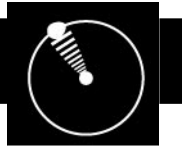
ORGANELLES



Centrifugation

J. A. Garner, University of Southern California, Keck School of Medicine, Los Angeles, CA, USA

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Introduction

Centrifugation techniques are commonly used to fractionate and separate intracellular organelles of many cell types. Here, common reasons for separation of those organelles, as well as brief description of different types of subcellular organelles, are presented. This is followed by a description of the physical principles upon which centrifugal separations are based, and common methods applied for this purpose. Last, a brief description of the types of equipment available for these separations, as well as the advantages and disadvantages of different tools, will be presented. The reader is directed to additional reviews in the Further Reading section for more detailed information. Another source of valuable information is the literature provided by manufacturers of centrifuges, rotors, and separation media.

Subcellular Organelles

Higher eucaryotic cells contain numerous intracellular (or subcellular) organelles (**Figure 1**). These are structurally distinct entities located within the boundaries of the cell's plasma membrane and are generally associated with one or more specific cellular functions. Indeed, one of the distinguishing features of eucaryotic cells as compared with prokaryotes, is that they have attained the ability to compartmentalize their cellular functions into these organelle 'packages'. This has allowed eucaryotic cells to evolve into larger cells, cells that can have highly specialized functional domains by limiting organelles to particular cellular regions, and eventually allowed the evolution of specific cell types, tissue types and complex organisms.

Subcellular organelles can generally be divided into two broad classes: the membrane-bounded organelles and cytoplasmic organelles. Membrane-bounded organelles are structures enclosed within one or more lipid-based membranes. These organelles include vesicular structures of different sizes, such as the cell's nucleus, mitochondria, lysosomes, peroxisomes, vacuoles, secretory vesicles, endosomes, and less well-defined vesicular structures. The Golgi apparatus and endoplasmic reticulum are large, complex structures that, during the process of disruption of the cell to release organelles, are usually broken and reform into smaller vesicles (termed 'microsomes') in a wide range of sizes. Certain plasma membrane fractions from certain cells may also be obtained during subcellular fractionation owing to the presence of highly specialized plasma membrane domains that may differ structurally and functionally (see Mircheff in the Further Reading).

Separate from the membrane-bounded organelles are the cytoplasmic organelles, distinct structural entities that are not enclosed within intracellular membranes. Examples of cytoplasmic organelles include the intracellular filament systems, such as microtubules, microfilaments, and intermediate filaments. Other cytoplasmic organelles are free ribosomes (not membrane-associated) and specialized cellular inclusions. Stretching the definition of cytoplasmic organelles slightly would allow the inclusion of multienzyme complexes found in the cytoplasm and separable by centrifugal and other techniques in this class.

Last, centrifugal techniques may be used to enrich parts of cells that have highly specialized functional domains. Examples of these subcellular fractions include synaptosomes (pinched-off neuron terminals and associated postsynaptic structures from the central nervous system) or the brush border of intestinal epithelial cells. These are complex parts of cells that are functionally distinct, and their isolation by centrifugal techniques allows for increased ease of their study.

For an in-depth description of the structure and function of all of the intracellular organelles described above, the reader is referred to the cell biology text by Alberts *et al*.

Purpose of Separating Subcellular Organelles

The separation of subcellular organelles by centrifugal techniques allows the enrichment (but not usually the purification) of these organelles. This enrichment may then serve as a starting point for use of other techniques that can more effectively isolate organelles. Obtaining an enriched sample may also be the first step towards biochemical purification of

