



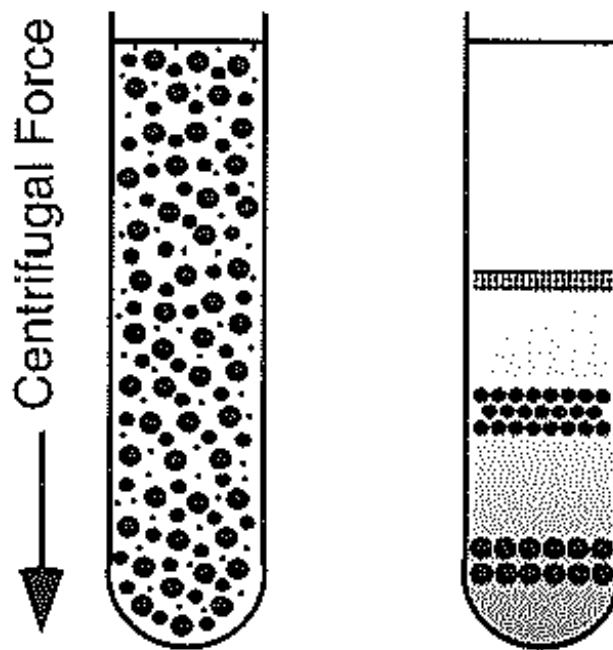
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- Isopycnic (Isodensity) density gradient centrifugation

*Principle:* It is also used to separate particle on the basis of buoyant density. Sample is mixed with gradient material such as cesium chloride to provide density equal to average density of the particle. This homogeneous suspension is spun and a gradient formed during the spin. Particles stop sedimenting when they reach their buoyant density.

*Examples:* Isolation of plasmid DNA in cesium chloride gradient.



1. Uniform mixture of sample and gradient

2. Under centrifugal force, gradient redistributes and sample particles band at their isopycnic positions

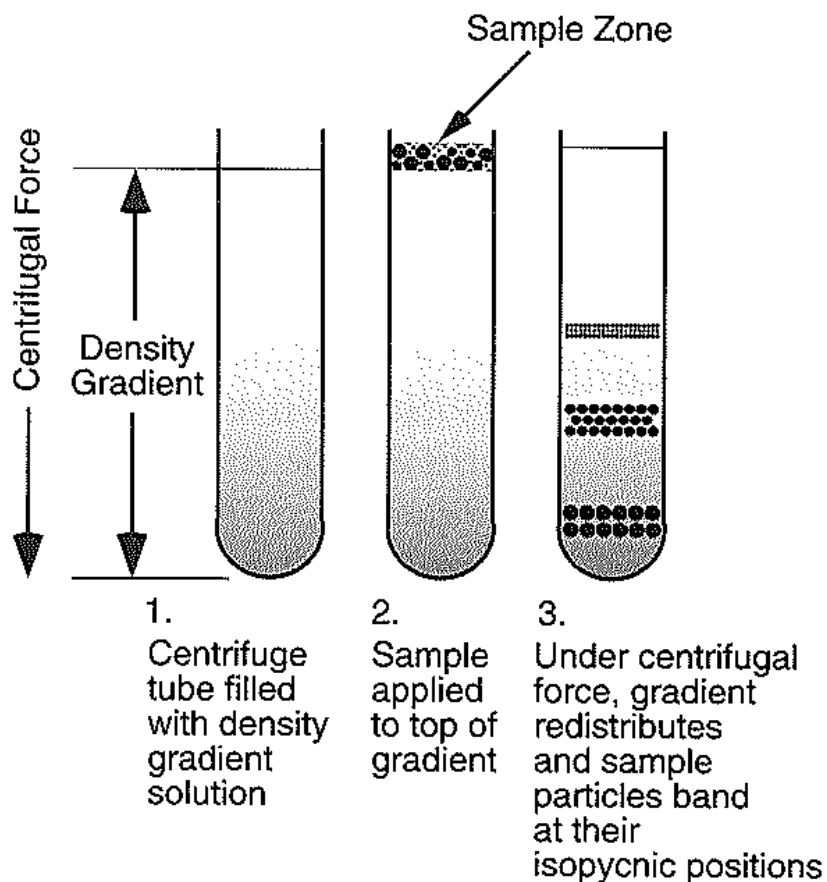
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- **Equilibrium density gradient centrifugation**

*Principle:* Like isopycnic density gradient centrifugation, used to separate particles on the basis of buoyant density. Equilibrium density gradient centrifugation is done with a preformed gradient instead of self-generated one. The sample is centrifuged in a density gradient of a medium of density higher than the density of the cells or particles until equilibrium is reached, at which each particle has migrated to a point in the gradient where it has the same density as the surrounding solution.

*Examples:* isolation of lymphocytes



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### Centrifuges

High speed and ultracentrifuges are built with refrigeration units, needed because of the heat generated by high-speed spins.

