Application Guide

Practical Techniques for Centrifugal Separations

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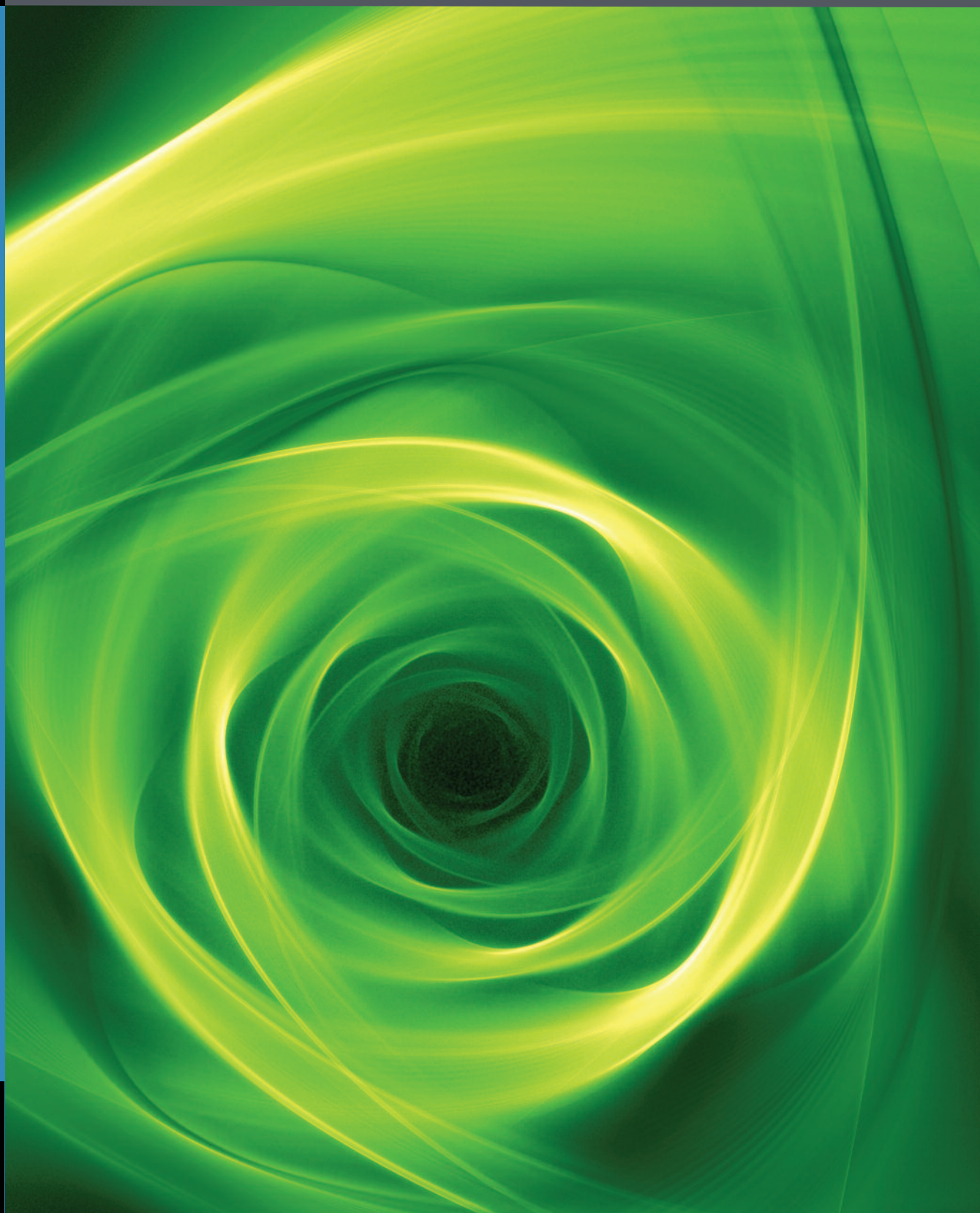

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Preface

This book presents essential techniques and new rotor technologies and accessories used in centrifugal separation. It is written for those who have training in biological sciences and who need to make the best use of the new techniques and accessories available today for separating and characterizing cellular bodies and macromolecular species. Also included in this book, is centrifugal theory, a table showing the densities of macromolecules in sucrose solution, and reference to studies involving many up-to-date applications and accessories used in existing centrifuges.

Health Caution

Some chemicals used in the procedures described in the following pages may be associated with biological hazards. The reader should be aware of the hazards associated with handling of all the chemicals and tissues used in any particular sample preparation. While the author has tried to indicate the hazards associated with different procedures described here, it is the responsibility of the reader to use safe work practices and to carefully follow the instrument manufacturer's instructions for operations of centrifuges, rotors and accessories.

ACKNOWLEDGEMENT

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I. Introduction to Centrifugation

A. Basic Concepts

A centrifuge is a device that separates particles from a solution through use of a rotor. In biology, the particles are usually cells, subcellular organelles, or large molecules, all of which are referred to here as particles.

There are two types of centrifuge procedures; one is preparative, the purpose of which is to isolate specific particles, and the other is analytical, which involves measuring physical properties of the sedimenting particles.

As a rotor spins in a centrifuge a centrifugal force is applied to each particle in the sample; the particle will then sediment at the rate that is proportional to the centrifugal force applied to it. The viscosity of the sample solution and the physical properties of the particles also affect the sedimentation rate of each particle.

At a fixed centrifugal force and liquid viscosity, the sedimentation rate of a particle is proportional to its size (molecular weight) and to the difference between the particle density and the density of the solution.

B. Basic Theory of Sedimentation

Some of the basic principles of sedimentation theory originated from Stokes' law. The following is the Stokes' law equation, derived from the settling of a sphere in a gravitational field. As the velocity of the sphere reaches a constant value, the net force of the sphere is equal to the force resisting its motion through the liquid; see equation 1. This resisting force is called the frictional or drag force.

$$\frac{1}{6}\pi d^3(p_p - p_l)g = 3\pi d\mu v$$

Equation 1

Where:

v = sedimentation rate, or velocity of the sphere
d = diameter of the sphere
ρ_p = density of the sphere
ρ_1 = density of the liquid
μ = viscosity of the liquid medium
g = gravitational force

Solving for v,

$$v = \frac{d^2(p_p - p_l)}{18\mu} \times g$$

Equation 2

From the Stokes' law equation, we conclude:

1. The sedimentation rate of a given particle is proportional to the size of that particle.
2. The sedimentation rate is proportional to the difference between the density of the particle and the density of the liquid medium.
3. The sedimentation rate is zero when the density of the particle is equal to the density of the liquid medium.
4. The sedimentation rate decreases as the viscosity of the liquid increases.
5. The sedimentation rate increases as the gravitational force increases.

Stokes' Law refers to "1-g" sedimentation, thus equation 3 can be written as follows:

$$v = \frac{d^2(p_p - p_l)}{18\mu} \times g = \frac{dr}{dt} \times g(981)$$

Equation 3

Where:
dr/dt = rate of movement of the particles (cm/sec)

The sedimentation rate, v, is useful in the characterizing a particle and can be determined in the centrifuge as shown in equation 4:

$$v = \frac{dr}{dt} = s \times \omega^2 r$$

Equation 4

Where:

s = sedimentation coefficient (expressed in seconds)*
r = distance between the particle and the center of rotation (cm)
ω = angular velocity (radians/sec)
dr/dt = rate of movement of the particles (cm/sec)

The sedimentation rate per unit of centrifugal force is called the sedimentation coefficient and can be written as follows:

** In Svedbderg's and Pedersen's book the Ultracentrifuge (1940), sedimentation coefficients are usually expressed in Svedberg (s), or 10^{-13} seconds. Thus, a particle whose sedimentation coefficient was measured at 10^{-12} seconds or 10 x10^{-13} seconds, is said to have a value of 10 S.*

$$s = \frac{1}{\omega^2 r} \times \frac{dr}{dt}$$

Equation 5

Using equations 4 and 5, we can see how the sedimentation coefficient relates to molecular weight (M), as shown by Svedberg and Pedersen (1940).

$$\frac{v}{\omega^2 r} = \frac{M(1-\bar{V}\rho_1)}{\int}$$

Equation 6

Where:

M = Mass or molecular weight of one particle

$\bar{v} = 1/\rho_p$

$\int$ = molar friction constant

Therefore,

$$s = \frac{M(1-\bar{V}\rho)}{N\int}$$

Equation 7

Where:

N = Avogadro's number

Svedberg's equation can be now written in terms of the sedimentation and diffusion coefficient as follows:

$$\frac{s}{D} = \frac{M(1-\bar{V}\rho)}{RT}$$

Equation 8

Where:

D = diffusion coefficient

R = universal gas constant

T = absolute temperature

This equation shows that the mass of a particle is proportional to its sedimentation coefficient and inversely proportional to its diffusion coefficient. For example, a sedimenting particle with a small molecular weight will have a correspondingly small sedimentation coefficient; it will therefore show a tendency to diffuse rapidly in solution of low viscosity.

The relative pelleting efficiency (sedimenting of particles to the bottom of the tube) of a rotor is expressed as its clearing factor, or K-factor. The formula for determining K-factor is as follows:

$$k = \frac{\ln(r_{max}/r_{min})}{\omega^2} \times \frac{10^{13}}{3600}$$

Equation 9

Where:

r_{max} = maximum radial distance measured to the bottom of the tube

r_{min} = radial distance measured to the meniscus at the top of the tube

ω = angular velocity in radians/sec (2πrpm/60, or 0.10472 x rpm)

The K-factor can be used in the following equation to estimate the time, T (in hours) required to pellet a particle to the bottom of the tube.

Note: Total run time calculated from the above formula may be longer for most metallic (aluminum and titanium) rotors although their K-factor may appear to have better sedimentation efficiency than the comparable carbon fiber rotor; however, the total calculated run times are shorter. The K-factor is determined for the rotor at maximum speed. Acceleration and deceleration rate are not included in the derivation of the K-factor formula.

$$T = \frac{K}{S}$$

Equation 10

Where:

s = sedimentation coefficient (in Svedberg's) of the particles

The new K-factor for the rotor can be calculated from the maximum speed (in rpm) for a speed other than the maximum using the following formula:

$$K = \frac{(\text{maximum rated speed of the rotor})^2}{(\text{actual run speed of the rotor})^2}$$

Equation 11

Where:

K_{new} = new K-factor for rotor at speed other than the maximum speed (i.e. reduced speed)

K_{max} = K factor for rotor at the maximum speed of the rotor

Run time can be calculated from data established in prior experiments when the K-factor and the run time of one rotor are known. For example, for rotors a and b, the formula below can be used:

$$T(a)/T(b) = K(a)/K(b)$$

Equation 12

Where:

T(a) = time to pellet in the "new" rotor
T(b) = time to pellet in the "old" rotor
K(a) = K-factor of the "new" rotor
K(b) = K-factor of the "old" rotor

In some instances the literature can supply the value of the relative centrifugal force (RCF) obtained from the centrifugation studies. The RCF value, when it is the sediment that is to be recovered, is the centrifugal force at a specific radial distance measured at the bottom of the tube (r_{max}). When it is the supernatant that is to be recovered, the radial distance is measured at the middle of the tube (average radius), or r (av). The formula for RCF is as follows:

$$RCF = \frac{r\omega^2}{g}$$

Equation 13

Where:

r_{max} or r (av) = radius (mm)
ω = angular velocity (radians/sec)
g = standard acceleration of gravity (9807 mm/s2)

After substitution equation 13 can be written as follows:

$$RCF = 1.12r\frac{(RPM)^2}{(1000)^2}$$

Equation 14

Or

$$RCF = RPM^2 \text{ x } 0.0112r/10{,}000$$

Equation 15

C. Simple Calculations for RCF from RPM and RPM from RCF

Some centrifuges have input displays that calculate RCF from RPM, or vice versa, when the maximum radial distance of the rotor used in the instrument is known. However, if one of these three parameters is unknown, a nomogram may be used to select the unknown RPM or RCF if the radial distance (r_{max}) is known; see Appendix 2, including a similar nomogram for superspeed and ultraspeed centrifuges.

Run time calculations for new studies can also be determined for a different rotor if RCF (*g*-force) and run time are known for a previous study using a different rotor.

Calculate run time (T) for the new study from the following relationship.

$$T = \frac{a \text{ x } b}{c}$$

Equation 16

Where:

a = run time from a previous study
b = *g*-force of rotor for a previous study
c = *g*-force for the new study

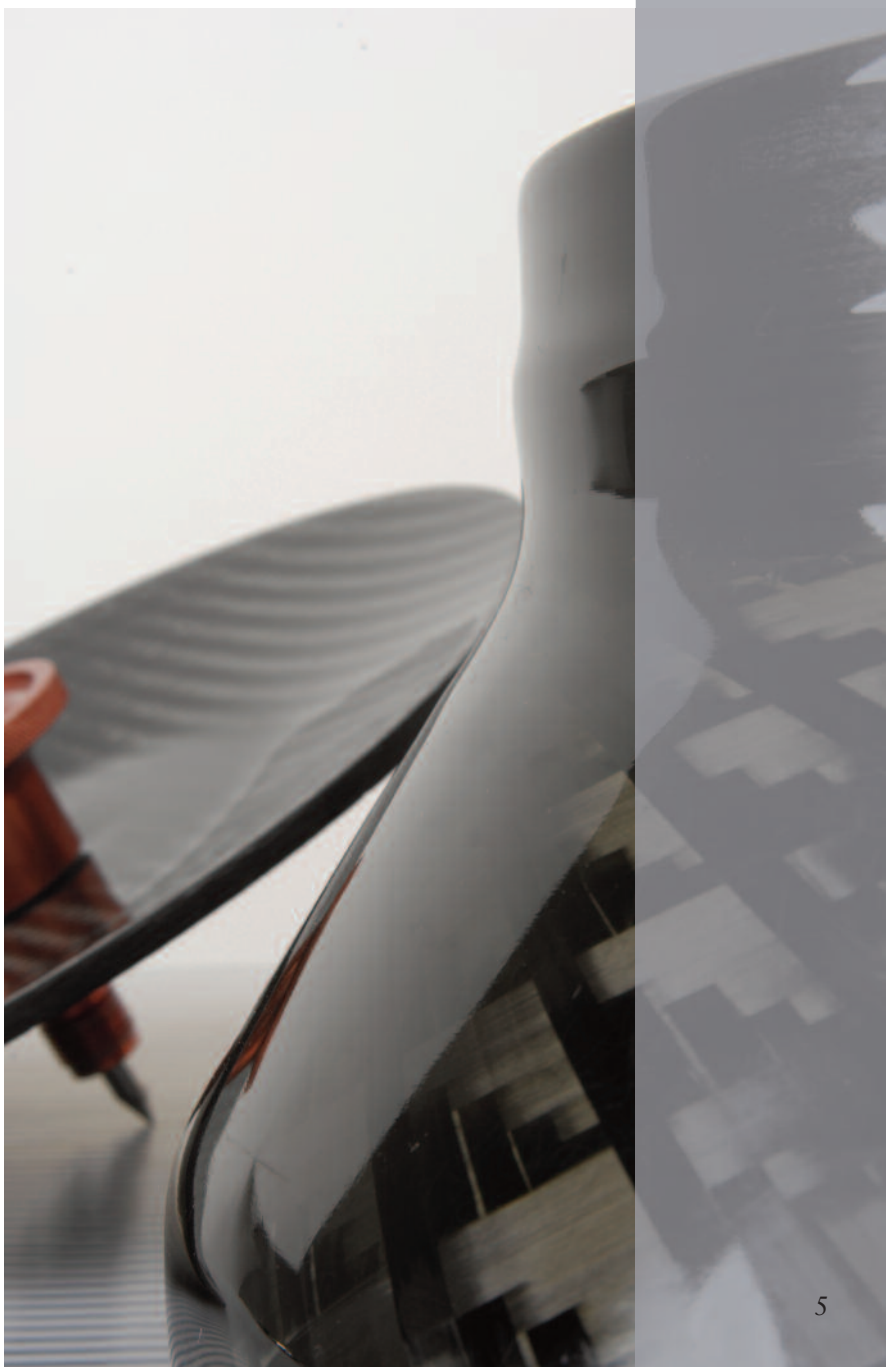


Figure 1: Fiberlite F13-14x50cy and F15-8x50c conical tube rotors.