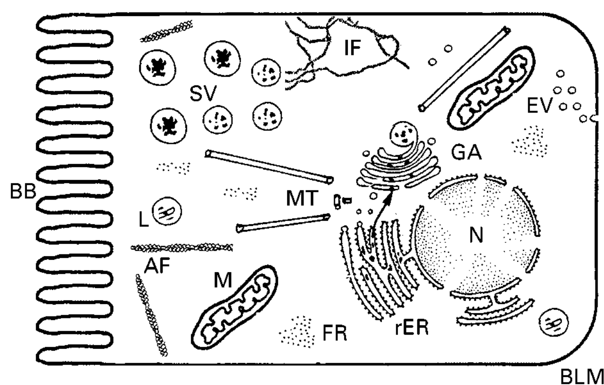

Figure 1 Diagram of a cell with subcellular organelles. A stylized diagram of an epithelial cell is shown with various common intracellular organelles drawn inside. Membrane-bounded organelles include the nucleus (N), the rough endoplasmic reticulum (rER), the Golgi Apparatus (GA), mitochondria (M), immature and maturing secretory vesicles (SV), endocytic vesicles (EV), and lysosomes (L). Cytoplasmic organelles include free ribosomes (FR), actin filaments (AF), microtubules (MT), and intermediate filaments (IF). Specialized regions of the plasma membrane include the brush border (BB) of the cell and the basolateral membrane of the cell (BLM).

individual proteins, glycoproteins, or other macromolecules that may be of considerable functional significance. Preparative centrifugation of lysed cells is a common technique that is used to perform the primary crude enrichment of these molecules.

A separate feature of interest to many cell and molecular biologists has to do with intracellular processing of macromolecules, whether they be destined for export from the cell or are specifically targeted to a particular domain of interest. Centrifugation techniques that allow separation of the various intracellular organelles such as the Golgi apparatus, the endoplasmic reticulum and various secretory vesicle pathways can be used to identify the compartment a particular molecule of interest is entering at various times after initiation of transcription. Combination of these centrifugal techniques with timed processing of a tagged protein or other cellular product can allow detailed elucidation of the intracellular route and compartments inhabited by that protein and its ultimate target location.

Homogenization of Cells to Free Subcellular Organelles

Organelles do not randomly float in the cytoplasm of cells; they are instead highly organized in a cytoplasmic matrix of very high protein concentration. The cytoplasmic matrix is a proteinaceous web or net of varying viscosity that organizes the organelles and maintains them within specialized functional domains of the cell. Before beginning to enrich for certain subcellular organelles, it is necessary first to break open the plasma membrane, and disrupt the matrix that holds the organelles. This is done by the process of homogenization of cells or tissues, and dilution of the cellular contents with at least 10 volumes of aqueous buffer prior to any centrifugation.

Tissue or cells of interest plus neutral aqueous buffer and protease inhibitors are placed in a ground glass/glass, glass/Teflon®, or Dounce cell homogenizer. A ground glass/glass homogenizer disrupts cells but also will likely damage nuclei. This causes the release of large amounts of highly viscous nucleic acids (which can severely compromise the subsequent isolation of subcellular organelles). A glass/Teflon® or Dounce homogenizer (using the loose pestle followed by the tight pestle) should break open cells but leave nuclei intact. The latter is the more desirable for subsequent subcellular fractionation of organelles.

If a large quantity of material must be processed, or a tissue of origin has large amounts of connective tissue within it (such as muscle) then other types of tissue or cell disruptors may be used. Examples are the Omnimixer® (Sorvall Instruments, Inc.) or Polytron® (Brinkmann Instruments, Inc.) in which a motor-driven propeller drives the sample through small apertures in the shaft to cause cell disruption.

Fractionation of Subcellular Organelles

The type of centrifugation technique used to separate organelles after homogenization will be determined by the physical properties of the desired organelles, and generally will depend on the sedimentation rate of the organelle of interest in a medium of defined density and viscosity. The sedimentation rate of any particular organelle obeys the principal of Stokes' law as it refers to the settling of a sphere in a gravitational field, as follows:

$$1/6\pi d^3(\rho_p - \rho_l)g = 3\pi d\mu v$$

where v = sedimentation rate or velocity of the sphere, d = diameter of the sphere, ρ_p = particle density, ρ_l = liquid density, μ = viscosity of liquid medium, and g = gravitational force.

'Sedimentation rate', or the rate at which an organelle moves through a medium of defined density and viscosity in a centrifugal field, is a function of the intrinsic buoyant density (density being mass per unit volume), the shape of the organelle in the medium, and the centrifugal force exerted on the or-

ganelle. The shape of the organelle plays a role in that a highly asymmetric organelle may sediment more slowly than a tight spherical organelle of the same mass. Sedimentation rate is determined by the following:

$$v = \mathrm{d}r/\mathrm{d}t = (s)(\omega^2 r)$$

where v = sedimentation rate, s = sedimentation coefficient in seconds or Svedberg units, r = the distance between the particle and the centre of rotation (cm), ω = the rotor speed in radians s^{-1}, and $\mathrm{d}r/\mathrm{d}t$ = the rate of movement of the particle (cm s^{-1}).

