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ULTRACENTRIFUGATION: PRINCIPLES AND APPLICATION

Centrifugation, and ultracentrifugation, is nowadays, at the core of the laboratory routine. They are used on a day-to-day basis in a wide range of experimental protocols, from concentrating solutions to isolating cells and subcellular components. In biology, the development of ultracentrifugation in the early 1900s, widened the possibilities of scientific research to the subcellular level, allowing for the separation of cellular components, such as organelles, lipid membranes, and even to purify proteins and ribonucleic acids (DNA and RNA).

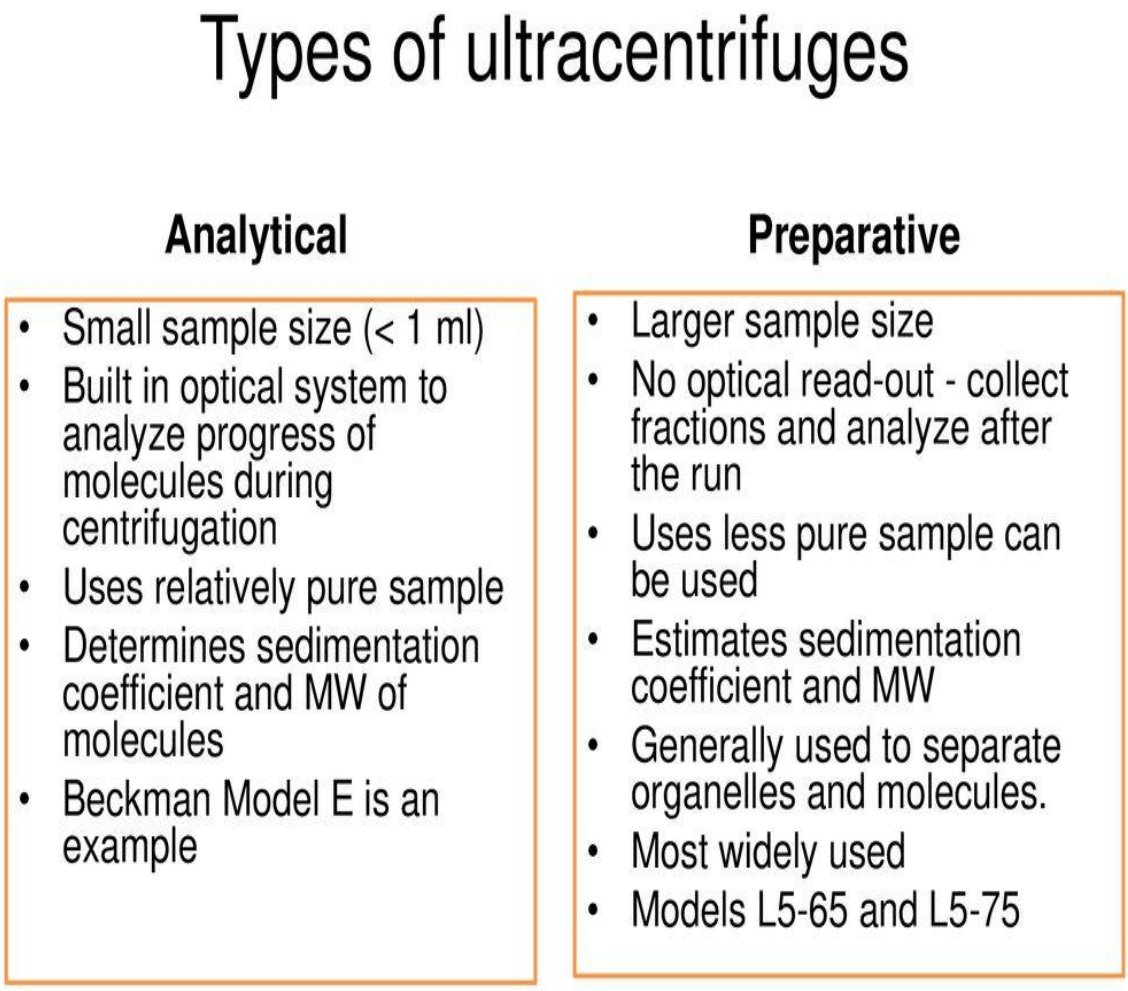
Principle of Ultracentrifuge :