II. Materials Used For Centrifuge Rotors

A. Aluminum Rotors

The stress on a rotor caused by centrifugal force is proportional to the speed squared. In order to obtain the high performance required for superspeed and ultraspeed centrifuge rotors, a range of titanium and aluminum alloys can be used; the choice of alloy depends on the exact type of rotor. In contrast, rotors for the lowspeed or tabletop centrifuges can be made of bronze, steel and steel metal (aluminum) or plastic such as polypropylene. However new advances (see Section C) in carbon fiber technology have made it possible to achieve high speeds typically reserved for metallic rotors.

Centrifuge rotors made of aluminum alloy must be treated with care because the material is particularly susceptible to corrosion. The greater corrosion of aluminum rotors is caused by acids, alkalis and high concentrations of salt solutions. However, even dilute solutions of salts in buffers can become concentrated as a result of evaporation and lead to corrosion of the rotors.

Aluminum rotors are usually anodized to decrease corrosion and wear, but this is not always the most effective method for protection of the metal. If the anodizing film is damaged in any way during centrifugation, any liquid present in the tube cavity of the rotor will be forced into the crystalline structure of the metal. This eventually leads to stress corrosion. Stress corrosion is the main cause of failure in aluminum rotors.

B. Titanium Rotors

Titanium rotors are much more resistant to corrosion because the titanium alloy is not affected by most acids, alkalis, or salt solutions. High performance titanium rotors can withstand more stress cycles at their full rated speed than aluminum rotors. However, repeated run cycles of acceleration and deceleration from maximum speed will eventually weaken the rotor, thus requiring de-ration (reduction of maximum speed) or retirement of the rotor. Additionally, the rotor overspeed disk (a decal that allows the centrifuge to detect the maximum speed of the rotor) must be changed after the rotor is de-rated to prevent the rotor from being used beyond its specifications; see the manufacturer's warranty for repeated run cycles.

C. Carbon Fiber Rotors

One of the major patented centrifugation innovations of the 1990s was the introduction of the carbon fiber rotor. Manufactured from carbon fiber material and molded into a typical configuration of the desired rotor, these rotors are available for a wide range of processing needs, from ultraspeed and superspeed rotors for floor model centrifuges to general purpose and microvolume rotors for tabletop models.

As a result of the use of carbon fiber composite materials, Thermo Scientific Fiberlite rotors provide unique advantages for centrifugation. These rotors are up to 60% lighter than comparable titanium rotors and 50% lighter than comparable aluminum rotors, making them easier to lift, carry and clean. In addition, Fiberlite rotors are able to reach maximum speed faster than comparable metallic rotors.

Because of the strength of the composite material (highest strength to weight ratio of all rotor materials), the lifespan of carbon fiber rotors far exceed that of metal rotors; to avoid rotor failure, most manufacturers publish recommended de-rating and retirement guidelines for metal rotors based on a certain number of runs or hours, which is not necessary with these Fiberlite® rotors.

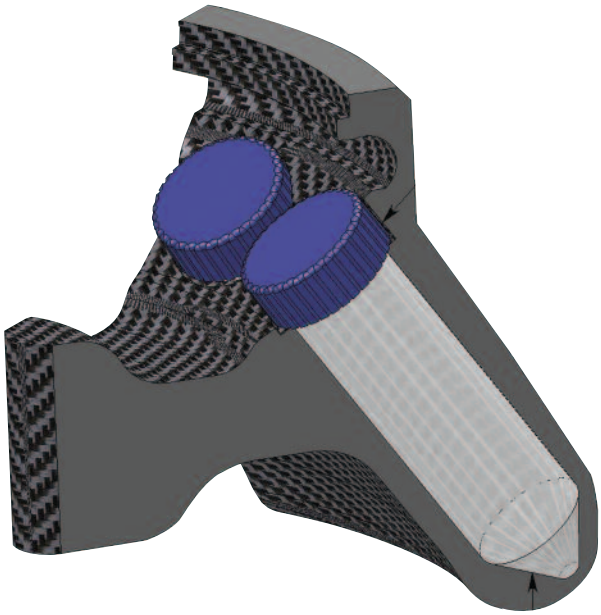


Figure 2: Through patented technology, Fiberlite rotor cavities are molded to the exact shape of many disposable conical tubes for maximum support; 50 mL conical tube shown here.

Unlike aluminum and titanium which are prone to corrosion and pitting, carbon fiber is corrosion-resistant. As a result, these rotors are more forgiving to exposure of regularly occurring laboratory corrosives, such as most organic solutions, and are also safe for use with most alkaline laboratory detergents and commercially available solutions for radioactive decontamination, which are also highly alkaline.

Fiberlite rotors are molded into a typical configuration and exact tolerances of the desired rotor. As a result of this unique rotor manufacturing technique, tubes and bottles are well-supported. Additionally, specially designed tissue culture rotors for disposable conical tubes have been developed, such as Fiberlite F15-8x50c and Fiberlite F13-14x50cy rotors; see Figure 1.

Since the rotors do not elongate or stretch during centrifugation, as metallic rotors would, disposable and non-disposable conical tubes have been shown to tolerate high g-forces, ~29,000 x g for example, without cracking. Fixed angle rotors for conical tubes can be used instead of swinging bucket rotors and fixed angle rotors with round bottoms for some pelleting studies. Since tubes in the swinging bucket rotors have hemispherical bottoms, the sediment is spread over a large surface of the bottom of the tubes. Conversely, with conical tubes in fixed angle rotors, the sediment is collected in the cone of the tubes thus sediment re-suspension can be done in a smaller volume of solution. The conical tube rotors have found use in centrifugal filtration methods with Centricon® and Amicon® Ultra devices.

Carbon fiber materials are heat insulators; not heat conductors. Biological samples are therefore kept at a constant temperature during centrifugation much longer than with metallic rotors. As result, the centrifuge refrigeration system may cycle less and could last longer, providing the end-user with heightened sample protection as well as savings in energy usage.

Fiberlite rotors are autoclavable and remain corrosion-free throughout their lifetime, providing further advantages to the user. Additionally the reduced weight of the rotors allows the drive system of the centrifuge to require less maintenance; see Table 1.

TABLE 1: COMPARISON OF ROTOR AND CARBON FIBER ROTOR MATERIAL CHARACTERISTICS

Aluminum	Titanium	Carbon Fiber
Highly susceptible to acid, alkali, salts corrosion	Moderately susceptible to acid, alkali, or salt corrosion	Corrosion-free
Anodizing may lead to stress corrosion	May require deration due to repeated run cycles	No deration due to high strength to weight ratio of rotor materials
		No stretching or elongation during centrifugation
Heavy material, uses increased energy to spin	Heavy material, uses increased energy to spin	Lightweight
		Heat insulating maintains sample temperature
		Environmentally friendly production process

III. Selection of Rotors for Centrifugal Separations: Applications for Rotors by Rotor Type

The selection of a rotor depends on sample volume, number of sample particles to be separated, the particle size and/or density, desired run time and desired quality of separation. The common rotors types – swinging bucket, fixed-angle, vertical and near-vertical rotors.

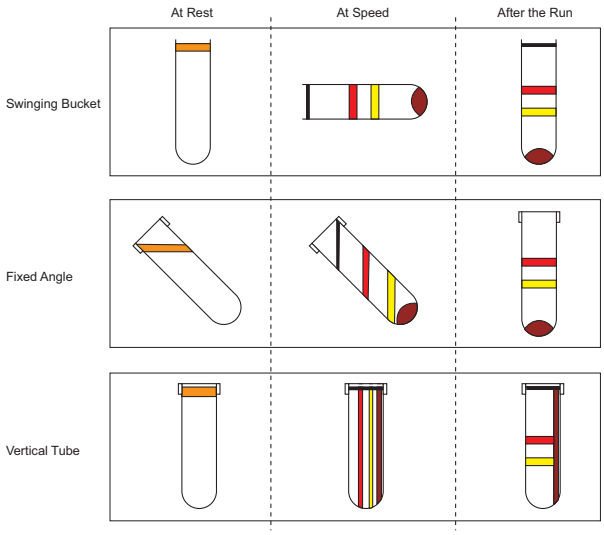


Figure 3: Sample orientation in common rotor types.

A. Swinging Bucket Rotors

Swinging bucket rotors are used for pelleting (sedimenting particles from solution), rate zonal studies (separation as a function of particle size and density), and isopycnic gradient studies (separation as a function of density only). While all three methods can be employed in this rotor, the method most often used in swinging bucket rotors is the rate zonal density gradient method in which the maximum resolution of sample components is needed. However, because of the length of the tubes in the horizontal position, the sample separation run times are very long for the rate zonal methods due to the increased distance the particles will travel for effective separation. Additionally, the separated zones remain in the same position in the tube during and after centrifugation.

The rate zonal method separates particles on the basis of size and sedimentation coefficient. During centrifugation, the total sample weight in tubes is concentrated on the bottom (max radius) of the buckets in the swinging-bucket rotors. This weight places excess stress on the buckets during centrifugation and can result in failure of the buckets.

B. Fixed Angle Rotors

Fixed angle rotors are made with different tube angles from the horizontal plane, ranging from 17 to 34 degrees in floor model centrifuges, while the angle may be up to 45 degrees in a tabletop model or microtube rotor. These angles permit the rotor to safely achieve the highest possible speed with the shortest path length or radial distance for short run times; see Figure 3. Fixed angle rotors are best used for pelleting, but they are also used for density gradient centrifugation. These are the most versatile centrifuge rotors.

In the fixed angle rotor, the particles move in a downward spiral manner (Coriolis effect) as they migrate towards the wall during pelleting/centrifugation until the initial sediment fills the hemispherical bottom of the tube (Berman 1966). The additional sedimenting particles are collected on the surface of the sediment at an angle coincident to the axis of rotation. Material collecting on the tube wall is observed only if the initial sample concentration of sedimenting particles in solution is extremely high.

Studies (Griffith 1996) show that there is negligible difference in the total sediment collected between rotors with different tube angles, similar tube size, and similar tube volumes under similar g-forces. An experiment was conducted which compared the maximum amount of sediment that can be collected in a rotor with an angle of 23 degrees versus the sediment collected in a rotor with a tube angle of 34 degrees. The concentration of the sample solution was high enough to permit the sediment to reach the tube cap. The sample volumes, tube sizes, and g-forces were similar for both rotors. The rotor with a tube angle of 23 degrees had total sediment of 23.3 g/mL, whereas the rotor with a tube angle of 34 degrees had total sediment of 28.2 g/mL. Next, the concentration of the sample solution in the tubes was reduced to an amount less than 50% of the previous run. No difference in sediment volume was observed between the two rotors. Although researchers rarely fill their centrifuge tubes to recover a full tube of sediment, this test was also done to observe that possibility. Additionally, the separated zones change their position in the tube during and after centrifugation. The solution volume between zones is greater to increase the separation between the separated components.

C. Vertical and Near Vertical Tube Rotors

Vertical tube rotors carry the centrifuge tubes at 0 degrees from the axis of rotation and near vertical rotors have angles less than 9 degrees. Therefore, these rotors have the shortest path length or radial distance; see Figure 3. During acceleration, the solution reorients and sedimentation is carried out across the diameter of the tube, instead of down the length of the tube as in fixed angle rotor or horizontally along the length of the tube as in the swinging bucket rotor. The shorter path length of the vertical tube rotor reduces the run time for isopycnic gradient studies. However, resolution is lost when the separation involves multi-component sample particles because the sediment collected on the tube wall remains there until the run is terminated. After the rotor decelerates to rest, any sediment that is pelleted during centrifugation falls off the tube wall and re-contaminates the separated sample zones in solution.

IV. Thermo Scientific Fiberlite Rotors

For ultimate system flexibility, Fiberlite rotors are compatible with virtually all centrifuge brands and are manufactured in three classes: (a) standard volume (b) micro-volume and (c) large volume rotors. Easily understand the type of rotor, maximum speed, and rotor volume by the nomenclature for most Fiberlite rotor models; see Figure 4.

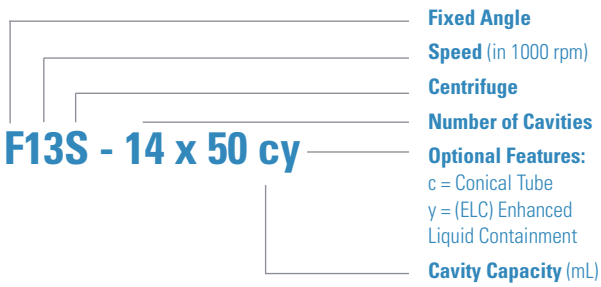


Figure 4. Fiberlite rotor model nomenclature.

A. Rotors for Superspeed and Tabletop Centrifuges

Many fixed angle rotors compatible with Thermo Scientific Sorvall, Heraeus, Jouan and Beckman®, and Eppendorf® centrifuges have been designed to increase capacity, maximum rpm and *g*-force. Among the standard pelleting separations, large volume rotors have enough *g*-force to process subcellular organelles, bacteria cells, and cell membranes.

