"Matching the correct rotor to each application type can be confusing, but in order to maximise experimental efficiency, it is important that this is performed as accurately as possible"

Technical Considerations for Ultracentrifugation

Initially developed in the 1920's, ultracentrifuges have the ability to generate forces thousands or millions of times stronger than the force of gravity. Further development of this class of devices permitted the fractionation of sub-cellular components which were previously visible only through the use of an electron microscope. As a result, modern ultracentrifugation can be used to determine the shape, size and weight of macromolecular complexes. Extremely high speeds can be obtained through the combination of specialised rotors, tubes and bottles. When spun in micro-volumes, particles can be separated at 150,000 rpm or in excess of 1,000,000 x g. This high-speed separation capability has made ultracentrifugation an ideal technique for applications such as cell biology (sub-cellular fractionation), proteomics (protein and lipoprotein purification and fractionation), genomics (RNA and DNA purification), microbiology (pelleting, virus purification/concentration), and nanotechnology (purification and separation of nanoparticles).

The size and density of the material to be separated forms the basis behind the theory of ultracentrifugation. Depending upon the application in question, differential or density gradient centrifugation should be used. Differential centrifugation enables the successive pelleting of particles, which decrease in sedimentation velocities. As a result, denser components pellet at the bottom of the tube and less dense components will remain in suspension. Density gradient centrifugation causes components to come to rest at points in the tube at which they are in density equilibrium with the surrounding solvent, and can be subdivided into: rate-zonal (separation based on molecular weight) and isopycnic (an equilibrium separation).

TUBE VARIATIONS

Due to the large number of variables associated with ultracentrifugation, each selection directly impacts on the next. As such, when choosing tubes and bottles, it is important to consider rotor and chemical compatibility.

Size and volume

The sample volume to be processed will naturally impact on the tube size required and in order to maximise efficiency, the tube must be compatible with the rotor type.

Tube volumes are generally referred to as either nominal or fill: the nominal volume is the maximum amount of liquid that may fit into the rotor cavity and the fill volume is the volume that manufacturers recommend the tube or bottle should hold. If not filled correctly, the centrifugal forces can adversely affect the tube material, causing it to warp and bend, resulting in a loss of volume.

Format

As shown in *Table 1*, multiple tube formats are available to meet the complete range of applications conducted within various laboratories.

Table 1. Tube format and rotor compatibility

Tube format	Rotor type			Application
	Fixed angle	***Swinging bucket***	***Vertical***	
Thin wall open top	No	Yes	No	Banding or pelleting
Thick wall open top	Yes	Yes	No	Pelleting small volumes when high g-forces are required
Thin wall sealed	Yes	Some tube formats	Yes	For use with delicate samples
Oak ridge style	Yes	No	No	Popular choice as suitable for a wide range of applications

Materials

Various samples will have different properties and as such, the effect of the tube material must be taken into consideration. Tubes are commonly available in polyethylene (PE), polypropylene (PP), polyallomer (PA) thin wall or thick wall, and polycarbonate (PC). These materials need to be chemically resistant, transparent, thin, and flexible. However, a balance needs to be obtained between being thin and tolerant, since some of the tubes will need to be piercable for sample extraction, as well as resistant to high pressures and temperatures to prevent the occurrence of deformation. Various tube materials and their properties are detailed in *Table 2* below.

Table 2: Centrifuge tube materials and properties

	Property				
Tube material	Clarity	Piercable	Sliceable	Autoclavable	Chemical resistance
PE	Transparent/ translucent	Yes	No	No	Good
PP	Transparent	No	No	Yes	Good
PA thin wall	Transparent	Yes	Yes	Yes	Good
PA thick wall	Transparent	No	No (yes for tubes Ø 5-13 mm)	Yes	Good
PC	Transparent	No	No (yes for tubes Ø 5-13 mm)	Yes	Good

ROTOR COMPATIBILITY

After tube or bottle selection, an appropriate rotor needs to be chosen and each rotor type has different operational characteristics at rest and at speed *(Figure 1)*. Of the three standard classes of rotor (swinging bucket, fixed angle and vertical/near vertical) fixed angle and swinging bucket styles are predominantly used in ultracentrifugation applications. Rotors have traditionally been made of steel, titanium or aluminum, but more recently Thermo Fisher Scientific has introduced Fiberlite carbon rotors, providing a lightweight and versatile performance with a robust, corrosion-free design.