The sedimentation rate of an organelle is proportional to the relative centrifugal force (RCF) on that organelle. It is determined by the following:

$$\mathrm{RCF} = 1.12r(\mathrm{rpm}/1000)^2$$

where rpm is the revolutions per minute, and r, or radius, is the distance from the centre of rotation to periphery of rotation. The maximum centrifugal force would be experienced at the bottom of the centrifuge tube (r_{max}), and the minimum centrifugal force would be experienced at the top of the centrifuge tube (r_{min}), and the average at r_{ave}.

The sedimentation rate is proportional to the size of that particle; hence, the sedimentation rate of a cell's nucleus is usually higher than that of a smaller mitochondrion or endocytic vesicle of similar density. Each population of organelles has an average buoyant density. However, this average value may be of limited value in that the range in variation in both structure and buoyant density for individuals within the population of organelles can be quite substantial. **Table 1** lists buoyant densities for common membrane-bounded subcellular organelles, and it demonstrates the similarity in buoyant density among membrane classes. As a result of this similarity – and this is an important point – it is virtually impossible to completely purify an individual organelle from all other organelles on the basis of its buoyant density alone, although a given type of organelle may be significantly enriched by these processes (which may be sufficient for certain studies).

Table 1 Densities of common membrane-bounded organelles in sucrose solutions[a]

Organelle	*Density (g cm $^{-3}$)*
Golgi apparatus	1.06–1.10
Plasma membrane fractions	1.16
Smooth endoplasmic reticulum	1.16
Mitochondria	1.19
Lysosomes	1.21
Peroxisomes	1.23
Soluble proteins	1.30
Ribosomes	1.60–1.75

[a]Data were compiled from Sober HA (ed.) (1968) *Handbook of Biochemistry*, 2nd edn. Cleveland: The Chemical Rubber Co. Also Birnie GD and Rickwood D (eds) (1978) *Centrifugal Separations in Molecular and Cell Biology*. London: Butterworths.

An exception to this rule can be found for certain organelles such as microtubules (major polymeric cytoskeletal structures in cells), which can be purified to near homogeneity by centrifugal techniques. However, in these cases, one must usually make use of techniques that take advantage of that organelle's unique biological properties. For example, microtubule polymers and tubulin (the constituent protein subunit) have substantially different buoyant densities. Sample conditions can be easily altered so that they either favour assembly of the tubulin into the polymer, which pellets during centrifugation, or disassembly of the polymer into tubulin subunits, which remain in solution. Thus microtubules can be purified away from other organelles by 'cycling' the tubulin between its assembled and disassembled states and using centrifugation to separate the fractions appropriately.

In addition to the intrinsic buoyant density of organelles, it should also be appreciated that the buoyant density of a particular organelle may be altered in response to the osmolarity of the medium. For example, the high osmolarity of sucrose solutions in a sucrose gradient can cause a depletion of water from mitochondria, resulting in their shrinkage and a concomitant increase in buoyant density. This is sufficiently significant to account for differences in mitochondrial fractionation patterns in sucrose solutions as compared with media of high density but low osmolarity such as Ficoll®. On occasion, this feature of certain intracellular organelles can be exploited to help in their enrichment.

Types of Centrifugation Used for Organelle Enrichment

The enrichment of subcellular organelles may be provided by any one or a combination of different centrifugal techniques, including differential, rate zonal, or isopycnic centrifugation. These types of centrifugation would be termed 'preparative', as their ultimate goal is the enrichment of organelles. Analytic ultracentrifugation, where the goal is to determine the physical nature of a particle, is discussed elsewhere in the chapter.