Conical tube rotors have enough *g*-force to clarify crude lysates from plasmid maxi protocols and provide conical tubes with full support. This unique feature of the carbon fiber conical tube rotors allows for the use of disposable conical tubes at higher *g*-force than in standard metal rotors. With the use of disposable conical tubes, DNA preparations can be done quicker and in a more cost-effective manner.

Fiberlite swinging-bucket rotors are also available to spin microplates, blood collection tubes, and conical tubes for clinical and research studies. The standard height of 96- and 384-well microplates will spin down liquids while the deep plates are used to pellet cells. Filter plates are used to process samples through filters while amplification strips and tubes are for nucleic acid clean-up. Tube adapters are used for clarifying physiological fluids for clinical diagnostic tests, collection of platelet rich plasma from whole blood and sedimentation of platelets from platelet rich plasma or for urine specimens.

B. Rotors for Superspeed and Ultracentrifuges

Studies of different populations of samples using small volumes (1.5 mL to 200 mL) have been reported. The studies involve the isolation of mini-preparations of DNA from bacterial colonies, clinical studies of plasma HIV-RNA assays from individuals infected with HIV, and lipoprotein subfraction isolation from plasma of individuals for VLDL, LDL, and HDL cholesterol quantitation. Other separations such as the purifying viruses, pelleting of mammalian cells, clarification of cell lysates, and the pelleting of subcellular organelles are also conducted at small volumes.

V. Separation Theory and Methods in Bioresearch Disciplines

A. Pelleting and Differential Centrifugation

The most common centrifugation method is differential centrifugation or pelleting. The pelleting method separates particles of different sedimentation coefficients. Under a constant centrifugal field, the largest particles in the sample travel to the bottom of the tube first. During pelleting in fixed angle rotors, as the particles migrate spirally down the tube until the initial sediment reaches the bottom of the tube. This result is known as the Coriolis effect (Berman 1966).

The differential centrifugation method is the successive pelleting of particles from the previous supernatant, using increasingly higher centrifugation forces. In this method, the centrifuge tube is initially filled with a uniform mixture of sample solution. Through each centrifugation process, two fractions are obtained: a pellet containing the sedimented material and a supernatant solution of unsedimented material. Any particular component in the mixture may end up in the supernatant or the pellet, or it may be distributed in both fractions, depending on its size and/or the conditions of centrifugation; see Figure 5.

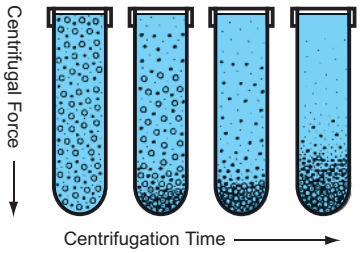


Figure 5. Differential centrifugation (pelleting).

The pellet is a mixture of all of the sediment components, and it is contaminated with whatever unsedimented particles were at the bottom of the tube initially. The only component of the sample which is in purified form is the one that sediments most slowly during the run; its yield, however, is very low. The two fractions from each run are recovered by decanting the supernatant solution from the pellet. This supernatant can be re-centrifuged at higher speeds to obtain a new pellet and supernatant. The pellet can be re-centrifuged after resuspension in a small volume of a suitable solvent – to "wash" or remove the sample solvent from the pellet.

Run times for pelleting can be shortened by using half filled thick-walled polycarbonate tubes, carrying smaller tube volumes. Short path length means less distance for particles to travel in the portion of the tube experiencing greater centrifugal forces, hence the shortened run time. As in all pelleting methods when using fixed angle rotors, the final stages of decelerating the rotor (e.g., from 800 rpm to rest) should be slow, in order to preserve the integrity of the sediment material owing to the Coriolis effect during centrifugation, especially when the sediment material is delicate or not noticeable (Berman 1966).

The pelleting method is most commonly performed in tabletop and superspeed centrifuges. As this is a preparative technique, large volume rotors have been used for such purposes as separating blood components and harvesting large amounts of sediment from tissue homogenate or cultured bacterial cells from growth media. Rotors processing tube volumes of 1.5 mL to 700 mL per tube with tabletop centrifuges have been used routinely for these studies. With the advent of Fiberlite rotors, total sample volumes of from 72 mL to 6000 mL per can be processed in a floor model centrifuge with volumes from approximately 36 mL to 800 mL in ultraspeed centrifuges.

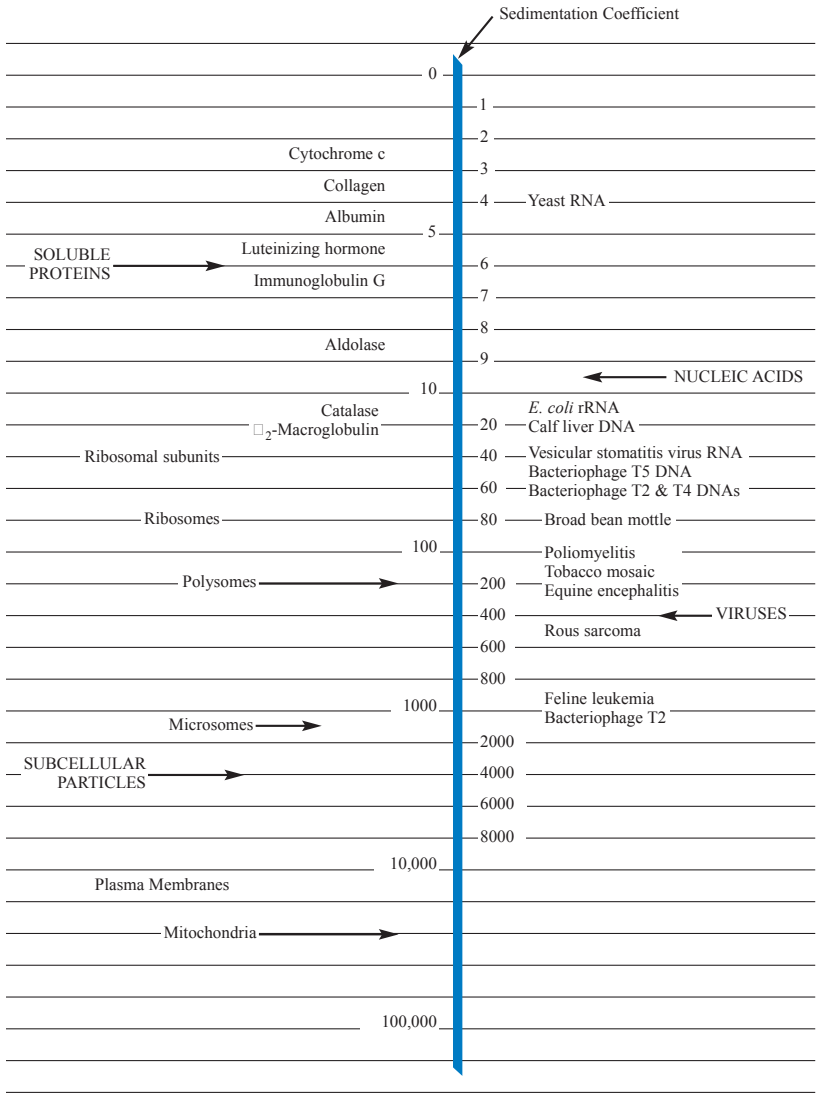
B. Density Gradient Centrifugation

Another method of separation is density gradient centrifugation. This method is somewhat more complicated than pelleting or differential centrifugation, but it has compensating advantages. The density gradient method permits for the complete separation of several or all components in a sample mixture, but also permits analytical measurements to be obtained. Often, changes in these measured parameters are used to study the effects of chemical, physical, or biological treatment of the sample material or the organism from which it was isolated.

Density gradient centrifugation was first used with swinging bucket rotors for separating or purifying viruses, subcellular particles and nucleic acids (Brakke 1958). A subsequent publication which provided a thorough discussion of this technique showed that fixed angle rotors could be effectively used to separate or purify these particles (Griffith 1982).

Density gradient methods are particularly suited to the study of viruses (Brakke 1960; Schumacher 1967). For example, viruses have been purified and their purity confirmed; infectivity has been associated with physical properties; aggregation, dissociation, and serological differences have been studied; sedimentation coefficients and buoyant densities have been measured.

Table 2: Sedimentation coefficients (in Svedbergs) for selected biological particles



Separation methods for biological research applications

Nucleic acids have also been studied extensively through density gradient methods (Vinograd 1963; Schumaker 1967; Parish 1972). DNA and RNA, as well as other poly nucleotides, have been separated and their sizes estimated from their sedimentation coefficients.

Cells and cell particulate fractions, including erythrocytes, spermatozoa, lymphocytes, bacteria, spleen cells, mitochondria, and microsomal and other supernatant fractions from mammalian tissue, have been isolated and purified (Skyes 1971; Birnie 1972). The density gradient method involves a supporting column of liquid with density, concentration, or viscosity that increases towards the bottom of the tube. This liquid column consists of a suitable low molecular weight solute in a solvent in which the sample particles can be suspended.

There are two methods of density gradient centrifugation: rate-zonal and isopycnic.

1. Rate-Zonal Centrifugation

In the rate-zonal method, a sample solution containing particles to be separated is layered on a pre-formed linear gradient column; see Figure 6. The sample solution creates a negative gradient at the top of the column thus preventing the sample from premature sedimentation.

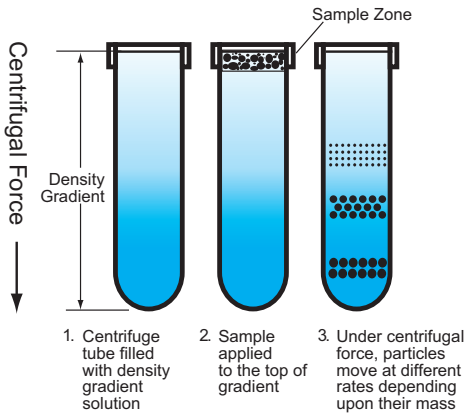


Figure 6: Rate zonal separation using pre-formed gradient.

Under centrifugal forces, the particles will sediment through the gradient column in separate zones, each zone consisting of particles characterized by their size and sedimentation rate; see Table 2.

To achieve a rate-zonal separation, the density of the sample particles must be greater than the density of the gradient column at any position along the column which the particles must travel. The run must be terminated before any of the separated zones reach the bottom of the tube.

Gradients are suited for separating whole cells and subcellular organelles from plant or animal tissue homogenate and for purifying some viruses. Table 3 gives a list of approximate densities of some biological particles. A discontinuous gradient is a gradient that is used for rate-zonal and isopycnic separations with approximately 4 steps. The materials used for discontinuous rate-zonal separations are viscosity gradient materials made with sucrose, Ficoll®, Percoll®, Nycodenz®, or OptiPrep®® solution. In some cases, a "cushion" a small volume of high density solution is placed at the bottom of the tube to act as a step in the gradient. This step may also be useful in effecting part of the separation. This cushion makes it easier to re-suspend any sedimented material at the end of the run and prevents damage to particles that may not withstand pelleting. It is known that some viruses may lose their viability when pelleted. Discontinuous gradient techniques for separating or purifying viruses, subcellular organelles and nucleic acid were first used in swinging bucket rotors by Brakke (Brakke 1958).

Table 3: Approximate densities of macromolecules in sucrose solutions

Macromolecules	Density (g/cm^3)
Golgi apparatus	1.06 - 1.10
Plasma membranes	1.16
Smooth endoplasmic reticulum	1.16
Intact oncogenic viruses	1.16 - 1.18
Mitochondria	1.19
Lysosomes	1.21
Peroxisomes	1.23
Soluble proteins	1.30
Plant viruses	1.30 - 1.45
Rhino- and enteroviruses	1.30 - 1.45
Nucleic acids, ribosomes	1.60 - 1.75
Glycogen	1.70

2. Preparation of Discontinuous or Step Gradients

To make a discontinuous gradient for separating subcellular organelles using Fiberlite rotors, Griffith suggested a simple layering method; see Figure 7 (Griffith 1986).

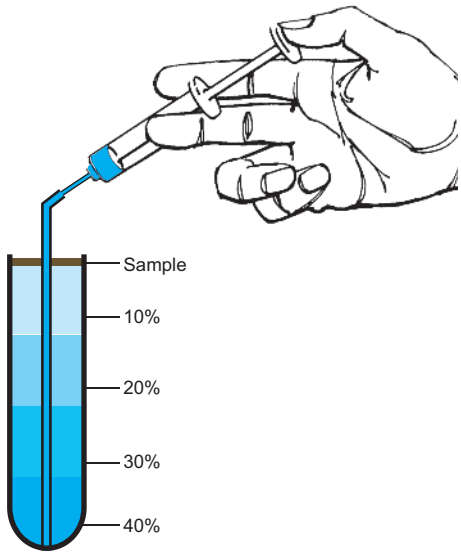


Figure 7: Hand layering method for discontinuous gradients.

Equal volumes of the appropriate concentrations of sucrose solutions, such as 10% w/w, 20% w/w, 30% w/w, and 40% w/w should be used. Gradients made with sucrose by the hand layering method described can be formed by other viscous materials as well. Percent weight/weight rather than percent weight/volume solutions are used for density gradient centrifugation methods because the sample particles are separated by density or size. A weight/weight solution is made by mixing a fixed weight (in grams) of solute 100 grams of solvent to give the required percent w/w solution. In contrast, a weight/volume solution is made by mixing a fixed weight (in grams) of solute 100 milliliters of solvent to give a required percent w/v solution.

For example 40% w/v solution is 40 grams solute is mixed in a 100 mL solvent, where as 40% solution w/w solution is 40 grams of solute mixed in 60 grams of solvent. Table 5 shows the difference in density between w/w and w/v solution of sucrose. The concentrations should be layered in the centrifuge tube using a pasture pipette that reaches to the bottom of the tube. The lightest concentration of the gradient e.g., 10% should be placed in the tube first. The 20% should be layered below the 10%. The 30% is then layered under the 20% concentration and finally the 40% concentration is layered below the 30%. Care must be taken not to disturb the interfaces between the layers by holding the tip of the pipette to the wall of the tube when removing or introducing it into the tube.

The gradient must be prepared with cold solutions and used immediately to prevent premature diffusion of the layers. The sample mixed with buffer is layered on the top gradient layer when all the components of the sample are to be separated. If the sample contains particles of a known density close to that of one of the layers of the gradient the sample solution should be mixed with that layer of the gradient concentration prior to layering; see Table 4.

Before applying the sample to the gradient, the sample concentration and volume must be determined. Svensson et. al. (1957), pointed out that the theoretical capacity of the gradient is a function of its density slope. Later, Brakke discovered experimentally that only a small percentage of the theoretical sample loads could be supported in swinging bucket rotor tubes (Brakke 1964). Based on Brakke's work it has been demonstrated that gradients in the swinging bucket or fixed angle rotors can support most samples if the ratio between sample concentration (% w/w) and starting gradient concentration (% w/w) is 1:10 w/w. If the sample concentration on the gradient is too high, streaming or turnover effect will result. Even if streaming is not evident, too high a sample concentration may overload the gradient causing broadening of the separated zones and loss of resolution. Additionally, if the sample concentration is too low, it may be difficult to identify some of the separated zones.