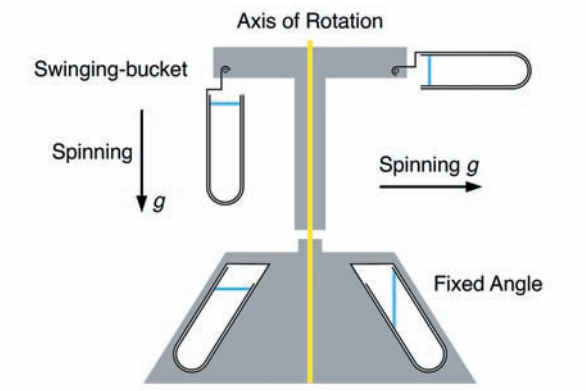

Figure 1. Rotor Characteristics. At rest, tubes are held parallel or at a fixed angle relative to the axis of rotation. At speed, tubes may move positioning depending upon rotor type.

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Swinging bucket rotors

Swinging bucket (SW) rotors are held in a horizontal position, relative to the axis of rotation during centrifugation. Due to this position, the path length that the particles need to travel is the equivalent of the full length of the tube *(Figure 2)*. A SW rotor system consists of a rotor body, which attaches to the centrifuge drive. The buckets are secured into the arms of the rotor body using trunnion pins.

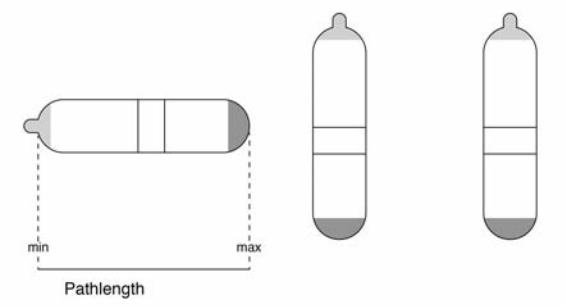

Figure 2. SW rotors - at speed, at rest inside the rotor, at rest outside the rotor

Fixed angle rotors

When using fixed angle (FA) rotors, the tubes are fixed between 20 and 45° relative to the axis of rotation. As seen in figure 3, the path length is therefore smaller than the length of the tube, making run times faster. During centrifugation, the gradient must re-orientate relative to the centrifugal force. The cavities in these rotors range in volume from 0.2 ml to 1 L, and speeds from single digits to 1,000,000 x g can be achieved, making them ideal for pelleting and the isopycnic banding of DNA.

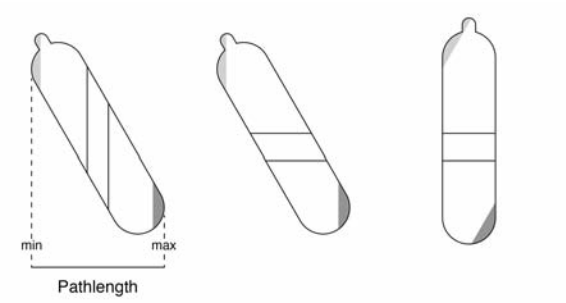

Figure 3: FA rotors – at speed, at rest in the rotor, at rest outside the rotor

Vertical tube rotors

Vertical tube rotors fix the tubes parallel to the axis of rotation, allowing bands to separate across the diameter of the tube, as shown in *Figure 4*. Although a reorientation of the gradient is required, the path length is the shortest possible. These rotors can therefore be used for density gradient separations where a short run time is required, but cannot be used for pelleting purposes, since the sediment will adhere to the tube wall. As specialised rotors, their most common use is for isopycnic separation, specifically for the banding of DNA in Cesium Chloride.

