

Ultracentrifuge

By- Dr. Ekta Khare

Ultracentrifuge

- Ultracentrifuge is a sophisticated and advanced centrifuge that operates at an extremely high speed and separates smaller molecules that cannot be separated from the traditional centrifuges.
- The speed of the rotors in ultracentrifuge can range from 60,000 rpm to 150,000 rpm.
- Ultracentrifuges are mostly operated in more facilitated laboratories to perform more advanced operations.
- These are larger in size and can operate samples either in batches or as a continuous flow system.
- Most ultracentrifuges are refrigerated in order to control the heat that might be generated due to the excessive speed.

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 smaller molecules move slower.
- At the same time, denser molecules are moved outwards to the periphery of tubes whereas less dense molecules are rotated towards the center of 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 suspended in the supernatant or float on the surface.

Types of Ultracentrifuge

- Based on the application and purpose, ultracentrifuge are of two types;
- **Analytical ultracentrifuge (AUC)**
- **Preparative ultracentrifuge**

Analytical ultracentrifuge (AUC)

- Analytical centrifuges, as the name suggests, are the ultracentrifuges that are used for the analysis of various particles present in the sample.
- Analytical ultracentrifugation (AUC) is a versatile and robust method for the quantitative analysis of macromolecules in solution.
- These ultracentrifuges have detection systems to monitor the spinning and position of the particles in real-time to determine the sedimentation coefficient that aids in the analysis of particles based on shape, size, and masses.
- Analytical ultracentrifugation for the determination of the relative molecular mass of a macromolecule can be performed by a sedimentation velocity approach or sedimentation equilibrium methodology.
- The hydrodynamic properties of macromolecules are described by their sedimentation coefficients.

... Analytical ultracentrifuge (AUC)

- The sedimentation coefficient can be used to characterize changes in the size and shape of macromolecules with changing experimental conditions.
- Three optical systems are available for the analytical ultracentrifuge (absorbance, interference, and fluorescence) that permit precise and selective observation of sedimentation in real-time.
- Based on the position, the sedimentation coefficients can be determined, which allows the determination of properties of different molecules.
- Analytical ultracentrifuge is most commonly used for determination of properties of biomolecules like proteins and nucleic acids.

Preparative ultracentrifuge

- Preparative ultracentrifuges are the centrifuges that are primarily used for the isolation and separation of particles in a sample by the process of centrifugation.
- In a preparative run of an ultracentrifuge, the contents of the tubes are analyzed after the centrifugation period, unlike the analytical centrifuge where the analysis is done during the centrifugation process.
- Preparative ultracentrifuges can be operated for different types of centrifugation processes like density gradient centrifugation, differential centrifugation, and isopycnic centrifugation.
- The particles in a sample are either separated on the basis of their density or their sizes.