The volume of the sample which can be applied to the gradient is a function of the cross sectional area of the gradient that is exposed to the sample. When there is too much sample volume there is not sufficient radial distance in the centrifuge tube for effective separation of components in a multi-component sample. Typical sample volumes with respect to centrifuge tube diameter are as follows:

Approximate Tube Diameter	Sample Volume
12.5 mm	0.2 mL per tube
15.0 mm	0.5 mL per tube
25.0 mm	1.0 mL per tube
35.0 mm	2.0 mL per tube

A subsequent study by Griffith showed that fixed angle rotor can be used to effectively separate and purify these particles using these gradient techniques (Griffith 1986).

After the gradient and sample is prepared and layered in the tubes, the filled tubes are capped and placed in a pre-cooled rotor when required. Slow acceleration to approximately 800 rpm is necessary to prevent premature mixing of the gradient steps or the sample-gradient interface (Griffith, 2009). This slow acceleration permits the sample and layers to be oriented parallel to the axis of rotation before the rotor is rapidly accelerated to its maximum speed for the separation. During acceleration the gradient changes its orientation and the layers diffuse to become linear; see Figure 8. As the interfaces between the layers become wider, diffusion between the layers enable the gradient to become linear. This occurs much faster in fixed angle rotors. At the end of the run the rotor should be decelerated slowly from 800 rpm to rest to prevent the separated zones from re-mixing.

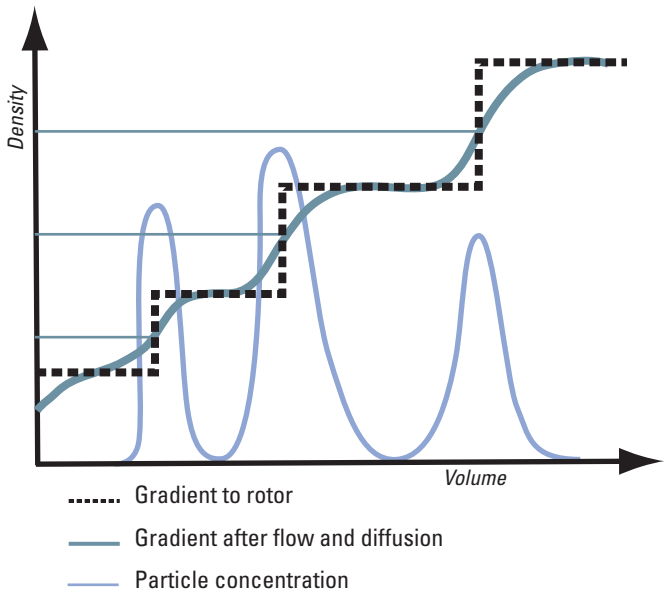


Figure 8: Gradient layering and diffusion during centrifugation.

As the sample particles migrate towards the tube wall they encounter the higher density of the gradient. This change in viscosity/density retards the sedimentation rate of the particles, thus preventing the sample particles from sliding down centrifugally along the tube wall before they are separated according to their size or density. Each particle will sediment or separate according to the calculated or appropriate run time before the sample component reaches the bottom of the tube. Therefore, the idea that the sample particles migrate towards the centrifuge tube wall and slide down to the bottom of the tube during density gradient separation in the fixed angle rotor is not accurate.

After centrifugation the rotor is decelerated rapidly to 800 rpm then slowly to rest to permit the tube contents to reorient without mixing. If the separated zones can be visually detected, a Pasteur pipette or a hypodermic syringe and needle can be used to carefully remove each zone.

Table 4: Density, refractive index, and concentration data for sucrose at 25°C, molecular weight = 342.3.

Density (g/cm^3)	Refractive index	% by Weight	mg/ml of solution*	Molarity
1.0021	1.3344	1	10.0	0.029
1.0099	1.3374	3	30.3	0.089
1.0179	1.3403	5	50.9	0.149
1.0259	1.3433	7	71.8	0.210
1.0340	1.3464	9	93.1	0.272
1.0423	1.3494	11	114.7	0.335
1.0507	1.3526	13	136.6	0.399
1.0592	1.3557	15	158.9	0.464
1.0678	1.3590	17	181.5	0.530
1.0765	1.3622	19	204.5	0.597
1.0854	1.3655	21	227.9	0.666
1.0944	1.3689	23	251.7	0.735
1.1036	1.3723	25	275.9	0.806
1.1128	1.3758	27	300.5	0.878
1.1222	1.3793	29	325.4	0.951
1.1318	1.3829	31	350.9	1.025
1.1415	1.3865	33	376.7	1.100
1.1513	1.3902	35	403.0	1.177
1.1612	1.3939	37	429.6	1.255
1.1713	1.3978	39	456.8	1.334
1.1816	1.4016	41	484.5	1.415
1.1920	1.4056	43	512.6	1.498
1.2025	1.4096	45	541.1	1.581
1.2132	1.4137	47	570.2	1.666
1.2241	1.4179	49	599.8	1.752
1.2351	1.4221	51	629.9	1.840
1.2462	1.4264	53	660.5	1.930
1.2575	1.4307	55	691.6	2.020
1.2690	1.4351	57	723.3	2.113
1.2806	1.4396	59	755.6	2.207
1.2924	1.4441	61	788.3	2.303
1.3043	1.4486	63	821.7	2.401
1.3163	1.4532	65	855.6	2.500
1.3286	1.4581	67	890.2	2.864

** Devide by 10.0 to obtain % w/v.*

Table 5: Density gradient methods in fixed angle rotors.

Experiments	Acceleration	Deceleration	Lipoprotiens recovered after floation
Run #1	Rapid to max speed	Rapid to rest	Mixed in top 20 ml
Run #2	Rapid to max speed	Rapid to 300 RPM ,then slow to rest	Mixed in top 10 ml
Run #3	Rapid to max speed	Rapid to 800 RPM, then slow to rest	Mixed in top 5-6 ml
Run #4	Rapid to max speed	Rapid to 1,000 RPM, then slow to rest	Mixed in top 5-6 ml
Experiments	**Acceleration**	**Deceleration**	**Membranes Recovered**
Run #5	Slow to 300 RPM, then rapid to max speed	Rapid to 800 RPM, then slow to rest	Sharp zone observed at the 25% and 40% sucrose interface
Run #6	Slow to 800 RPM, then rapid to max speed	Rapid to 800 RPM, then slow to rest	Sharp zone observed at the 25% and 40% sucrose interface

C. Estimation of Run Times for Sucrose Gradients in Rate Zonal Separations

An estimation of the run time for particles separated in sucrose gradients, for example, rate zonal separation by size, is required because the run has to be completed before the sedimenting particles reach the bottom of the centrifuge tube. However, in an isopycnic separation, the particles band within the gradient because the separation occurs from a difference in particle density.

Mc Ewen developed a method for the estimation of total run time for sample particles sedimenting in sucrose gradients (Mc Ewen 1967). The run time estimation was based on tables of sucrose concentration with respect to particle densities and sedimentation coefficients (S-values). The particle densities used were 1.3 g/mL for proteins and some plant bacterial viruses. The densities of 1.5 g/mL were for ribosomes and ribo-nucleoproteins and densities of 1.7 g/mL for nucleic acids.

Later, Griffith discovered by using the K-factor for the rotors, run times can be estimated for most particles when sucrose gradients of 10% w/w to 40% w/w were used. Run times for proteins and some viruses with sedimentation coefficients of 5 S to 20 S in swinging bucket rotors were observed in 16 hours at 300,000 x g (Griffith, 1986). When fixed angle and vertical tube rotors were used with the same gradient the run time for the same sample was 3 hours at the same g-forces.

Ribo-nucleoproteins, ribosomes and nucleic acids with densities 1.5 g/mL and 1.7 g/mL respectively have S-values from 20 S to 100 S. These particles, which sediment in the same sucrose gradients as the proteins, had run time estimates of 0.5 hours in fixed angle and vertical tube rotors and 2.5 hours in swinging bucket rotors at 250,000 x g. Rickwood (1983) has published an abundance of evidence from studies on fractionation of subcellular organelles. The much reduced osmolarity of iodinated density gradients offer greater resolution that can be achieved with sucrose gradients. Run times for these particles are 2.5 hours for fixed angle and vertical tube rotors and 5 hours for swinging bucket rotors at 250,000 x g. The discontinuous gradient range for sucrose was 20% w/w to 50% w/w.

VI. Isopycnic Gradient Centrifugation

Isopycnic gradient technique differs from the method of rate zonal centrifugation as it is used to separate particles on the basis of density rather than size. In the isopycnic method, the density gradient column encompasses the whole range of densities of the sample particles; see table 3. Each particle sediments to a position in the centrifuge tube where the gradient density is equal to the particle density. The particle is buoyant at that position and remains there; see Figure 9. The isopycnic technique therefore separates particles into zones solely on the basis of their different densities, independent of time. In many density gradient experiments, elements of both rate-zonal and isopycnic principles enter into the final separation. For example, the gradient may be of such a density range that one component sediments to the bottom of the tube while another component sediments to its isopycnic position and remains there.

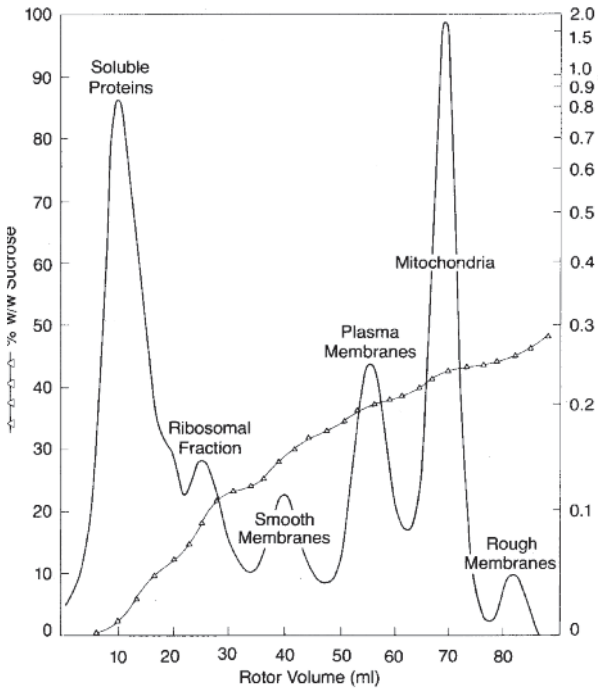


Figure 9: Separation of subcellular organelles from rat liver homogenate.

A. Selection of Gradient Material for Viscosity Gradients

Gradients are suited for separating whole cells and subcellular organelles from plant or animal tissue homogenate and for purifying some viruses. Table 4 gives a list of commonly used gradient material with their solvents and densities at 20°C. For a detailed review of the characteristics of many of these materials, please see Ifft et al. (1961) and Hu et al. (1962).

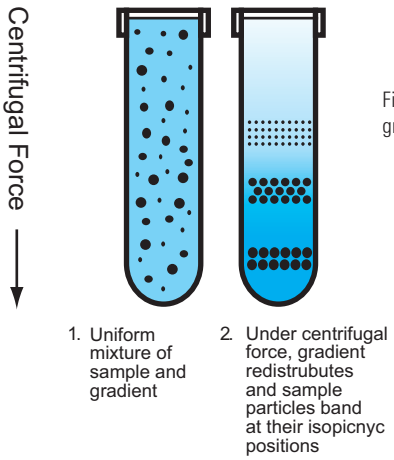


Figure 10: Isopycnic gradient separation.

B. Self-generating Gradients

In an isopycnic procedure using salt solutions it is not always convenient to form a gradient mechanically and then layer the sample solution on top of the salt solution. The low viscosity of the salt solution will not support the sample solution on the meniscus of the gradient and the sample will disperse throughout the gradient. It is sometimes easier to start with uniform concentration of sample and salt gradient material; see Figure 10. Under centrifugal forces the gradient material sediments or redistributes to form the required concentration and density change of the gradient from the top of the tube to the bottom. During centrifugation, the sample particles which were initially distributed throughout the gradient will either sediment or float to their isopycnic or buoyant position. This is an example of a self-generating gradient.

The self-generating gradient technique may require long hours of centrifugation for the gradient material to form. For example, isopycnic banding of DNA takes approximately 36-48 hours in a self-generating CsCl gradient using swinging bucket and fixed angle aluminum rotor (Anderson 1962). Without calculating the higher force fields and run times required to redistribute or to form the required gradient with salt solutions in high energy titanium fixed angle rotor, researchers follow published procedures in which swinging bucket rotor or lowspeed aluminum fixed angle rotors were used for CsCl DNA separations. Table 6 gives the density refractive index and concentration data for CsCl solution at 25° C that is typically used by researchers in the determination of a protocol for self generating gradients.

To circumvent the long run time typically associated with self generating gradients, Little and McRorie showed that Beckman high energy titanium fixed angle rotors type 70.1 Ti, type 75 Ti and type 80 Ti can produce a run time of 4.5 -0.5 hours using 8.0 mL tubes which is a similar run time for vertical or near vertical tube rotors using 5.0 mL tubes (Little and

Table 6: Commonly used gradient material.

Materials	Solvent	Maximum Density at 20° C
High viscosity, low density		
Ficoll	H_2O	1.17
Glycerol	H_2O	1.26
Silica sols	H_2O	1.30
Sucrose (66%)	H_2O	1.35
Albumin	H_2O	1.35
Sucrose (65%)	H_2O	1.37
Diodon	H_2O	1.37
Sorbitol	H_2O	1.39
Meltrizamide	H_2O	1.46
Low viscosity, low density		
Lithium chloride	H_2O	1.33
Sodium formate	H_2O	1.40
Potassium acetate	H_2O	1.41
Rubidium chloride	H_2O	1.49
Potassium formate	H_2O	1.57
Rubidium chloride	H_2O	1.63
Potassium formate	H_2O	1.63
Low viscosity, high density		
Lithium bromide	H_2O	1.83
Rubidium formate	H_2O	1.85
Cesium chloride	H_2O	1.98
Cesium acetate	H_2O	2.00
Cesium formate	H_2O	2.10

McRorie 1989). Both types of rotors used plasmid DNA in CsCl solution for separation studies. The authors demonstrated that the short path length of the Beckman 4.2 mL g-MAX™ tube can be used at higher speeds to reduce the run time without CsCl precipitation. Three rotors were used at 65,000 rpm; Beckman Types 70.1 Ti, 75 Ti, and 80 Ti. Good separation occurs in 4-5 hours with the short path length Beckman g-MAX tube. Similarly, Griffith showed that the Fiberlite F65L-6x13.5 mL rotor using similar short path length tubes accomplished the same run times with similar samples (Griffith 2007).

