# Centrifugation Techniques

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# Centrifugation Techniques

Introductory article

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Centrifugation is the use of the centrifugal forces generated in a spinning rotor to separate biological molecules primarily on the basis of either their size or their density.

Article Contents



## Basic Principles

Centrifugation is used to separate all types of particle based upon their sedimentation properties. The sedimentation properties of particles depend on a number of different factors including size, density and shape. However, both density and shape vary significantly depending on the composition of the solution in which the particles are suspended. Particles are separated primarily on the basis of either their density (isopycnic separations) or size (differential pelleting and rate-zonal separations); in the latter case the density does affect sedimentation but, in most cases, to a much lesser extent than size.

In centrifugation it is important to differentiate between the speed of centrifugation (revolutions per minute, RPM) and the relative centrifugal force (RCF or *G*) since these are often confused. The centrifugal force generated by a centrifuge can easily be calculated from the equation:

$$\mathrm{RCF} = 11.18 \times R \times (\mathrm{RPM}/1000)^2$$

where $R$ is the distance from the centre of rotation in centimetres; that is, the centrifugal force increases as the particles move down the centrifuge tube. As a general rule, the greater the centrifugal force the shorter the separation time. However, centrifugation also generates hydrostatic forces within the solution and so excessive centrifugal forces can disrupt some biological particles such as ribosomes.

The other very important aspect in optimizing centrifugal separations is the choice of rotor. Centrifuge rotors can be divided up into five different types and of these the most frequently used are fixed-angle and swinging bucket rotors. As a general guide, fixed-angle rotors should be used for efficient pelleting and isopycnic centrifugation of macromolecules, while swinging bucket rotors are primarily used for isopycnic gradients for cells and organelles and rate-zonal centrifugation. The other three types of rotor are vertical rotors that can be used for gradient separations where no pelleting occurs, zonal rotors that are used for large-scale gradient separations, and analytical rotors where instead of tubes the samples are centrifuged in sector-shaped cells with transparent sides. The efficiency of rotors for pelleting particles is expressed in terms of their *k*-factors with the most efficient rotors having the smallest *k*-factors.

## Pelleting and Differential Pelleting

Pelleting is the separation of particulate and nonparticulate material and it is one of the simplest, most frequently used centrifugation techniques, typically as part of a procedure for harvesting cells or the isolation of precipitated material. Differential pelleting is a refinement of the simple pelleting technique and this methodology is based on the fact that there can be quite large differences between the sedimentation properties of different types of particles. Therefore, by sedimenting a complex mixture of particles using a series of increasing centrifugal fields, it is possible to obtain partially purified fractions of various subcellular fractions. As a very approximate guide to the conditions needed to sediment various biological particles the following can be used:

As a general rule, differential centrifugation produces enriched fractions rather than purified fractions; for example, the 'nuclear pellet' obtained by differential centrifugation almost always contains mitochondrial material that has co-sedimented with the nuclei. Similarly, the 'mitochondrial pellet' always contains material from lysosomes and peroxisomes. Hence differential centrifugation is usually carried out as an early step in the purification of subcellular components, often prior to further purification involving the use of rate-zonal or, more frequently, isopycnic gradient centrifugation.

## Gradient Media

A wide range of compounds have been used to form gradients for rate-zonal and isopycnic separations. In the case of gradients for rate-zonal centrifugation usually sucrose or, less frequently, glycerol are used to form the gradients. However, the range of compounds used for gradient media for isopycnic separations is much wider and the choice of medium depends on the type of separation. For macromolecules, such as DNA, RNA and proteins, concentrated solutions of salts such as CsCl, $Cs_2SO_4$, KI,

KBr, NaI and RbCl are used and the gradients self-form during centrifugation. Strong salt solutions disrupt most types of biological complexes and so for organelles and membrane separations it is traditional to use sucrose gradients although better separations can often be obtained using nonionic iodinated gradient media such as Nycodenz or Optiprep (both manufactured by Axis Shield PoC AS, Oslo, Norway) that have higher densities and lower viscosities than sucrose solutions. Gradients for separating cells, especially animal cells, need to be isotonic throughout and so self-forming Percoll gradients are often used together with an osmotic balancer. However, Percoll is not ideal because of its colloidal nature and usually better separations can be obtained using the nonionic gradient media such as Nycodenz or Optiprep.

## Formation of Gradients

Both isopycnic and rate-zonal separations involve fractionating particles on a gradient. For isopycnic separations gradients can be continuous or discontinuous but usually continuous gradients are preferred since they are less likely to produce artefactual separations as a result of the presence of interfaces within the gradient. In the case of isopycnic separations of macromolecules, self-forming gradients of concentrated salt solutions are usually used and in this case the gradient is formed by the centrifugal field during centrifugation as the gradient medium (e.g. CsCl) starts to sediment. However, when using self-forming gradients it is necessary to ensure that the centrifugation conditions used optimize the shape and resolution of the gradient. Self-forming gradients will give better resolution when centrifuged in a fixed-angle or near-vertical rotor. Preformed gradients are prepared by either diffusion of a step gradient made up of 3–4 solutions for several hours or by using one of the two-chamber gradient mixers that are available commercially. Preformed gradients can also be prepared by freezing and thawing a uniform solution but the disadvantage of this technique is that it is difficult to control or predict the exact shape of the gradient.

