**CENTRIFUGATION**

**Introduction**

Biological centrifugation is a process that uses centrifugal force to separate and purify mixtures of biological particles in a liquid medium. It is a key technique for isolating and analysing cells, subcellular fractions, supramolecular complexes and isolated macromolecules such as proteins or nucleic acids. The development of the first analytical ultracentrifuge by Svedberg in the late 1920s and the technical refinement of the preparative centrifugation technique by Claude and colleagues in the 1940s positioned centrifugation technology at the centre of biological and biomedical research for many decades. Today, centrifugation techniques represent a critical tool for modern biochemistry and are employed in almost all invasive subcellular studies. While analytical centrifugation is mainly concerned with the study of purified macromolecules or isolated supramolecular assemblies, preparative centrifugation methodology is devoted to the actual separation of tissues, cells, subcellular structures, membrane vesicles and other particles of biochemical interest.

**Types of centrifuges**

Centrifugation techniques take a central position in modern biochemical, cellular and molecular biological studies. Depending on the particular application, centrifuges differ in their overall design and size. However, a common feature in all centrifuges is the central motor that spins a rotor containing the samples to be separated. Particles of biochemical interest are usually suspended in a liquid buffer system contained in specific tubes or separation chambers that are located in specialised rotors. The biological medium is chosen for the specific centrifugal application and may differ considerably between preparative and analytical approaches. As outlined below, the optimum pH value, salt concentration, stabilising cofactors and protective ingredients such as protease inhibitors have to be carefully evaluated in order to preserve biological function. The most obvious differences between centrifuges are:

• The maximum speed at which biological specimens are subjected to increased sedimentation;

• The presence or absence of a vacuum;

• The potential for refrigeration or general manipulation of the temperature during a centrifugation run; and

• The maximum volume of samples and capacity for individual centrifugation tubes.

Many different types of centrifuges are commercially available including:

• Large-capacity low-speed preparative centrifuges;

• Refrigerated high-speed preparative centrifuges;

• Analytical ultracentrifuges;

• Preparative ultracentrifuges;

• Large-scale clinical centrifuges; and

• Small-scale laboratory microfuges.

**Types of rotors**

To illustrate the difference in design of fixed-angle rotors, vertical tube rotors and swinging-bucket rotors, figure below outlines cross-sectional diagrams of these three main types of rotors. Companies usually name rotors according to their type of design, the maximum allowable speed and sometimes the material composition. Depending on the use in a simple low-speed centrifuge, a high-speed centrifuge or an ultracentrifuge, different centrifugal forces are encountered by a spinning rotor. Accordingly different types of rotors are made from different materials. Low-speed rotors are usually made of steel or brass, while high-speed rotors consist of aluminium, titanium or fibre-reinforced composites. The exterior of specific rotors might be finished with protective paints. For example, rotors for ultracentrifugation made out of titanium alloy are covered with a polyurethane layer. Aluminium rotors are protected from corrosion by an electrochemically formed tough layer of aluminium oxide. In order to avoid damaging these protective layers, care should be taken during rotor handling.



**Figure**: Design of the three main types of rotors used in routine biochemical centrifugation techniques. Shown is a cross-sectional diagram of a fixed-angle rotor (a), a vertical tube rotor (b), and a swinging-bucket rotor (c).

**PREPARATIVE CENTRIFUGATION**

**Differential centrifugation**

Cellular and subcellular fractionation techniques are indispensable methods used in biochemical research. Although the proper separation of many subcellular structures is absolutely dependent on preparative ultracentrifugation, the isolation of large cellular structures, the nuclear fraction, mitochondria, chloroplasts or large protein precipitates can be achieved by conventional high-speed refrigerated centrifugation. Differential centrifugation is based upon the differences in the sedimentation rate of biological particles of different size and density. 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. Following the initial sedimentation of the largest particles of a homogenate (such as cellular debris) by centrifugation, various biological structures or aggregates are separated into pellet and supernatant fractions, depending upon the speed and time of individual centrifugation steps and the density and relative size of the particles. To increase the yield of membrane structures and protein aggregates released, cellular debris pellets are often rehomogenised several times and then recentrifuged. This is especially important in the case of rigid biological structures such as muscular or connective tissues, or in the case of small tissue samples as is the case with human biopsy material or primary cell cultures.