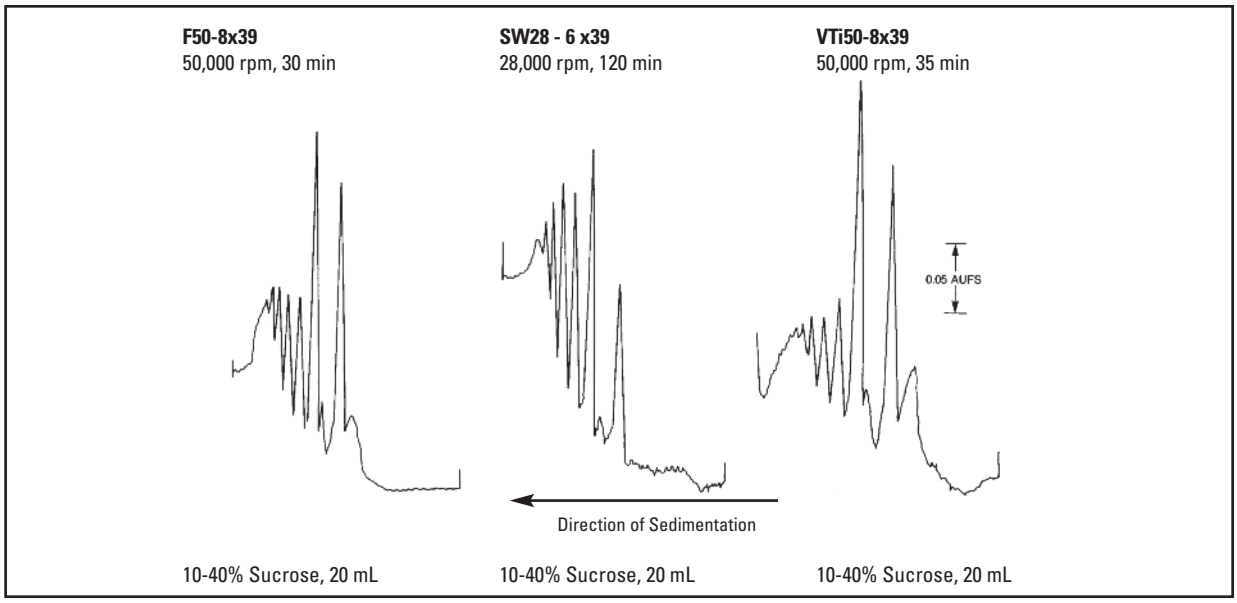


Figure 11: Rate zonal separation of polysomes from rat liver (sucrose gradient).

VII. Typical Separations of Common Sample Materials

This section contains some typical separations of common samples using the techniques previously discussed.

A. Density Gradient Separations in Vertical Tube, Near Vertical, Fixed Angle and Swinging Bucket Rotors: A Comparative Study

To compare the efficiency of separation of samples in vertical, near vertical, fixed angle and swinging bucket rotors, polysomes from rat liver were separated by density gradient methods (Griffith 2007). Rate zonal centrifugation with a sucrose gradient was used to separate the polysomes, while isopycnic separations were used to separate *Micrococcus luteus* and lambda (λ) phage DNA; see Figure 11. It should be noted that because of the short path length of the vertical tube rotor, all of the polysomes were not separated as clearly as in the swinging bucket and fixed angle rotors. The sucrose gradient concentrations were the same in the three rotors and run times were calculated from the K-factor of the rotors.

M. luteus and λ phage DNA were mixed in a solution of 1.65g/mL CsCl and used in the three rotors to show the difference in separation. The reported density of the λ phage is 1.71 g/mL and 1.73 g/mL for M. luteus. The separation of the DNA's with the fixed angle rotor was better than with the vertical tube and swinging bucket rotors; see Figure 12. In the swinging bucket rotor the gradient does not reorient to permit the inter-zone CsCl solution to separate the DNA's further than they were during the run in both the fixed angle and vertical tube rotors. It should be noted that the zones in the vertical tube rotor were separated in a larger volume of solution. Therefore, during reorientation the DNA zones were wider than in the fixed angle rotor across the diameter of the tube when both tubes were held upright. The data shows that there was better base line separation in the fixed angle rotor than in the vertical tube rotor.

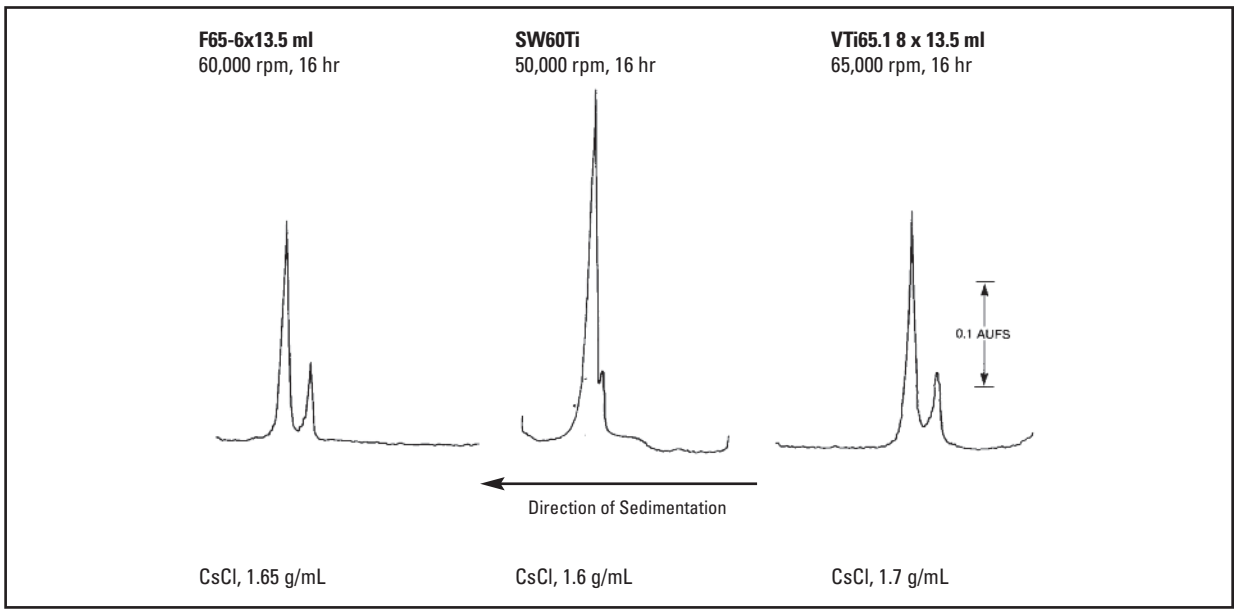


Figure 12 : Isopycnic separations of DNA from *M. luteus* and λ phage.

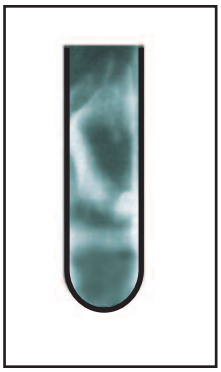


Figure 13. RNA Contaminant falling off the wall of a vertical rotor tube.

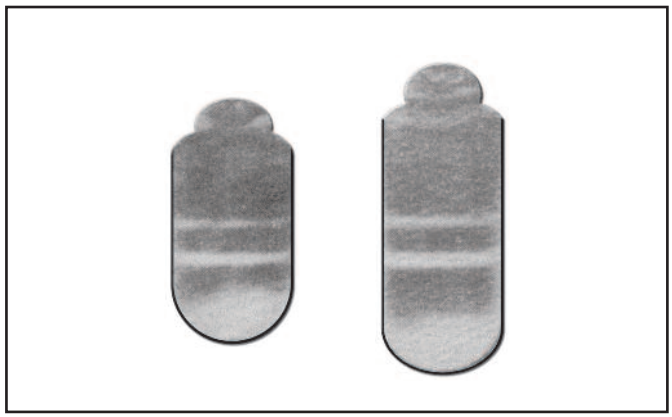


Figure 14: A separation of plasmid DNA using short path length polyallomer tubes in an ultraspeed Fiberlite fixed angle rotor.

B. Nucleic Acid Isolation in Fixed Angle, Vertical and Near Vertical Tube Rotors and RNA Sample Contamination

DNA is commonly extracted by homogenizing tissues, cells, subcellular fractions, bacteria or viruses by treatment with detergent such as Triton® X-100 or sodium dodecyl sulfate (SDS) with or without lysozyme. In some cases less gentle methods, such as boiling or alkali treatment may be suitable for cell disruption for the isolation of small plasmids (Maniatis et al, 1982).

The material may then be extracted with phenol or chloroform. The aqueous phase contains the DNA after low speed centrifugation. Any detergent used in the isolation procedure can be removed by dialysis. The DNA in the solution may then be precipitated with ethanol and the precipitate collected as sediment using a tabletop microcentrifuge at 13,000 x g.

Maniatis et al. (1982) reported that DNA could be isolated via CsCl/EtBr isopycnic gradients using an ultracentrifuge at a force field of 200,000-300,000 x g for 16 hours (overnight). Much of the preliminary sample preparation can be eliminated when this method is used. The starting tissue is ground in SDS solution and solid CsCl is added releasing the DNA. When this mixture is centrifuged using a fixed angle rotor at high speed, the proteins form a precipitate at the meniscus, and the RNA (because it is denser than the CsCl) pellets to the bottom of the tube. The isolated DNA is banded as a zone in the middle of the tube and can be collected by puncturing the tube with a hypodermic needle and syringe at the area where the DNA zone is visible.

Studies have shown that when plasmid DNA with high RNA concentration is harvested from bacterial cell lysates the RNA from the sample sediments on the tube wall when vertical and near vertical tube rotors are used. At the end of such a run this RNA falls from the tube wall and re-contaminates the purified DNA zones.

The RNA contaminant is observed falling off the wall of the tube after a run when using the vertical tube rotor; see Figure 13. This RNA re-contaminates the purified plasmid DNA.

When fixed angle rotors are used, RNA contaminants are pelleted at the bottom of the tube. Minor quantities of contaminating RNA greatly reduce the specific activity of ^{32}P-end-labeled DNA required for Maxam-Gilbert DNA sequencing as discussed by Maniatis et al.. (1982). The shortest single run with vertical or near vertical tube rotors for such studies is 4-5 hours. This is similar to run times completed with high-energy titanium fixed angle rotors or Fiberlite carbon fiber rotors with short path length tubes. Most investigators prefer fixed angle rotors because in general they are more versatile than vertical and near vertical tube rotors.

Griffith (1984) reported that plasmid DNA can be separated in 16 hours at 250,000 x g with 13.5 mL tubes in a metal ultracentrifuge rotor. The same sample can be separated in 5 hours when a half filled polycarbonate tubes are used in the Fiberlite F65L-6x13.5 mL rotor at 65,000 rpm (350,000 x g) (Griffith 2005). Overnight runs enable fellow researchers to use the centrifuge for short preparative runs during the day. Alternatively, short path length polyallomer tubes can be used to shorten the run times to as little as 4- 5 hours (Griffith 1986); see Figure 14.

The Qiagenn® Plasmid Midi and Maxi Kits can be used for the isolation of up to 100-500 µg of high- or low-copy plasmid or cosmid DNA using a protocol modified (Griffith 2009). The protocols within these kits begin with a modified alkaline lysis procedure, followed by DNA binding to Qiagen Anion-Exchange Resin under appropriate low-salt and pH conditions. Next, molecular components such as RNA, proteins, dyes and low-molecular-weight impurities are removed by a medium-salt wash. The final steps include the elution of plasmid DNA

in a high-salt buffer and concentration and desalting by isopropanol precipitation. The procedure was carefully followed through the first six steps. Precipitation of the genomic DNA, proteins, cell debris and SDS in the crude lysate was enhanced by using Buffer P3. Instead of loading the crude lysate directly onto a Qiagen column as described in the procedure, the lysate containing the plasmid DNA was transferred to 50 mL disposable conical tubes and placed in a Fiberlite F13-14x50cy rotor. The tubes were spun at 25,000 x g for 30 min at 4 °C. Removal of the particulates from the crude lysate by centrifugation simplified the collection of plasmid DNA by allowing for faster and more consistent use of the gravity fed Qiagen columns.

After centrifugation, the Qiagen protocol was followed and the plasmid DNA from the cleared lysate was collected and eluted from a Qiagen column. The DNA was then precipitated with 70% ethanol at room temperature then centrifuged at 25,000 x g for 10 min. The plasmid DNA pellet was air dried for 5- 10 min and re-dissolved in a suitable volume of buffer. In the above described procedure, Fiberlite rotors with disposable conical tubes were used at the maximum speeds in superspeed centrifuges.

Like DNA, RNA may be isolated from the cells and subcellular fractions, bacteria or viruses by extraction. SDS is often combined with phenol for this procedure because SDS dissociates RNA from proteins. The aqueous phase containing the RNA can be separated from the phenol phase by low speed centrifugation using a tabletop microcentrifuge. Any DNA present will also be extracted along with RNA but it can be eliminated by treatment with deoxyribonuclease.

Glisin et al. (1974) reported a rapid method for isolating RNA using guanidine isothiocyanate (GTC). The guanidine solution is added to the tissue or cell pellet and material is homogenized. The resulting solution is layered over approximately 2 mL of saturated solution of CsCl (5.7 M) in a 10-12 mL centrifuge tube of the fixed angle rotor. Approximately 200,000 x g for 5-7 hours is sufficient to pellet the RNA through the dense cushion of cesium chloride solution. Alternatively the run time of 16 hours can be used. During centrifugation the RNA forms pellet at the bottom of the tube, while most of the DNA floats upward in the cesium chloride solution. The supernatant is discarded and the RNA is recovered.

C. Pelleting Bacteria Using Large Volume Carbon Fiber Rotors

Bacterial cells grown in tissue culture media for extracting nucleic acids is frequently harvested with conventional superspeed centrifuges using metal fixed angle rotors, which carry bottles with volumes up to 500 mL. These rotors weigh approximately 40-49 pounds (19-22 kg). The total run time to pellet the bacteria and solids in these heavy metal rotors can be up to 20 minutes per run.

Fiberlite large volume carbon fiber rotors accept bottles up to 1000 ml in volume to sediment solids such as bacteria, yeast, and other protein precipitates from fermenters or bioreactors. These rotors are lighter weight and range from 20 to 35 pounds (10 - 15 kg). Because large volume Fiberlite rotors are lighter weight, superspeed centrifuges such as the Thermo Scientific Sorvall Evolution RC and Sorvall RC-6 Plus centrifuges, can sediment large volumes of solids in 8 to 10 minutes. When considering the time required for acceleration and deceleration, the lighter weight allows the large volume samples to experience the maximum g-force for a longer period of time resulting in a more efficient separation in less time.