- The ultracentrifuge works on the same principle as all other centrifuges. The working of an ultracentrifuge is based on the sedimentation principle, which states that the denser particles settle down faster when compared to less dense particles under gravity.
- However, the sedimentation of particles under gravity would take a larger amount of time, and that is why an additional force is applied to aid the sedimentation process.
- In an ultracentrifuge, the sample is rotated about an axis, resulting in a perpendicular force, called centrifugal force, that acts on different particles on the sample.
- The larger molecules move faster, whereas the smaller molecules move slower.
- At the same time, denser molecules are moved outwards to the periphery of the tubes whereas the less dense molecules are rotated towards the center of the tube.
- Once the process is completed, the larger and more dense particles settle down, forming pellets at the bottom of the tube. In comparison, the smaller and less dense particles remain either in the suspended in the supernatant or float on the surface.

Types of Ultracentrifugation :

There is, currently on the market, a wide variety of ultracentrifuges. The choice among different brands and models must consider the type of experimental applications to be performed, the availability of different rotors (making it possible to adapt the ultracentrifuge to different experimental settings) and the temperature range. **In that sense, two types of ultracentrifuges are available: analytical and preparative. Analytical ultracentrifugation** is used in the study of purified macromolecules or supramolecular assemblies, while **preparative**

ultracentrifugation is used in the actual separation of tissues, cells, subcellular components and other biochemically interesting particles.



- **Differential centrifugation** is used to separate the components of a solution based on differences in the sedimentation rate of the different components of the mixture. As explained above (*see section 2*: *The Principle of Ultracentrifugation*), the sedimentation properties of a substance depend on its size and density but also on the density of the solvent.
    1. In medical and biology labs, crude tissue homogenates containing organelles, membrane vesicles, and other structural fragments are divided into different fractions by the stepwise increase of the applied centrifugal field. Furthermore, differential centrifugation is also routinely used in the isolation of non-living substances, like nanoparticles, colloids, and viruses The general principle of differential centrifugation is outlined in Figure 1.