**Density-gradient centrifugation**

To further separate biological particles of similar size but differing density, ultracentrifugation with preformed or self-establishing density gradients is the method of choice. Both rate separation and equilibrium methods can be used. In the figure below, the preparative ultracentrifugation of low- to high-density particles is shown. A mixture of particles, such as is present in a heterogeneous microsomal membrane preparation, is layered on top of a preformed liquid density gradient. Depending on the particular biological application, a great variety of gradient materials are available. Caesium chloride is widely used for the banding of DNA and the isolation of plasmids, nucleoproteins and viruses. Sodium bromide and sodium iodide are employed for the fractionation of lipoproteins and the banding of DNA or RNA molecules, respectively. Various companies offer a range of gradient material for the separation of whole cells and subcellular particles, e.g. Percoll, Ficoll, Dextran, Metrizamide and Nycodenz. For the separation of membrane vesicles derived from tissue homogenates, ultra-pure DNase-, RNase and protease-free sucrose represents a suitable and widely employed medium for the preparation of stable gradients. If one wants to separate all membrane species spanning the whole range of particle densities, the maximum density of the gradient must exceed the density of the most dense vesicle species. Both step gradient and continuous gradient systems are employed to achieve this. If automated gradient makers are not available, the manual pouring of a stepwise gradient with the help of a pipette is not so time-consuming or difficult. In contrast, the formation of a stable continuous gradient is much more challenging and requires a commercially available gradient maker.

Following pouring, gradients are usually kept in a cold room for temperature equilibration and are moved extremely slowly in special holders so as to avoid mixing of different gradient layers. For rate separation of subcellular particles, the required fraction does not reach its isopycnic position within the gradient. For isopycnic separation, density centrifugation is continued until the buoyant density of the particle of interest and the density of the gradient are equal.



**ANALYTICAL CENTRIFUGATION**

As biological macromolecules exhibit random thermal motion, their relative uniform distribution in an aqueous environment is not significantly affected by the Earth’s gravitational field. Isolated biomolecules in solution only exhibit distinguishable sedimentation when they undergo immense accelerations, e.g. in an ultracentrifugal field. A typical analytical ultracentrifuge can generate a centrifugal field of 250 000 g in its analytical cell. Within these extremely high gravitational fields, the ultracentrifuge cell has to allow light passage through the biological particles for proper measurement of the concentration distribution. The availability of high-intensity xenon flash lamps and the advance in instrumental sensitivity and wavelength range has made the accurate measurement of highly dilute protein samples below 230nm possible. Analytical ultracentrifuges such as the Beckman Optima XL-A allow the use of wavelengths between 190nm and 800 nm. Sedimentation of isolated proteins or nucleic acids can be useful in the determination of the relative molecular mass, purity and shape of these biomolecules. 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 and can be determined from the rate that a concentration boundary of the particular biomolecules moves in the gravitational field. Such studies on the solution behaviour of macromolecules can give detailed insight into the properties of large aggregates and thereby confirm results from biochemical analyses on complex formation. The sedimentation coefficient can be used to characterise changes in the size and shape of macromolecules with changing experimental conditions. This allows for the detailed biophysical analysis of the effect of variations in the pH value, temperature or co-factors on molecular shape.

Analytical ultracentrifugation is most often employed in

• The determination of the purity of macromolecules;

• The determination of the relative molecular mass of solutes in their native state;

• The examination of changes in the molecular mass of supramolecular complexes;

• The detection of conformational changes; and in

• Ligand-binding studies.