## Isopycnic Centrifugation

In the case of isopycnic separations, particles are separated purely on the basis of their density and size only affects the rate at which particles reach their equilibrium position in the gradient. This method is very useful for isolating membranes and organelles that have very diverse sizes, but having the same functions they have the same composition and so they have very similar densities. The density of particles is very dependent on the nature of the gradient medium; for example, DNA has a density of about $1.7\,g\,mL^{-1}$ in CsCl gradients while in Nycodenz gradients DNA bands at about $1.11\,g\,mL^{-1}$. Traditionally, sucrose gradients have been used for subcellular fractions such as mitochondria and ionic gradient media (e.g. CsCl) have been used for nucleic acids. Gradients for isopycnic centrifugation for larger particles such as cells and organelles are usually preformed while, in the case of macromolecules and other small particles self-forming gradients are used; in self-forming gradients the gradient is formed during centrifugation by the sedimentation of the gradient medium. The choice of rotors for isopycnic centrifugation depends on the nature of the sample, for larger particles such as organelles it is best to use a swinging bucket rotor while in the case of macromolecules better results in terms of gradient capacity and resolution should be obtained using a fixed-angle rotor. When samples are separated by isopycnic centrifugation it is not necessary to load the sample at the top of the gradient, instead the sample can be loaded at the bottom of the gradient (flotation separations) or mixed throughout the gradient. Flotation isopycnic separation methods are used particularly for the separation of different types of lipoproteins as well as for the subfractionation of different types of membranes.

## Rate-zonal Centrifugation

Rate-zonal centrifugation is used to separate particles primarily on the basis of their sedimentation, usually in a sucrose gradient although in some cases glycerol gradients have been used. Sedimentation is determined primarily by a combination of size and conformation with more compact molecules sedimenting faster than the same size of molecule in an extended conformation. The sedimentation of particles is measured in 'Svedbergs' usually abbreviated as 'S'. Note that the S-values of particles are not simply additive, for example the 80S eukaryotic ribosome is made up of two subunits of 60S and 40S.

In analytical centrifugation sedimentation is carried out in a homogeneous solution but in preparative centrifuges, usually preformed, continuous linear sucrose gradients are used as these are close to being isokinetic, that is particles move at the same speed throughout the gradient. The gradient stabilizes the liquid column against convection currents and allows more material to be loaded on to the top of the gradient solution as a stable layer or zone. Typically, the gradients used are 5–20% (w/w) or 10–40% (w/w) sucrose and these should be centrifuged in either a swinging bucket rotor or, if no pelleting is likely to occur, a vertical rotor. Some biological samples, such as RNA, have a tendency to aggregate and in such situations denaturing gradients can be used where the solvent used is formamide or DMSO (dimethylsulfoxide) instead of water, since both of these solvents disrupt aggregates of

RNA and other molecules held together by hydrogen bonds.

However, the use of rate-zonal centrifugation has declined following the widespread use and development of high-resolution electrophoresis methods which generally offer greater flexibility of separations of many proteins and nucleic acids with much greater resolution than can be obtained by using any of the rate-zonal centrifugation methods.

## Analytical Centrifugation

Svedberg developed the first ultracentrifuge which was an analytical ultracentrifuge and it, like all subsequent analytical centrifuges, had the unique feature that the sample was centrifuged in a cell with transparent sides and so it is possible to view and analyse the sedimentation process during centrifugation. The sedimentation rate of a particle in an analytical centrifuge can be measured very accurately by using light absorption, schlieren optics or Rayleigh interference optics and this information can be processed to provide quantitative analyses of macromolecules in terms of their shape and physical size in solution. Sedimentation in an analytical centrifuge is particularly useful for studying macromolecules such as polysaccharides and other types of macromolecule that cannot be analysed by the usual analytical methods such as gel electrophoresis. In addition, analytical centrifugation is especially useful for studying the molecular interactions of macromolecules. Hence, although analytical centrifugation requires the purchase of an expensive purpose-built machine and a specialist operator, the unique information that this methodology can provide has ensured that it has continues as an essential centrifugation technique.

## Care of Centrifugation Equipment

One of the major problems when using centrifuges is the corrosion of centrifuge rotors, particularly those made of aluminium alloys. Rotors made of aluminium alloys are very susceptible to severe corrosion even when left to soak in water overnight. Solutions left in aluminium rotors can cause internal corrosion of the metal alloy. After use always rinse, drain and dry rotors to avoid corrosion and the build up of contamination. Always follow the manufacturers' recommendations regarding the care of centrifuge rotors. If at all possible use titanium or carbon composite rotors that are not affected by corrosion by aqueous solutions.

### Further Reading

Fisher D, Francis GE and Rickwood D (1998) *Cell Separation: A Practical Approach*. Oxford: Oxford University Press.

Graham JM and Rickwood D (1997) *Subcellular Fractionation: A Practical Approach*. Oxford: IRL Press at OUP.

Rickwood D (1984) *Centrifugation: A Practical Approach*, 2nd edn. Oxford: IRL Press at OUP.

Rickwood D (1992) *Preparative Centrifugation: A Practical Approach*. Oxford: IRL Press at OUP.