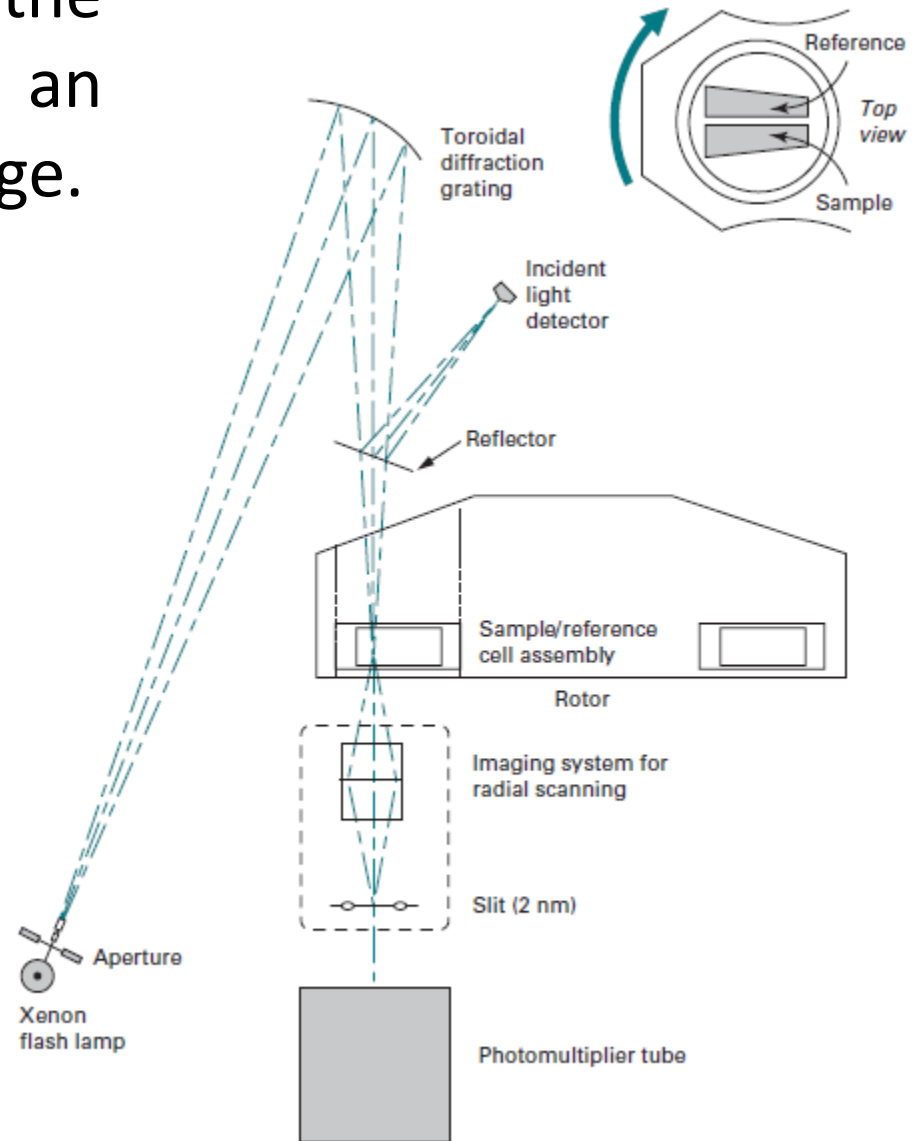
... Preparative ultracentrifuge

- In density gradient centrifugation and isopycnic centrifugation, the particles of a sample are separated on the basis of their density.
- Different particles present in a sample are isolated in the form of bands in distinct levels where the density of the particle equals the density of the medium.
- In differential centrifugation, however, the particles are separated by applying different speeds of the rotors. Larger particles settle down under lower speeds while smaller particles require higher speed for separation.
- Because particles are separated on the basis of density and size, preparative ultracentrifuges can be used for the determination of the density and size of different particles.

Instrumentation/ Parts of Ultracentrifuge

- Ultracentrifuges are provided with a variety of parts and components perform different functions.
- The rotors are an essential part of any ultracentrifuge. Ultracentrifuges use all three types of rotors, namely, vertical rotors, swinging bucket rotors, and fixed-angle rotors.
- The swinging bucket rotor is the most commonly used rotor in ultracentrifuge because this yields the highest concentration of particles. This is because the direction of centrifugal force in vertical rotors aligns with the position of the tubes.
- The drive is the power unit which spins the rotor holding cells or tubes which contain the solution of molecules or suspension of particles.
- Besides the interchangeable electric drive and rotors, it includes analytical rotors accommodating up to four cells, temperature devices with range and control from 0° to 40°, cells with thicknesses covering a factor of 10 in the sensitivity.
- Analytical ultracentrifuge also has a two-dimensional comparator with a printing desk calculator, interference, and absorption optical systems. The optical systems are necessary for the real-time analysis of molecules.
- Additionally, a gradient-forming device, hand refractometers, and a recording spectrophotometer with flow cell and fraction collector might also be present in a preparative ultracentrifuge.

Schematic diagram of the optical system of an analytical ultracentrifuge.



Uses of Ultracentrifuge

- Preparative ultracentrifuges are used in biology for a pelleting fraction of cell organelles like mitochondria, ribosomes, and even viruses.
- Density gradient centrifugation uses cesium 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.