Differential centrifugation (**Figures 2** and **3**) uses gross differences in buoyant density to sequentially pellet or sediment organelles of decreasing buoyant density. Starting with low centrifugal force spins for

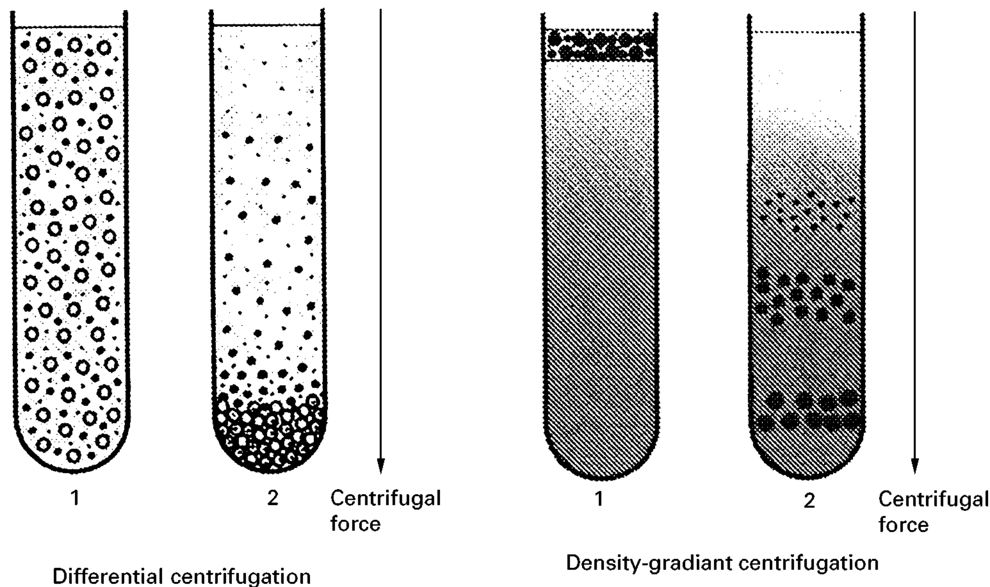

Figure 2 The principles of differential and density-gradient centrifugation. Differential centrifugation operates on the principle that denser particles sediment more rapidly and at lower g forces than lighter particles. Density-gradient centrifugation allows a mixture of particles or organelles of different densities to be separated: they band in a density gradient at the zone of the medium that has the same density as the particles.

short times, to remove the densest organelles from a sample homogenate, increased centrifugal force and length of time pellets lighter organelles. The first material pelleted from a homogenate at approximately 1000–1500 × the force of gravity, g, for 10–15 min are nuclei, along with cellular and extracelluar debris remaining in the homogenate. The supernate from this spin would be subsequently spun at approximately 10 000 × g for 20–30 min, to pellet mitochondria, lysosomes, and organelles of similar buoyant density. Microsomes, or small vesicles, pellet from the supernate of the mitochondrial spin after centrifugation at approximately 100 000 × g for several hours. Centrifugation speeds and times may be considerably lower for specific microsomal fractions, as determined empirically.

As mentioned above, use of these differential centrifugation steps is not sufficient to purify individual organelle types, primarily because of the range in size of the population. In addition, the mechanics of differential centrifugation allow for substantial trapping of lighter organelles within the pellets of heavier organelles. This latter can, to a certain extent, be compensated for by vigorous resuspension of the pellet and repetition of the centrifugation step. Effective resuspension of pellets into homogenous samples is imperative if those pellets are to be either subjected to further gradient fractionation or alternative treatment. If lumps are present, the sample will not fractionate according to individual organelle buoyant density. Use of a 3–10 mL syringe attached to a 4-inch, 14-gauge blunt-ended Popper Laboratory Pipetting cannula (Fisher Scientific, Inc.) allows thorough resuspensions. The same tool may be used in layering sample on gradients or retrieving fractions from the centre of density gradients as described below.

Each of the resuspended pellets and supernates obtained in the scheme described above (**Figure 3**) can be subjected to further centrifugation, usually by isopycnic or rate-zonal separation on density gradients, to more completely separate desired populations of organelles.

Isopycnic Centrifugation

In isopycnic centrifugation, using a continuous gradient, a fairly homogeneous population of organelles will ‘band’ in the gradient at their actual buoyant density over several hours. This method of separation is independent of time, and relies solely on the actual buoyant density of the particle. Banded organelles or particles can be recovered from the gradient by subjecting it to fractionation (either by punching a hole in the bottom of the tube and draining it, or by taking specific volumes of fractions from the top of the gradient). If bands are visible, they may be removed individually with a syringe with a long needle.