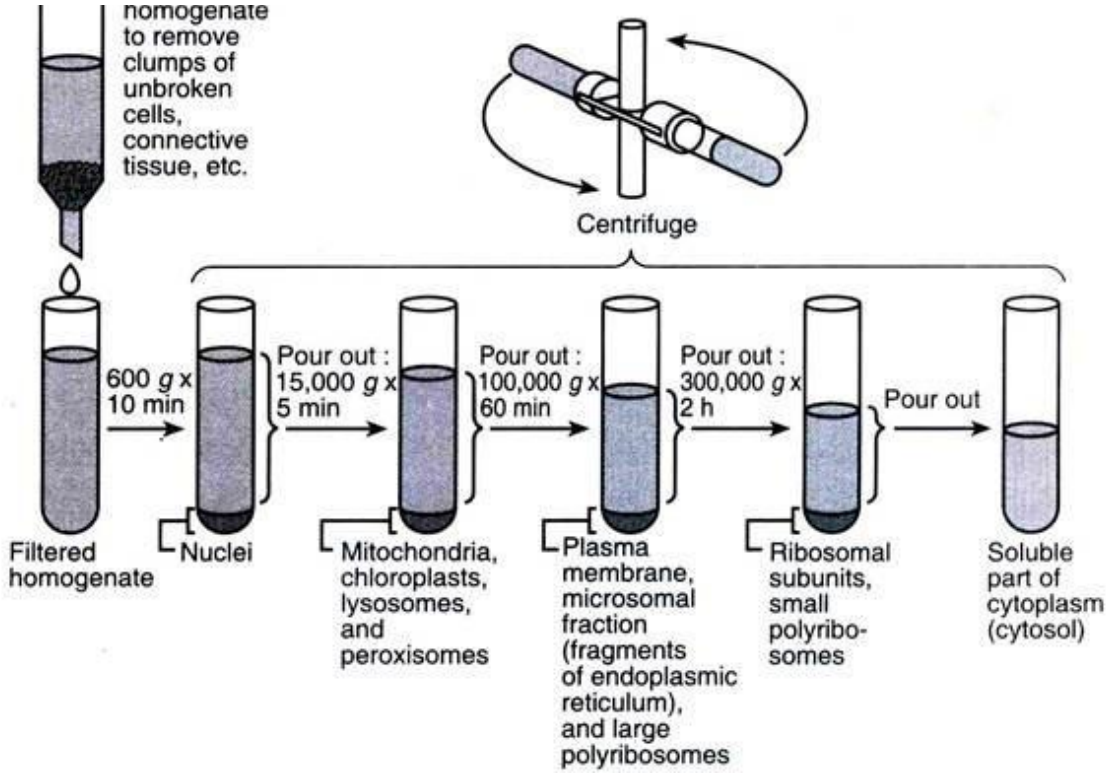


Figure 1: General principle of differential centrifugation, applied to subcellular fractionation (image credit: (Kumar))

Density gradient centrifugation ----It is ideal when the goal is to isolate particles of similar sizes, but different densities. In this case, it is possible to establish density gradient solutions with increasing concentrations of specific materials, in the spinning tubes (Figure 2). Cesium salt gradients are used in the separation of DNA, and sucrose gradients are used in subcellular fractionation to isolate organelles and multiprotein complexes, like ribosomes.

Roughly, there are two types of density gradient centrifugation: rote-zonal centrifugation and isopycnic centrifugation (also called equilibrium centrifugation), which differ in the way particles are separated across the gradient (see Figure 2).