- **Benchtop centrifuge.** Also known as multipurpose centrifuge.

*Uses:* Pellet cells and bacteria, phenol extractions.

*Speed:* 17000 *g*/14000 rpm (revolution per minute)

- **Clinical centrifuge**

*Uses:* Serum, urine, cells, and blood sedimentation

*Speed:* 4600 *g*/6000 rpm

- **Microfuge**

*Uses:* Mini-phenol extraction and ethanol precipitations.

*Speed:* 16000 *g*/14000 rpm

*Tubes:* Eppendorfs, 0.5 ml-2.0ml.

- **High-speed centrifuge.** Also known as high performance centrifuge.

*Uses:* Large-volume ethanol precipitations, pelleting bacteria, protein precipitations.

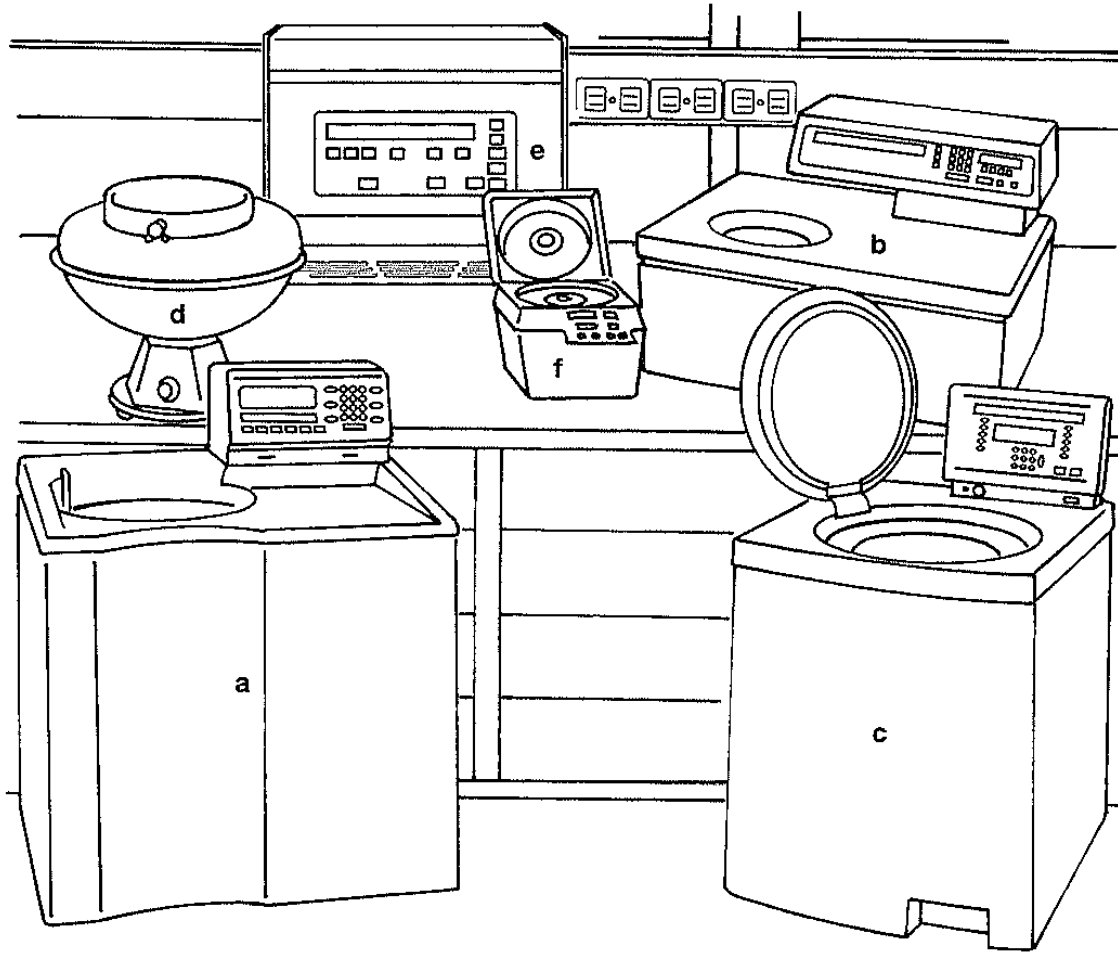
*Speed:* 75000 *g*

- **Ultracentrifuge**

*Uses:* Virus concentration, membrane subcellular fraction isolation, DNA and RNA isolation.

*Speed:* 800,000 *g*/120,000 rpm

*Tubes:* Polyallomer, nitrocellulose



**FIGURE 5.**

Commonplace centrifuges are the ultracentrifuge, floor (*a*) and bench (*b*) models; the high-speed (*c*); clinical (*d*); general purpose (*e*); and microfuge (*f*).