Ultracentrifugation :Principle types and application

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Ultracentrifugation is a specialized technique used to spin samples at exceptionally high speeds. Current ultracentrifuges can spin to as much as 150 000 rotations per minute (rpm) (equivalent to 1 000 000 g). However, extreme centrifugal forces may cause overheating, so to avoid sample damage, ultracentrifuges are equipped with vacuum systems that keep a constant temperature in the centrifuge's rotor .

Centrifugation, and ultracentrifugation, is nowadays, at the core of the laboratory routine. Benchtop centrifuges are essential devices in any biology or chemistry laboratory, and they are used on a day-to-day basis in a wide range of experimental protocols, from concentrating solutions to isolating cells and sub cellular components. Ultracentrifugation widened the applications of *benchtop centrifugation*, allowing the isolation of smaller sized particles, and the study of purified molecules and molecular complexes . In biology, the development of ultracentrifugation in the early 1900s, widened the possibilities of scientific research to the subcellular level, allowing for the differential separation of cellular components, such as organelles, lipid membranes, and even to purify proteins and ribonucleic acids (DNA and RNA).

2. The Principle of Ultracentrifugation

The basis of ultracentrifugation is the same as *normal* centrifugation: to separate the components of a solution based on their size and density, and the density (viscosity) of the

medium (solvent). A general principle, (ultra)centrifugation abides by the following rules:

- the denser a biological structure is, the faster it sediments in a centrifugal field.
- the more massive a biological particle is, the faster it moves in a centrifugal field.
- the denser the biological buffer system is, the slower the particle moves in a centrifugal field.
- the greater the frictional coefficient (i.e., the friction between the component and the neighbouring environment) is, the slower a particle moves
- the greater the centrifugal force is, the faster the particle sediments
- the sedimentation rate of a given particle will be zero when the density of the particle and the surrounding medium is equal.

Types of Ultracentrifugation:

Analytical ultracentrifuges are equipped with optical detection systems that allow the researcher to follow the centrifugation process in real-time. These systems may use ultraviolet (UV) light absorption or refracting index interference (RII) optical detection systems (ultracentrifuges may be equipped with one or both types of optical systems) . While UV detection directly measures the absorbance (abs) of a substance at a specific wavelength, RII measures changes in the refraction index (radiation direction) of a given substance, compared to the solvent it is dissolved in . The purpose of analytical centrifugation is different from other types of centrifugation. Although component isolation is possible with analytical centrifugation, the goal of this technique is to obtain data to characterize the sample that is spun (sedimentation velocity, viscosity, concentration, etc.). With analytical centrifugation, it is possible to follow the variations in sample concentration as a function of the applied centrifugal force. This technique is used in two main experimental settings:

sedimentation velocity and sedimentation equilibrium studies, which are key in macromolecular characterization.
Preparative ultracentrifuges are mostly used to process biological samples for further analysis. The most common application of preparative ultracentrifugation is in tissue and sub cellular fractionation, to isolate increasingly smaller components of the biological samples. For that, two main centrifugation methods are used: differential and density-gradient centrifugation.

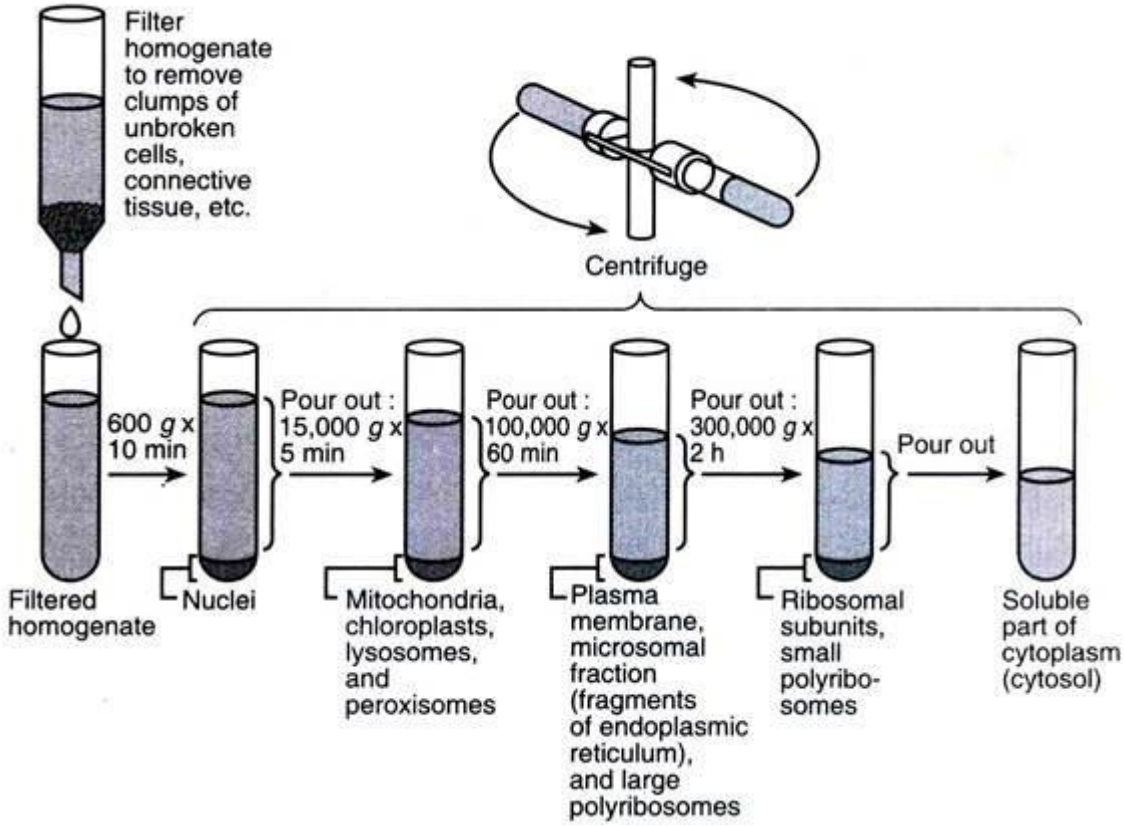

Figure: General principle of differential centrifugation, applied to subcellular fractionation.

Applications of Analytical and Preparative Ultracentrifugation

Analytical ultracentrifugation

- determination of the purity (including the presence of aggregates) and oligomeric state of macromolecules, by recording sedimentation velocity data
- determination of the average molecular mass of solutes in their native state
- Study of changes in the molecular mass of supramolecular complexes,
- using either sedimentation velocity, sedimentation equilibrium (or both)
- the detection of conformation and conformational changes

Preparative ultracentrifugation

- subcellular fractionation
- affinity purification of membrane vesicles
- separation of DNA components
- colloid separation
- virus purification

Thank you