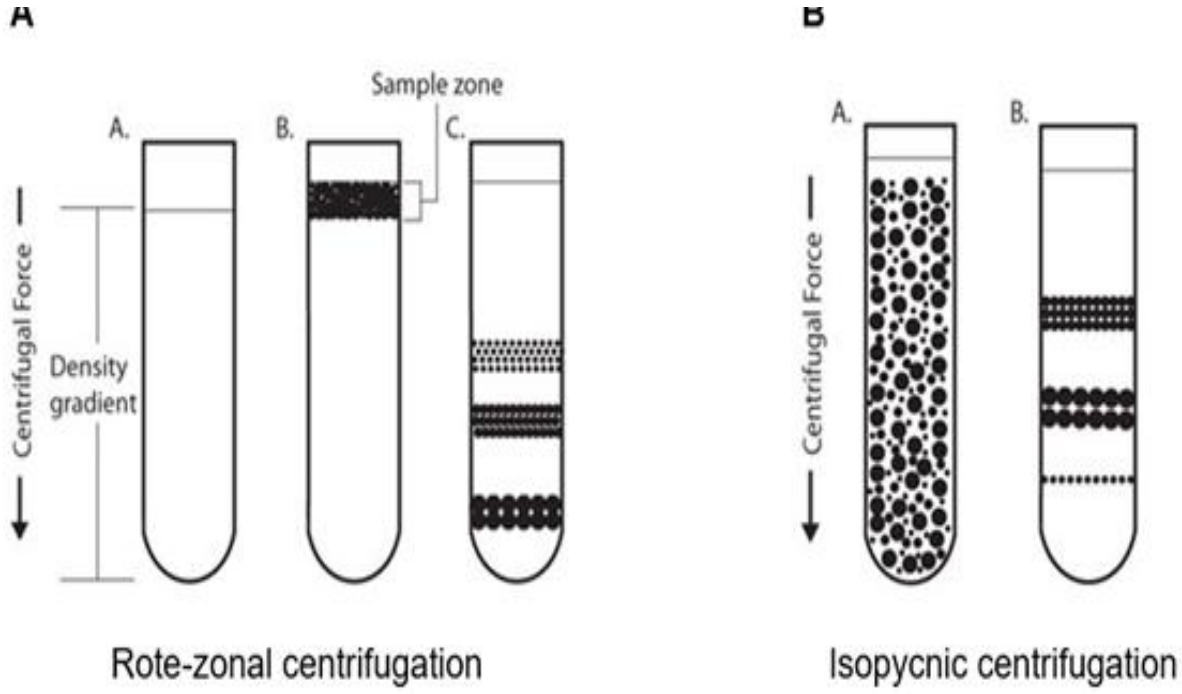


Figure 2: Types of preparative centrifugation. A – Rote-zonal centrifugation B – Isopycnic centrifugation.

A. **Rote-zonal centrifugation** – particle separation depends mostly on particle mass. Zones, or bands, are generated, each containing a particle fraction of a specific mass. However, care must be taken when performing rote-zonal centrifugation. Because the mass of the particles is higher than the density of the solvent, if they are centrifuged for too long, all particles will eventually deposit in the bottom of the tube.

B. **Isopycnic (equilibrium) centrifugation** –In isopycnic separation, particles are mixed with the gradient solution, and during centrifugation, they will move until they reach the gradient phase which equals their density (isopycnic or equilibrium point). Because the density of the gradient medium is always higher than the density of particles, these will never sediment, independently of the centrifugation time. Continuous gradients may be used in isopycnic centrifugation, however, discontinuous gradients in which particles form bands at the interface between the density gradient layers are more suitable for the separation of some biological samples, like the separation of lymphocytes from whole blood.

Uses of Ultra-Centrifuge:

- Preparative ultracentrifuges are used in biology for a pelleting fraction of cell organelles like mitochondria, ribosomes, and even viruses.
- Density gradient centrifugation uses caesium salt gradients for the separation of nucleic acids like DNA and RNA.
- Analytical ultracentrifuge allows the detection and characterization of macromolecular conformational changes due to changes in pH, temperature, and other environmental factors.
- AUC also allows the determination of stoichiometries of various macromolecules like molecular masses, size, etc.

- Analytical ultracentrifuges also help to differentiate between the assembly and disassembly of various biomolecular complexes.
- Ultracentrifuges are also used in the determination of densities of various macromolecules.

Besides, it also allows the purification of various biological Crude extracts.

The Ultracentrifuge: How to Use and How to Care Modern ultracentrifuges are heavy, sturdy equipment that requires certain *know-how* for proper usage and care.

1. **Rotor balance.** As in all centrifuges, sample spinning requires a proper balance of the weight inside the rotor. Moreover, in all ultracentrifuges, the rotor is encapsulated in a strong heavy metallic cage, to avoid vibrations and projections that could damage the sample and endanger the operator. Yet, it is of vital importance that the ultracentrifuge is properly loaded, according to the manufacturer's instructions.
2. **Sample position in rotor.** All rotor positions must be filled. Even when there are only a few tubes, the rest of the positions must be occupied with blank samples of equivalent weight. To ensure the proper function of the ultracentrifuge, care measures must be undertaken regularly. Apart from safety, proper loading of the rotor avoids excessive vibration, which can cause damage to the device.
3. **Centrifuge cleaning.** Maintenance and cleaning of the rotor must be done with non-abrasive detergents to avoid corrosion. Rotor cleaning is especially important to ensure that there are no remnants of the samples that were centrifuged, and therefore, should always be performed after **spinning.**
4. **position in rotor.** All rotor positions must be filled. Even when there are only a few tubes, the rest of the positions must be occupied with blank samples of equivalent weight. To ensure the

proper function of the ultracentrifuge, care measures must be undertaken regularly. Apart from safety, proper loading of the rotor avoids excessive vibration, which can cause damage to the device.