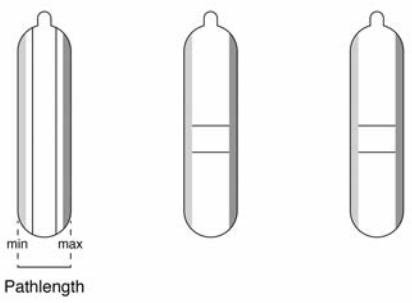

Figure 4. Vertical rotors – at speed, at rest inside the rotor, at rest outside the rotor

Near-vertical tube rotors

Tubes are held at a narrow angle of <10° relative to the axis of rotation *(Figure 5)*, providing a shorter path length and consequently, shorter run times. This allows for components that do not band under separation conditions to pellet at the bottom or float at the top of the tube, away from the band of interest. These rotors are ideal for density gradient separations where short run times are vital.

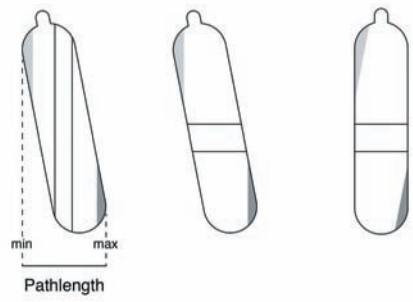

Figure 5. Near vertical rotors – at speed, at rest inside the rotor, at rest outside the rotor

Matching the correct rotor to each application type can be confusing, but in order to maximise experimental efficiency, it is important that this is performed as accurately as possible. In general:

- Sample pelleting: FA rotors enable efficient reorientation of the sample solution in the tube, for a fast run time
- Isopycnic separation: FA rotors combine a shallow density gradient with reorientation. Thus, the width of sample bands decreases, while the distance between the bands increases for an easy extraction
- Rate-zonal separation: SW rotors provide a long path length for extended run times and excellent sample band resolution
- Isopycnic or rate-zonal separation requiring short run times: vertical rotors provide a shorter path length for fast run times

PURIFICATION PROTOCOLS

Choosing the correct density gradient media is also important, especially for the purification of cells, viruses, subcellular membranes and macromolecules. Sucrose is the most commonly used gradient media for rate-zonal centrifugation as well as for the separation of cellular organelles and viruses, whereas alkali metal salts, including CsCl, are most often used for isopycnic centrifugation and the purification of nucleic acids and other macromolecules. Cells and individual organelles are most effectively purified using iodinated media, e.g. nycodenz. Colloidal silica (percoll) and polysaccharides (Ficoll) are effective across the range of cells, viruses and organelles.

INCREASING PELLETING EFFICIENCY

The clearing factor (k-factor) is one of the most important parameters for consideration when selecting a rotor. It is essentially a calculation of the relative pelleting efficiency and can be used to estimate the time required to pellet a particle of a known sedimentation coefficient (S) to the bottom of a tube [1]. The value of k depends on the maximum angular velocity of a centrifuge, as well as the minimum and maximum radius of the rotor:

$$k = 2.53\times10^{11}\left(\frac{\ln(r_{max} - r_{min})}{rpm^2_{max}}\right)$$

$$t = \frac{k}{S}$$

(r=radius in cm, rpm = revolutions per minute, rmax = maximum radial distance a particle can be from the rotor's axis, rmin = minimum radial distance a particle can be from the rotor's axis of rotation, t = time (hours), S = sedimentation coefficient in Svedberg units)

Once the centrifugation time has been calculated using this equation, the formula below can be used to calculate the run time for a new rotor.

$$T_a/T_b = k_a/k_b$$

(Ta = run time for the new rotor; Tb = run time for the original rotor, ka = k factor for the new rotor, kb = k factor for the original rotor)

These formulae can also be manipulated to increase rotor efficiency and speed-up the complete process. Since the k factor varies directly and exponentially with the ratio of the radii of a given sample, decreasing the sample volume will in turn increase efficiency.

[1]. Schieber G L & O'Brien T W. Extraction of proteins from the large subunit of bovine mitochondrial ribosomes under non-denaturing conditions. The Journal of Biological Chemistry 1982:257(15); 8781 – 8787.

CONCLUSION

There are a great number of technical considerations which need to be understood and taken into account when selecting appropriate centrifugation techniques and accessories.

By accurately matching these options to the different application types, functionality and experimental precision can be maximised. Since each option has a subsequent impact on the selection of the next component, the experiment as a whole needs to be taken into consideration when each choice is made.

In essence, it is vital that researchers are fully aware of all available options and are able to make an informed choice so that optimal resulting data are gained from each use.