Standard methods for pelleting are as follows: Inoculate Luria-Bertani broth (LB) with *Escherichia coli (E. coli)* containing the plasmid pBR322 and incubate overnight at 37 °C with vigorous shaking. Polycarbonate (PC) or polypropylene (PP) bottles can be used in all large volume rotors including the Fiberlite F10-6x500y, Fiberlite F9-4x1000y and the Fiberlite F8-6x1000y large volume rotors. The bottles can be filled with the bacteria containing medium and placed in the appropriate rotors.

The suggested set run times for the conventional superspeed centrifuges is 8 minutes with the Fiberlite F10-6x500y rotor and 10 minutes with the Fiberlite F9-4x1000y or F8-6x1000y rotor. However, the run times can be up to 20 minutes when heavy metal rotors are used.

Bacterial counts of the incubated overnight culture and that of the supernatants obtained after centrifugation can be taken to ascertain the pelleting efficiency of the rotors. All counts can be made using an electronic particle counter.

In all studies the pellets obtained were compact and allowed for easy removal after the supernatant was poured off from the bottles.

This data shows that a substantial increase in throughput efficiency over the use of metal fixed angle and heavier rotors can be achieved. This is made possible by combining standard centrifuges with the lightweight Fiberlite carbon fiber rotors. In addition the light weight of the Fiberlite large volume carbon fiber rotors makes it easier to remove rotors from the centrifuge for storage or cleaning when these rotors are used in a multiuse or student laboratory.

D. Separation of Whole Cells

Cell separation and cell harvesting methods have been reported for a variety of cell types: blood, cultured tumor cells, bacteria, algae, yeasts and cells of tissues such as brain, lymph node and spleen.

The density of most mammalian cells falls between 1.06 and 1.12 g/mL. Some cells with large amounts of cytoplasm may have densities as high as 1.29 g/mL. Tabletop centrifuges (general purpose and microcentrifuges) are used for differential centrifugation (pelleting) of small amounts of these cells.

Leif (1970), Harwood (1974), and Pretlow and Pretlow (1974) described methods of cell identification by density gradient centrifugation in floor model centrifuges. In these studies the authors report that tissue culture cell suspension and blood cells can be loaded directly onto density gradients whereas intact tissue first must be gently dispersed by mechanical or enzymatic means.

Rate zonal density gradients are commonly formed from media such as albumin, colloidal silica, Ficoll, Metrizamide, Nycodenz or OptiPrep. Some of these gradient materials can also be used for isopycnic separation of cells. When either isopycnic or rate zonal gradients can achieve the desired separation, reports show that preference is usually given to rate zonal method which exposes the cells to lower centrifugal force and shorter centrifugation run times.

E. Use of the Centrifuge for Nuclei Preparation

Nuclei are very large organelles composed of a nuclear membrane, nucleoplasm, chromatin material, and nucleolus. Any procedure to isolate nuclei must break the cell but not the nucleus. This procedure is accomplished by homogenizing the cells in buffer containing cations to stabilize the nuclear membrane to prevent the release of DNA. The homogenate is filtered then centrifuged at 5000 x g for 5 min to pellet the nuclei, cells and large debris.

Since cytoplasmic contamination of the nuclei will interfere with analysis of nuclear proteins, many procedures require resuspension of the nuclear pellet and layering it over a dense cushion of sucrose. Nuclei are denser than other cell components; therefore they will sediment through the sucrose as a pellet, while the other cell components will be trapped above the sucrose barrier. To prepare a large amount of nuclei from liver homogenate or other cell suspensions using the Fiberlite F40L-8x100 rotor follow the protocol suggested below:

For each 100 mL polycarbonate oak ridge centrifuge bottle, resuspend the pellet from 8g of homogenate in 20 mL of 8 % w/w sucrose (0.25M). Place 6 mL of 60% w/w sucrose (2.25 M) in each oak ridge bottle. Layer 20 mL of the re-suspended pellet on top of the sucrose solution using a hypodermic syringe with an 18-gauge needle or a Pasteur pipette. Take care to minimize disturbing the interface between the sucrose solution and the sample suspension.

Place the Fiberlite F40L-8x100 rotor in the ultracentrifuge such as Thermo Scientific Sorvall WX Series. Place the loaded bottles into the rotor and seal with the rotor lid. Set the centrifuge to accelerate to speed at slow acceleration (setting 2) to 40,000 rpm or 38,500 rpm. Set the run time for 30 min at 4°C. At the end of the run, decelerate the rotor slowly (setting 2). Remove the tubes from the rotor and the nuclei will be found as a pellet at the bottom of the bottles.

F. Isolation of Plasma Membranes by Differential Centrifugation

Differential centrifugation is commonly used to isolate subcellular components from tissue homogenate. This method can quickly eliminate many unwanted components from a large amount of starting material. Once collected, the components of interest may be analyzed or purified further by density gradient centrifugation using discontinuous gradients.

Of the many procedures reported in the literature for isolation of plasma membranes all employed differential centrifugation to first separate the membranes from most other component. After the sample tissue is homogenized and screened, the filtrate is centrifuged at low speed to remove the nuclei and other large debris, leaving the plasma membranes fragments and mitochondria in the supernatant. Other methods centrifuge at higher speeds to pellet membranes, mitochondria and nuclei together. There may follow several centrifugations to "wash" the pellet of contaminating materials.

In another method for plasma membrane purification, Barden et al. (1983) reported an isolation called density perturbation employing a change in the membrane density. Plasma membranes contain various protein receptors each binding a specific ligand, which is in turn coupled to a high-density particle. The increase in membrane density, which results from the binding of the particular ligand-high density particle complex to the receptor facilitates the separation of the membranes from other cell components by discontinuous gradient centrifugation.

Discontinuous step gradients often follow for further separation of the plasma membranes. The nuclei will pellet while the plasma membranes and mitochondria band together at one of the density steps. Another step gradient can be used to band the membranes and pellet the mitochondria. Dense sucrose layers are used for the gradients because membranes which band at a density of 1.18 g/mL are impermeable to sucrose, but mitochondria which would otherwise band at the same density may be permeable due to the high g-forces used in centrifugation. The density alteration in the mitochondria resulting from the uptake of sucrose permits the separation to take place.

The Fiberlite F21S-8x50y carbon fiber rotor can be used in conjunction with the Sorvall(R) Evolution RC or Sorvall RC-6 Plussuperspeed centrifuges to isolate plasma membranes from tissue homogenate using the suggested guidelines below:

Centrifuge the post mitochondria supernatant at 50,000 x g for 2 hr to pellet the membranes. Mix the pellet with 40% w/w sucrose (1.375 M). Form a 5 step discontinuous gradient, consisting of 8.0 mL per step in a 50 mL oak ridge bottle using the following concentrations: 10, 25, 30, 40 % w/w sucrose. Load 8 mL of sample in the bottom of the bottle after it is adjusted to 40% w/w sucrose. Place the sample in the Fiberlite F21S-8x50y rotor and spin at 21,000 rpm with slow acceleration and deceleration for 3.5 hr at 4 °C.

The plasma membranes will be located at the 25/10 % interface. The endoplasmic reticulum will be found at the 25/30 % interface and the lysosomes will be found at the 30/40% interface. Mitochondria and peroxisomes can be found in the 45% layer at the bottom of the bottle.

G. Isolation of Lipoproteins

Lipoprotein classification is important to human cholesterol studies. The various classes of serum and plasma lipoproteins differ from one another in both densities and sedimentation; differential flotation is the most commonly used method to separate them. Usually the starting solution is first centrifuged at approximately 15,000 x g for 30 min at the normal serum density to allow large chylomicrons to float to the top. A subsequent step will allow for the separation of very low-density lipoprotein (VLDL) which is removed at the end of the centrifugation. The VLDL cholesterol is then quantified meanwhile the low-density lipoproteins (LDL) and the high-density lipoproteins (HDL) remains in solution in the tube. The remaining solution in the tube is adjusted with salts (sodium chloride, sodium bromide or potassium bromide) to the density needed to float the next classes of lipoprotein. Using the Fiberlite F50L-24x1.5 rotor in an ultracentrifuge (i.e. Sorvall WX Series), the following protocol can be used for the isolation of VLDL proteins:

Place a volume of sample with chylomicrons removed into a 1.5mL polyallomer microcentrifuge tube. Place in the Fiberlite F50L-24x1.5 rotor and place in the ultracentrifuge. Spin the sample at maximum speed and force of the rotor (50,000 rpm and 281,000 x g) for 5 hours. The VLDL will float to the top. The remaining sample can be recentrifuged at progressively longer times for isolation of the LDL and HDL lipoproteins.

Bronzert and Brewer (1977) reported a micro method employing an air driven tabletop ultracentrifuge for quantifying cholesterol in plasma lipoprotein fractions. The method was later modified for use with a tabletop microcentrifuge. The plasma is separated from red blood cells with a pasture pipette. After low speed centrifugation using the table top centrifuge at 10,000 x g for 5 min at 4°C, cold heparin manganese solution is added to plasma. This solution is kept cold for 30 min to precipitate both VLDL and LDL. The remaining sample is then centrifuged at 15,000 x g for 30 min after which the HDL is quantified directly in the tube from the recovered supernatant. Using the quantified values of the precipitated VLDL and the total cholesterol in the starting plasma sample an estimate of the LDL cholesterol can be determined.

H. Rapid Isolation of Synaptosomes from Rat Brain Tissue

When brain or other nerve rich tissue is homogenized, the disrupted nerve endings form vesicles called synaptosomes. These synaptosomes consist of the plasma membranes and the organelles of the nerve endings (i.e. mitochondria and synaptic vesicles). The synaptic vesicles are small secretory vesicles, which store the various neurotransmitters. Isolation of synaptosomes, synaptic vesicles and synaptic membranes, are usually separated with Ficoll, Percoll, Metrizamide or Nycodenz gradients because of the osmotic shock of the synaptosomes by sucrose gradients.

The Fiberlite F21-8x50y rotor can be used in conjunction with superspeed centrifuges, such as the Sorvall Evolution RC and Sorvall RC-6 Plus centrifuges, for the procedure described below:

Prepare a discontinuous Ficoll gradient consisting of 12 mL per step with the following concentrations: 4, 6, and 13% w/v (Dodd et al. 1981). Prior to the run, homogenize the brain tissue in isotonic sucrose solution and spin at 40,000 x g for 1 hr in the Fiberlite F21-8x50y fixed angle rotor. Re-suspend the pellet in 2% Ficoll and layer 4 mL of this sample on top of the starting gradient. Using slow acceleration and deceleration profiles, set the superspeed centrifuge to run the carbon fiber rotor at 20,000 rpm (X g) for 2 hr at 4 °C.

The synaptosome band will be visible at the 6/13% interface. The sediment consists of connective tissue and other debris. A hypodermic syringe with a long needle can be used to remove the separated synaptosome band.

I. Isolation of Virus

Viruses and bacteriophage are usually isolated from their host cell by differential centrifugation. Cells are homogenized and centrifuged. After centrifugation at 15,000 x g for 10 min the debris is pelleted and the virus can be collected from the supernatant. Alternatively, the virus can be concentrated by pelleting in a fixed angle rotor at 100,000 x g for 30 min. Some viruses lose their biological activity as a result of pelleting. This problem can be avoided by sedimenting the virus on to a dense sucrose cushion. Isopycnic gradient is the most frequently used method for final purification of virus. The following gradient materials may be used CsCl, potassium tartrate, sucrose, or iodinated media such as Metrizamide or Nycodenz. The last two are especially useful for purifying viruses that lose infectivity in CsCl or when the medium (such as potassium tartrate) may be toxic to tissue culture cells. Vanden Berghe (1983) discusses the effects of various density gradient media on animal virus infectivity as well as media toxicities to cultured cells. A discontinuous gradient can be used for the preservation of infectivity in viruses during centrifugation. Infectivity is preserved when glycerol is used as a negative gradient during density gradient separation. The discontinuous gradient preparation is modified from the method of Talbot (1977). Samples may be placed in the Fiberlite F21-8x50y rotor and spun in a superspeed centrifuge, such as the Sorvall RC-6 Plus or Evolution RC, at 20,000 rpm for 2.5 hr at 4 °C; see Table 7.

J. Nucleic Acid DNA

DNA is commonly extracted by homogenizing tissues, cells and subcellular fractions, bacteria or viruses by treatment with detergent such as Triton X-100 or sodium dodecyl sulfate (SDS) with or without lysozyme. In some cases less gentle methods cells disruption such as boiling or alkali treatment may be suitable for the isolation of smaller plasmids, as reported by Maniatis et al.. (1982)

The material may then be extracted with phenol or chloroform, and the aqueous phase containing the DNA collected after low speed centrifugation. Any detergent used in the isolation procedure can be removed by dialysis. The DNA in the solution may then be precipitated with ethanol and the precipitate collected as sediment using a tabletop micro centrifuge at 13,000 x g.

TABLE 7. MODIFIED METHOD FOR PREPARING A DISCONTINUOUS GRADIENT WITH GLYCEROL AND POTASSIUM TARTRATE AT DIFFERING CONCENTRATIONS.

DISCONTINUOUS GRADIENTS
Purification of Viruses
*Preservation of Infectivity Using Glycerol-Potassium Tartrate Gradients**

Glycerol%	+	K-Tartrate%
30	+	5
20	+	15
15	+	30
5	+	50

Run time 2.5 hr. at 20,000 rpm in F21B-8x50 mL 4 deg C
* Talbot, J. Gen. Virology 36:345 (1977)

Maniatis et al.. (1982) reported that DNA could be isolated via CsCl/EtBr isopycnic gradients using an ultra speed centrifuge at a force field of 200,000-300,000 x g for 16 hours (overnight). Much of the preliminary sample preparation can be eliminated when this method is used. The starting tissue is ground in SDS solution and solid cesium chloride is added releasing the DNA. When this mixture is centrifuged using a fixed angle rotor at this ultra speed, the proteins form a precipitate at the meniscus, and the RNA (because it is denser than the cesium chloride) pellets to the bottom of the tube. The isolated DNA is banded as a zone in the middle of the tube and can be collected by puncturing the tube with a hypodermic needle and syringe at the area where the DNA zone is visible.

K. Separation of Sarcoplasmic Reticulum

Previous studies by Kai, Y. et al., reported that glycolytic enzymes were associated with sarcoplasmic reticulum membrane vesicles and metabolism through these enzymes was capable of supporting 45Ca transport (Kai et al, 1995). Sealed right-side-out SR vesicles were isolated by step sucrose gradient using a swinging bucket rotor to purify the SR vesicles from rabbit skeletal and cardiac muscle. Intravesicular 45Ca transport was measured after the addition of glycolytic substrates and cofactors specific for each of the glycolytic reactions were studied or after the addition of exogenous ATP and was expressed as transport sensitive to the specific Ca^{2+}-ATPase inhibitor thapsigargin.