Rate-zonal Centrifugation

In rate-zonal centrifugation, the sample to be analysed is layered on a preformed density gradient and subjected to centrifugal force for a defined length of

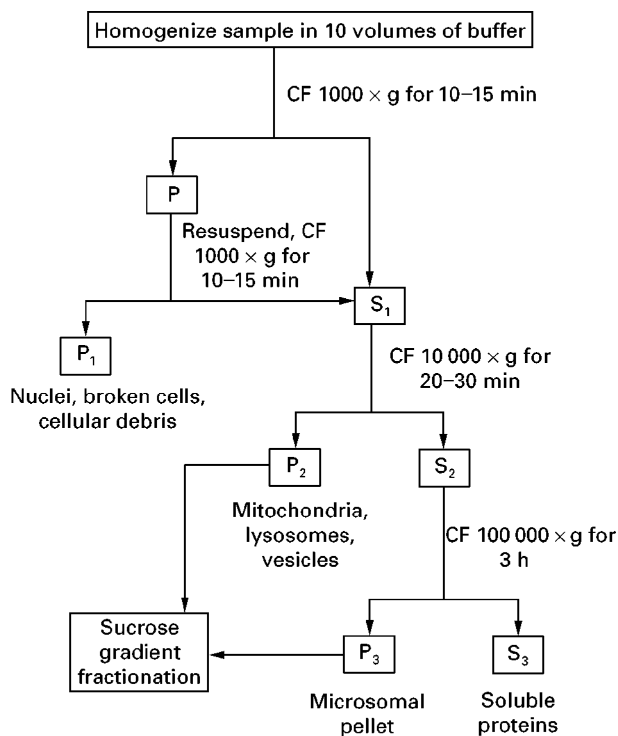

Figure 3 Differential centrifugation. A flow chart showing a common scheme for the differential centrifugation of a cellular homogenate. The first pellet is resuspended and recentrifuged, and the two supernates are combined as S_1. Both the microsomal and mitochondrial pellets may be further fractionated on sucrose density gradients or by other mechanisms. CF = centrifuge.

time. The particles sediment through the gradient at a rate that is a function of their sedimentation coefficient. Once sufficient separation of the desired organelles is achieved, the run is terminated, and once again, banded organelles can be obtained by fractionation of the gradient.

Depending upon the medium used, density gradients may either be self-generated, or may be formed within the centrifuge tube prior to sample administration (see later). Media that can generate their own gradient in a centrifugal field are usually mixed with samples at a density equivalent to the mid-density of the desired gradient, prior to gradient formation. Alternatively, preformed density gradients can be generated from a two-chambered gradient mixer, and sample layered on top of, or within, the gradient prior to centrifugation (**Figure 4**). Preformed gradients may also be made by layering decreasing densities of medium in 'steps' rather than a continuous gradient in a tube, then allowing diffusion over time to produce the gradient.

Equipment

Preparative or high-speed centrifuge A high-speed centrifuge (e.g. Beckman J21) will spin samples at speeds up to 20 000 rpm, with g forces of up to 48 000 (JA-20 rotor). With other rotors, both speed and centrifugal force vary. Rotors for use in this centrifuge can vary substantially in radius and volume of sample carried. High-speed centrifuges are generally refrigerated but do not achieve a vacuum. In differential centrifugation, the process of cellular fractionation up to the mitochondrial spin is usually performed in a high-speed centrifuge.

Preparative ultracentrifuge A preparative ultracentrifuge (e.g. Beckman L8-80M®) is a centrifuge capable of spinning a sample at up to 80 000 rpm, and, depending again upon the rotor radius, at maximal centrifugal forces in the hundreds of thousands × g. Ultracentrifuges spin rotors under vacuum, limiting frictional forces upon the rotor, and are refrigerated. There are numerous rotor types, varying in style and volume, that can be used in an ultracentrifuge. Centrifugal force placed upon an organelle is an important aspect of its fractionation, and, as described earlier, centrifugal force varies with the radius of the circle in which the sample is spun. Since rotors have varying radii, a simple nomogram can be used (**Figure 5**) to determine the rpm necessary to achieve the same centrifugal force in two different rotors.