The authors found that the entire chain of glycolytic enzymes from aldolase, including aldolase, GAPDH, phosphoglycerate kinase (PGK), phosphorglyceromutase, enolase, and pyruvate kinase (PK), was associated with SR vesicles from both cardiac and skeletal muscle.

In the centrifugation method for purifying the SR membranes, the Fiberlite carbon fiber fixed angle F50L-8x39 can be used with a differential centrifugation method to collect the supernatant containing the SR vesicles and purify the SR membrane vesicles by density gradient centrifugation. The total run time for the SR preparation and purification is less than 3.5 hours when the F50L – 8 x 39 rotor was used with a Thermo Scientific Sorvall Ultracentrifuge. A total of 18 hours with a swinging bucket rotor with similar tube volumes were needed to accomplish the same purification of the SR membranes (Kai et al., 1995).

For SR Vesicle preparation carefully remove cardiac and skeletal muscle from rabbits prepare the sarcoplasmic reticulum by the method of Chu et al (Chu et al., 1988). Dounce homogenize the tissue in 0.29 mol/L sucrose and 10 mmol/L imidazole/HCl buffer without KCl, pH 6.8, for 15 seconds at 22 000 rpm. Centrifuge the homogenate was at 8000 rpm (~11,000 x g) for 15 minutes and discard the pellet. Centrifuge the supernatant 50 000 rpm (~250,000 x g) for 20 minutes. Harvest and resuspend the pellet before loading on top of a five – step sucrose gradient, 45% w/w, 40% w/w, 35% w/w, 30% w/w, and 25% w/w (Griffith 2006). Centrifuge the gradient/sample solution for 2.0 hours at 50,000 rpm (~250,000 x g) with slow acceleration and slow deceleration instrument setting #3 to prevent mixing of the gradient and separated membranes before and after the run. The SR fraction can be visually observed at the interface between 30% and 35% gradient steps. Collect the fraction and sediment for 30 minutes at 50,000 rpm (~250,000 x g) (Griffith 2007). Re-suspend pellet in 10 mmol/L imidazole/HCl and 0.29 mol/L sucrose buffer and store at -70°C. After purification the sarcoplasmic reticulum was tested. The enzymes GAPDHPGK was observed bound to both cardiac and skeletal muscle of the sarcoplasmic reticulum. This info was consistent with the work of Chu et al (Griffith 2007). Ca2+-ATPase activity and sarcoplasmic reticulum Ca2+ uptake can be supported solely by provision of glycolytic substrates and cofactors without the addition of exogenous ATP. This information was consistent with previous works (Chu et al, 1998).

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IX. Appendices

Appendix 1: Chemical Resistance

The charts on the next three page indicate the general chemical resistances of various materials to a number of chemicals. Both the materials and the chemicals are commonly used in experiments involving centrifuges. By reading down a larticular column, the reader can determine the resistance of that material to each chemical – either satisfactory (S), marginally satisfactory (M), unsatisfactory (U) or unknown (blank). The chemicals are listed by their most common name within six categories: gradient forming media, acids, salts, solvents, bases and others. This chart has no guarantee for tubes or bottles. The user should text them under actual conditions of use because chemical resistance varies with speed, temperature, etc.

Based on internal testing, the following are recommendations for tube/chemical compatibility. Performance is not guaranteed. You should test them under actual conditions of use, because chemical resistance varies with speed, temperature, etc.

Chemical Resistance Charts

PA (Polyallomer)	SS (Stainless Steel)	TI (Titanium Alloy)	NBR (Nitrile-Butadione)
PC (Polycarbonate)	PET (Polyethylene Terephthalate)	PPO (Polyphenylene Oxide)	NY (Nylon)
PE (Polyethylene)	AL (Aluminum Alloy)	PAL (Polyacetal) (Delerin)	SI (Silicon Rubber)
PP (Polypropylene)	CF (Carbon Fiber)	CR (Neoprene Rubber)	VI (Victon, Fluorine Rubber)

S Satisfactory	U Unsatisfactory	M Marginal	- No Test Made

CHEMICALS	PA	PC	PE	PP	SS	PET	AL	CF	TI	PPO	PAL	CR	NBR	NY	SI	VI
GRADIENT MEDIUM																
Cesium Acetate	S	S	S	S	M	-	M	S	S	S	S	S	S	S	S	S
Cesium Bromide	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Cesium Chloride	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Cesium Formate	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Cesium Iodide	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Cesium Sulfate	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Dextran (Sulfate)	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Ficoll-Paque	S	S	S	S	M	-	M	S	S	S	S	S	S	S	S	S
Glycerol	S	S	S	S	S	S	M	S	S	S	S	S	S	S	S	S
Metrizamide	S	S	S	S	M	-	M	S	S	S	S	S	S	S	S	S
Potassium Bromide	S	S	S	S	M	S	U	S	S	S	S	S	S	S	S	S
Rubidium Bromide	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Rubidium Chloride	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Sodium Bromide	S	S	S	S	M	S	U	S	S	S	S	S	S	S	S	S
Sodium Iodide	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Sucrose	S	S	S	S	S	S	M	S	S	S	S	S	S	S	S	S
Sucrose, alkaline	S	U	S	S	M	U	M	S	S	S	S	S	S	S	S	S
ACIDS																
Acetic Acid (5%)	S	S	S	S	M	S	S	S	S	S	M	S	M	S	S	M
Acetic Acid (60%)	S	U	M	M	U	U	S	M	S	S	U	M	M	-	M	U
Acetic Acid (glacial)	S	U	M	M	U	U	S	U	S	S	U	U	U	U	U	U
Aqua Regia	U	U	U	U	U	-	U	U	S	U	U	U	U	-	M	M
Boric Acid	S	S	S	S	S	S	U	S	S	S	U	S	S	S	S	S
Chromic acid (10%)	S	M	S	S	U	S	U	S	S	S	U	U	U	U	M	S
Chromic acid (50%)	M	U	S	S	U	-	U	U	S	U	U	-	-	-	-	S
Citric Acid	S	S	S	S	S	S	S	S	S	M	U	S	S	M	S	S
Formic Acid (50%)	S	M	S	S	U	S	U	M	S	S	U	S	U	U	S	U
Hydrochloric Acid (10%)	S	M	S	S	U	S	U	S	S	S	U	S	S	-	S	S
Hydrochloric Acid (35%)	S	U	S	S	U	U	U	M	M	S	U	M	M	U	U	M
Hydrofluoric Acid (10%)	S	M	S	S	U	-	U	S	U	-	U	S	U	S	-	-
Hydrofluoric Acid (50%)	M	U	S	S	U	-	U	M	U	U	U	S	U	-	U	M
Lodoacetic Acid	S	M	S	S	S	M	S	S	S	S	S	M	-	M	M	M
Lactic Acid	S	S	M	S	S	-	-	-	S	S	-	-	-	-	-	S
Mercaptoacetic Acid	M	U	U	U	S	U	U	S	S	S	S	M	-	U	U	S
Nitric Acid (10%)	S	S	S	S	M	S	M	S	S	S	U	U	U	M	M	S
Nitric Acid (50%)	S	M	M	M	M	U	M	U	S	S	U	U	U	M	U	S
Oleic Acid	S	S	S	S	U	S	S	S	S	S	U	U	-	S	M	M
Oxalic Acid	S	S	S	S	U	-	M	S	M	S	U	S	M	S	U	S
Perchloric Acid	M	U	M	M	U	U	U	U	S	S	U	-	U	U	U	S
Phosphoric Acid (10%)	S	S	S	S	M	S	U	S	U	S	U	S	S	U	U	S
Phosphoric Acid (50-85%)	M	U	S	S	M	S	U	S	U	M	U	U	S	U	U	S
Piric Acid	M	U	S	S	U	-	U	M	U	S	U	U	S	U	U	S
Stearic Acid	S	S	S	S	S	S	S	S	S	S	S	S	S	S	M	S
Sulfuric Acid (10%)	S	S	S	S	U	S	U	M	U	M	U	S	S	S	U	S
Sulfuric Acid (50%)	S	U	S	S	U	S	U	U	U	U	U	S	-	M	U	S
Sulfuric Acid (98%)	S	U	M	S	M	U	U	U	U	M	U	U	U	U	U	S
Tartaric Acid	-	-	-	-	-	-	-	S	-	-	-	S	S	-	S	-
Trichloroacetic Acid	S	M	S	S	U	U	U	S	U	S	U	U	-	U	U	U

Chemical Resistance Charts (continued)

PA (Polyallomer)	SS (Stainless Steel)	TI (Titanium Alloy)	NBR (Nitrile-Butadione)
PC (Polycarbonate)	PET (Polyethylene Terephthalate)	PPO (Polyphenylene Oxide)	NY (Nylon)
PE (Polyethylene)	AL (Aluminum Alloy)	PAL (Polyacetal) (Delerin)	SI (Silicon Rubber)
PP (Polypropylene)	CF (Carbon Fiber)	CR (Neoprene Rubber)	VI (Victon, Fluorine Rubber)

S Satisfactory	U Unsatisfactory	M Marginal	- No Test Made

CHEMICALS	PA	PC	PE	PP	SS	PET	AL	CF	TI	PPO	PAL	CR	NBR	NY	SI	VI
SALTS																
Aluminium Chloride	S	S	S	S	U	S	U	M	U	S	U	S	S	M	M	S
Ammonium Acetate	S	S	S	S	S	S	S	S	S	S	S	S	-	S	S	S
Ammonium Carbonate	S	U	S	S	S	S	S	S	S	S	S	S	U	S	S	S
Ammonium Chloride	S	-	-	-	-	-	-S	S	-	-	S	S	S	-	S	-
Ammonium Phosphate	S	M	S	S	M	-	U	S	S	S	S	S	S	S	S	S
Ammonium Sulfate	S	S	S	S	M	S	S	S	S	S	S	S	S	S	S	U
Barium Salts	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Calcium Chloride	S	M	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Calcium Hypochlorite	S	M	S	S	U	-	U	M	S	S	M	M	M	S	S	S
Ferric Chloride	M	S	S	S	U	-	U	S	S	S	M	S	S	S	S	S
Guanidine Hydrochloride	S	S	S	S	U	S	U	S	S	S	S	S	-	S	S	S
Guanidine Thiocyanate	S	-	S	S	-	U	-	-	S	-	-	-	-	-	-	-
Magnesium Chloride	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	S
Nickel Salts	S	S	S	S	S	S	M	S	S	S	S	S	-	S	S	S
Potassium Acetate	S	M	S	S	S	-	M	-	S	-	-	-	-	-	-	-
Potassium Carbonate	S	S	S	S	S	S	M	S	S	S	S	S	-	S	S	S
Potassium Chloride	S	S	S	S	U	S	U	S	S	S	S	S	-	S	S	S
Potassium Iodide	S	S	S	S	S	S	M	S	S	S	S	S	-	S	S	S
Potassium Permanganate	S	S	S	S	M	S	S	S	S	S	S	M	-	U	M	S
Silver Nitrate	S	S	S	S	M	S	U	S	S	S	S	M	-	S	U	S
Sodium Bicarbonate	-	-	-	-	-	-	-	S	-	-	S	S	S	-	S	S
Sodium Carbonate	S	S	S	S	S	S	M	S	S	S	S	S	S	S	S	S
Sodium Chloride	S	S	S	S	M	S	U	S	S	S	S	S	S	S	S	S
Sodium Dichromate	S	S	S	S	-	-	M	-	S	-	-	-	-	S	-	-
Sodium Nitrate (10%)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	U	S
Sodium Phosphate	-	-	-	-	-	-	-	S	-	-	S	S	S	-	U	S
Sodium Sulfate	S	S	S	S	M	S	U	S	S	S	S	S	-	S	S	S
Sodium Thiosulfate	-	-	-	-	-	-	-	S	-	-	S	S	S	-	S	S
Zinc Chloride	S	S	S	S	U	S	M	S	S	S	U	S	S	S	S	S
Zinc Sulfate	S	S	S	S	S	S	U	S	S	S	S	M	S	S	S	S
BASES																
Ammonium Hydroxide (10%)	S	U	S	S	S	-	U	S	S	S	U	S	U	S	S	S
Ammonium Hydroxide (28%)	S	U	S	S	S	U	U	M	S	S	U	S	-	S	S	U
Aniline (10%)	U	U	U	M	S	U	S	M	S	U	S	U	U	U	S	S
Potassium Hydroxide (5%)	S	U	S	S	M	M	U	S	M	S	U	S	-	S	M	S
Potassium Hydroxide (45%)	S	U	S	U	M	U	U	S	U	S	U	S	S	U	U	M
Pyridine (50%)	M	U	S	S	U	U	U	U	U	U	M	U	U	S	S	U
Sodium Hydroxide (1%)	S	U	S	S	S	M	U	S	S	S	U	S	-	S	M	S
Sodium Hydroxide (10%)	S	U	S	S	S	M	U	S	S	S	U	S	S	S	U	S
Sodium Hydroxide (50%)	M	U	S	M	S	U	U	S	M	M	U	-	-	S	U	U

Chemical Resistance Charts (continued)

PA (Polyallomer)	SS (Stainless Steel)	TI (Titanium Alloy)	NBR (Nitrile-Butadione)
PC (Polycarbonate)	PET (Polyethylene Terephthalate)	PPO (Polyphenylene Oxide)	NY (Nylon)
PE (Polyethylene)	AL (Aluminum Alloy)	PAL (Polyacetal) (Delerin)	SI (Silicon Rubber)
PP (Polypropylene)	CF (Carbon Fiber)	CR (Neoprene Rubber)	VI (Victon, Fluorine Rubber)

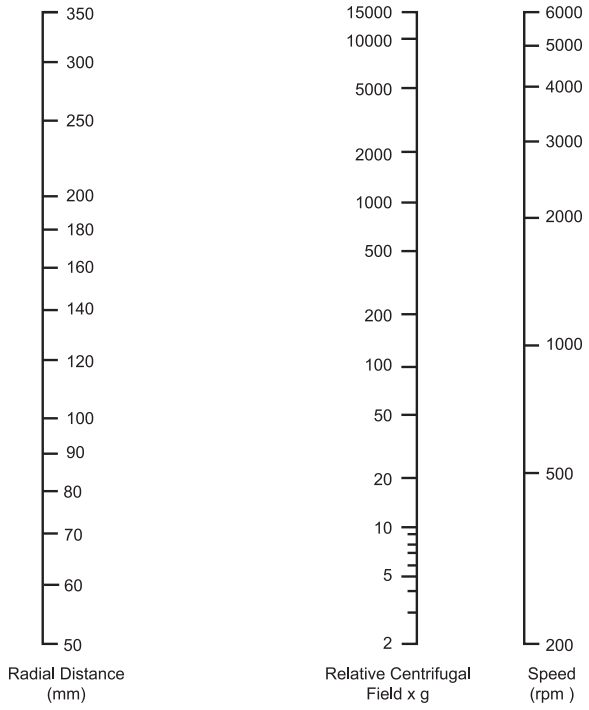
S Satisfactory	U Unsatisfactory	M Marginal	- No Test Made

CHEMICALS	PA	PC	PE	PP	SS	PET	AL	CF	TI	PPO	PAL	CR	NBR	NY	SI	VI
SOLVENTS																
Acetone (50%)	U	U	S	U	S	U	S	M	S	U	M	U	U	M	S	U
Amyl alcohol	S	M	U	U	-	-	S	M	S	M	S	S	S	S	S	M
Benzene	U	U	U	U	S	U	S	U	S	U	M	U	U	S	S	S
Carbon Tetrachloride	U	U	U	U	M	U	U	U	U	U	M	U	M	S	S	S
Chloroform	U	U	U	U	S	U	U	M	U	U	M	U	U	S	S	S
Cresol	-	U	U	U	S	U	S	M	S	U	S	U	U	U	U	U
Diethyl Ether	U	U	U	U	S	U	S	U	S	U	S	U	U	S	S	U
Diethyl Ketone	U	U	U	M	M	-	S	M	S	-	M	U	M	S	S	U
Dimethyl Sulfoxide	M	U	S	S	S	U	S	S	S	S	S	U	-	S	S	U
Dimethylformamide	M	U	S	S	S	U	S	M	S	U	S	S	-	S	S	U
Ethyl Acetate	U	U	M	U	M	U	M	M	S	U	M	M	U	S	S	U
Ethyl Alcohol (50%)	S	U	S	S	S	M	S	S	S	S	M	S	-	S	S	S
Ethyl Alcohol (95%)	S	U	S	S	S	U	S	M	S	S	M	S	S	S	S	S
Ethylene Glycol	S	U	S	S	M	-	S	S	S	S	S	S	S	S	S	S
Isopropyl Alcohol	S	U	S	S	S	-	S	S	U	S	S	M	-	S	S	S
Methyl Alcohol	S	U	S	S	S	M	S	M	S	S	M	S	S	S	S	U
Methyl Ethyl Ketone	U	U	U	U	S	U	S	U	S	U	M	U	U	S	S	U
Methylene Chloride	U	U	U	U	M	U	U	S	U	U	S	U	-	M	M	M
N-Butyl Alcohol	M	M	M	S	-	-	M	M	S	M	S	S	S	S	S	S
Phenol (1%)	S	U	S	S	S	U	U	M	S	M	S	U	U	U	U	S
Toluene	U	U	U	U	S	U	S	M	U	U	M	U	U	S	S	S
Xylene	U	U	U	U	S	U	S	M	S	U	M	U	U	U	U	S
OTHERS																
Culture Media	S	S	S	S	-	-	S	-	-	M	-	-	-	-	-	-
Diethylpyrocarbonate	S	U	S	S	S	U	S	S	S	U	S	S	-	S	S	S
Ethylene Oxide Vapor	S	M	S	S	S	-	S	U	S	-	-	U	U	S	S	U
Formaldehyde (40%)	S	S	S	S	M	S	M	S	S	S	S	S	S	S	S	U
Hydrogen Peroxide (3%)	S	S	S	S	S	S	S	M	S	S	M	S	U	S	U	S
Hydrogen Peroxide (5%)	S	S	S	S	S	S	S	M	U	U	U	M	U	U	U	M
Kerosene	U	U	U	U	S	U	S	S	S	U	S	U	S	S	S	S
Liquid Paraffin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2-Mercaptoethanol	S	M	S	S	S	U	S	M	S	S	S	U	-	S	S	S
Milk	S	S	-	-	-	-	S	-	-	M	-	-	-	-	-	-
Petroleum	M	M	U	U	U	S	-	S	S	S	S	S	S	S	S	S
Serum	S	S	-	-	-	-	S	-	-	M	-	-	-	-	-	-
Sodium Hypochlorite (5%)	S	U	M	M	S	S	M	U	S	S	U	U	M	S	S	S
Tris Buffer (neutral)	S	S	S	S	S	S	S	S	S	U	S	S	-	S	S	S
Triton X-100	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Vegetable Oils	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Urea	S	S	S	S	S	S	S	S	S	S	S	S	-	S	S	S

Appendix 2: Nomogram for Speed Selection of Rotors

A. Tabletop centrifuges

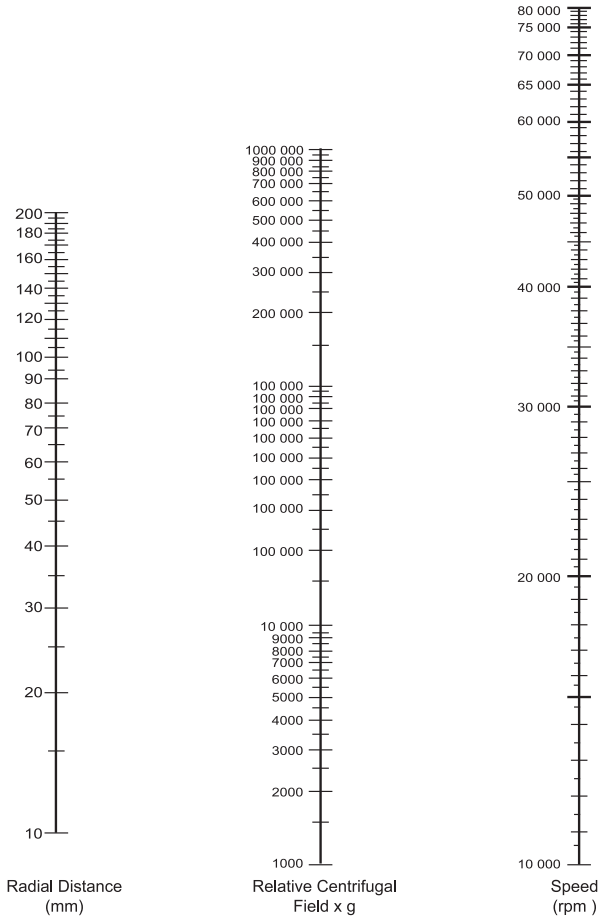
The centrifugal force at a given radius is a function of run speed. To obtain a desired rotor force, align a straight-edge through known values in any two columns. Read the required value from the third column intersect.



A. Nomogram for Speed Selection for Tabletop Rotors

B. Superspeed/Highspeed and Ultraspeed Centrifuges

Align a straight-edge through known values in two columns; read the desired value where the straight-edge intersects the third column.



B. Nomogram for Speed Selection for Superspeed Rotors

C. Appendix 3: Glossary of Terms Used in Centrifugation

ACCELERATION TIME of a rotor to its maximum speed varies with the rotor, the centrifuge, and the acceleration program selected by the user.

ANGULAR VELOCITY (symbol: ω, omega) is the rotational velocity of a body about an axis, expressed in radians per second:
ω = 2rpm/60, or
ω = 0.10472 x rpm
where RPM is the speed of rotation in revolutions per minute (rpm)

AVERAGE RADIUS (symbol: $_{rav}$) of a rotor is the distance from the center of rotation to the midpoint of the centrifuge tube during centrifugation.

AVOGADRO'S NUMBER (symbol: N) is the number of molecules in one gram molecular weight, or one mole, of a substance. It is equal to 6.023 x 10^23.

BANDING is the redistribution and concentration of particles at their buoyant densities during isopycnic centrifugation.

BUOYANT DENSITY is the effective density of a particle, as determined by isopycnic centrifugation in a specific gradient medium.

CENTRIFUGAL FORCE (symbol: *g*) is exerted on a rotating body or particle, tending to pull it away from the center of rotation.

CLEARING is the removal, by sedimentation in a centrifugal field, of particles from a supernatant.

CLEARING FACTOR (symbol: K) is a constant, different for each rotor, used to compare the relative efficiency of rotors for pelleting operations:
K = T/S,
where T is the clearing time of a specified particle in hours and S is the sedimentation coefficient of that particle in Svedbergs

CLEARING TIME is the time required to sediment a particle in aqueous solution to the bottom of a centrifuge tube:
T = K/S,
where T is the clearing time in hours, K is the clearing factor for a specific rotor, and S is the sedimentation coefficient of the particle in Svedbergs

CUSHION is a layer of dense solution placed at the bottom of a centrifuge tube to prevent damage to or pelleting of particles.

DECELERATION TIME of a rotor from its maximum speed varies with the rotor, the centrifuge, and the deceleration program selected by the user.

DENSITY (symbol: ρ, rho) is the mass per unit volume of a substance, often expressed in g/ml.

DENSITY GRADIENT CENTRIFUGATION is separation performed in a supporting column of solution in which the density and solution concentration increase toward the bottom of the centrifuge tube.

DERATION of rotors, after a specified amount of use, guards against the effects of metal fatigue.

DIFFERENTIAL CENTRIFUGATION separates particles on the basis of their size. By a series of centrifugations at various speeds and times, different sized particles are sedimented and collected from an initially homogenous suspension.

DISCONTINUOUS, OR STEP, GRADIENT is one composed of layers, with abrupt changes in density and/or concentration from one layer to the next.

EQUILIBRIUM, OR ISOPYCNIC, GRADIENT CENTRIFUGATION separates particles on the basis of their buoyant densities. A gradient range is selected to encompass the densities of all particles to be banded. Equilibrium gradients may be self-generated by centrifugal force acting on a solution of uniform starting density, or they may be preformed by layering to achieve equilibrium more rapidly.

FLOATATION COEFFICIENT (symbol: s) is analogous to the sedimentation coefficient for particles that float rather than sediment. *g* is the symbol for the standard acceleration of gravity. Centrifugal force is also expressed in terms of *g*.

GRADIENT LAYERING is the process of making a preformed discontinuous gradient by means of a mechanical pump or a Pasteur pipette.

ISOKINETIC GRADIENT is one in which all particles of equal density move at a constant rate at all distances along the length of the centrifuge tube. Isokinetic gradients are convex in shape, but some linear gradients are also isokinetic (e.g., 5-20% sucrose solution used in swinging bucket rotors).

ISOPYCNIC GRADIENT CENTRIFUGATION. See EQUILIBRIUM GRADIENT CENTRIFUGATION.

K-FACTOR. See CLEARING FACTOR.

LINEAR GRADIENT is one in which a plot of density (or c oncentration) vs. distance along the centrifuge tube yields a straight line.

MAXIMUM RADIUS (symbol: r_{max}) of a rotor is the distance from the center of rotation to the bottom of the centrifuge tube during centrifugation.

MINIMUM RADIUS (symbol: r_{min}) of a rotor is the distance from the center of rotation to the inside (centripetal) top edge of the centrifuge tube during centrifugation.

MOLECULAR WEIGHT (symbol: M) is the sum of the atomic weights of all the atoms in a molecule.

n is the symbol for REFRACTIVE INDEX.

NOMINAL TUBE VOLUME is rhat which a centrifuge tube would hold if completely filled. The actual fill volume under operating conditions, however, will be somewhat less.

OMEGA (ω) is the symbol for ANGULAR VELOCITY.

OVERSPEED DISK is a ring with alternating light and dark sector-shaped stripes that is attached to the bottom of each preparative ultracentrifuge rotor. An essential part of the photoelectric overspeed detection system, it should be replaced if damaged or when the rotor is derated.

PELLETING is the process of sedimenting material to the bottom of a centrifuge tube.

PELLICLE is a film or floating layer at the top of a centrifuge tube, following centrifugation.

PREFORMED GRADIENT is one in which the gradient is created prior to centrifugation by a gradient generator or by hand layering using a Pasteur pipette.

r is the symbol for RADIUS of a rotor (distance from the rotor axis). $_{rav}$ is the symbol for the AVERAGE RADIUS of a rotor (distance to the middle of the tube).

r_{max} is the symbol for the MAXIMUM RADIUS of a rotor (distance to the bottom of the tube).

r is the symbol for the MINIMUM RADIUS of a rotor (distance to the mIll meniscus at the top of the tube).

RADIUS (symbol: r) of a rotor is the distance from the center of rotation.

Continues on next page.

RATE-ZONAL CENTRIFUGATION is the separation of particles by differences in their size and sedimentation rates, using a preformed linear or discontinuous density gradient.

RCF is the symbol for RELATIVE CENTRIFUGAL FIELD.

REFRACTIVE INDEX (symbol: n) is the ratio of the velocity of light (at a particular wavelength) in a vacuum to that in a medium, such as water.

RELATIVE CENTRIFUGAL FIELD (symbol: RCF) is the ratio of a centrifugal field, at a specific speed and a specific radius, to the earth's field of gravity:
$RCF = 1.12\ r \times (RPM/1000)^2$,
where r is the radius in millimeters and RPM is the speed of rotation in revolutions per minute (rpm)

REORIENTATION of the solution within a centrifuge tube changes its position in all fixed angle, vertical tube, and near vertical tube rotors. During centrifugation, the solution orients perpendicular to the axis of the rotor; it reorients to its original position during deceleration and at rest, when the rotor has stopped.

RESOLUTION, as applied to density gradient centrifugation, refers to the volume of gradient medium between bands or zones of separated components.

rho (ρ) is the symbol for DENSITY.

ROTOR CAPACITY is the maximum volume of liquid (both sample and gradient) that a rotor can carry with all tubes full.

s is the symbol for SEDIMENTATION COEFFICIENT.

S20w is the symbol for the STANDARD SEDIMENTATION COEFFICIENT.

S is the symbol for SVEDBERG (1 S = 1 Svedberg unit = 1×10^{-13} seconds).

SEDIMENTATION COEFFICIENT (symbol: s) is the sedimentation velocity per unit of centrifugal force:
$s = dr/dt \times 1/\omega 2r$),
where s is the sedimentation coefficient in seconds, r is the distance from the center of rotation (radius) in centimeters, T is the time in seconds, and ω is the angular velocity of the rotor in radians per second.

SELF-GENERATING GRADIENT is one that is generated during centrifugation by redistribution and sedimentation of an initially uniform concentration of the gradient medium and sample
solution.

SHORT COLUMN METHOD is the practice of partially filling centrifuge tubes in order to reduce the sedimentation path-length, thereby reducing the run time. Short path-length tubes, such as Beckman G-max tubes, are designed to serve this purpose.

STANDARD SEDIMENTATION COEFFICIENT (symbol: S20, w) is one corrected to sedimentation in water at 20°C.

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