Rotors

The rotor used in a protocol is chosen on the basis of the type of centrifugation being performed (e.g. rate zonal or density gradient), the g forces necessary, and the capacity of the rotor tubes. The manufacturer's literature is an excellent source for further information about the specifics of individual rotor types. Three different types of rotors are commonly used in the fractionation of subcellular organelles: swinging-bucket rotors, fixed-angle rotors, and vertical or near-vertical tube rotors (**Figure 6**). Continuous-flow rotors may also be used for certain large-scale applications.

In fixed-angle or vertical tube rotors, the sample orientation is fixed in space, and the liquid contents reorient during acceleration and deceleration. The angle at which fixed-angle rotors hold the tubes can vary among rotors up to completely vertical in vertical tube rotors. Fixed-angle rotors are excellent for pelleting material, since the pellets move both downward and outward to accumulate in a small restricted zone at the outer base of the tube. Fixed-angle and vertical tube rotors are often used for isopycnic runs. They are capable of superior separation of sample densities because of the large cross-sectional area of material banded, and the change in orientation of the sample during acceleration and deceleration does not

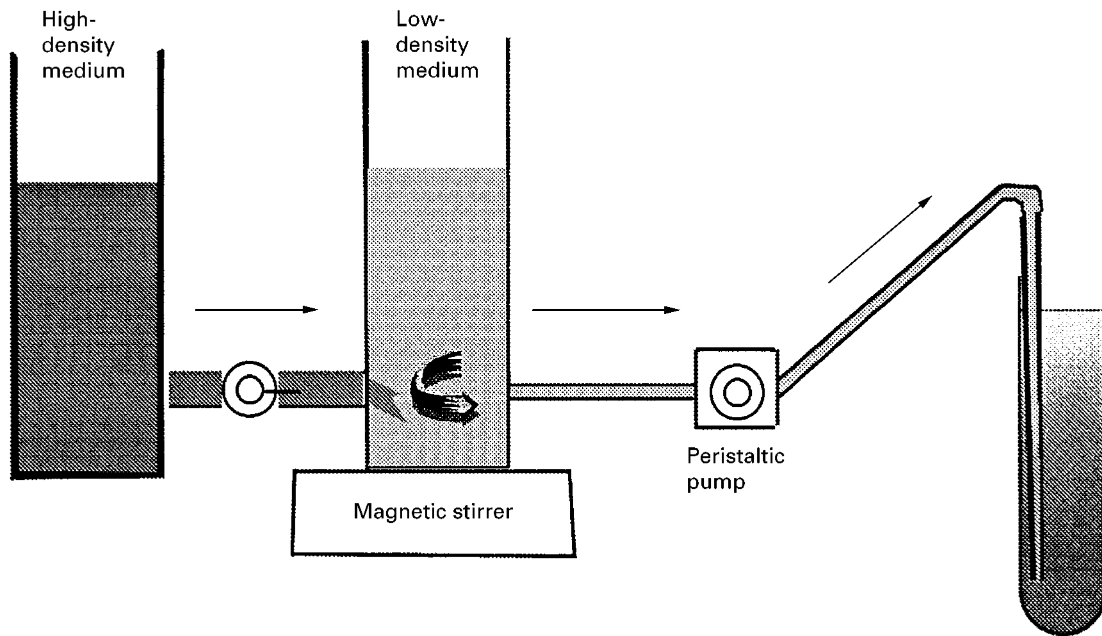

Figure 4 Generation of density gradients. A defined volume of high concentration medium is put in one chamber that is connected through a stopcock with a second chamber. A defined volume of low concentration medium is put in the second chamber. The second chamber contains a mixing bar. Fluid is drawn out of the second chamber into the bottom of the centrifuge tube by gravity or peristaltic pump, and concurrently drawn from the first chamber into the second. The medium concentration thus increases continuously, with the heavier medium displacing the lighter medium upwards as it enters the bottom of the gradient tube. Sample is usually carefully layered in lighter medium on the top of the gradient, with particles travelling to their buoyant density after being subjected to centrifugal force. Samples may be alternatively layered within the central part of the gradient, then organelles travel up or down the gradient to reach their own buoyant density.

appear to affect the relative separation of materials. Fixed-angle and vertical tube rotors can achieve equilibrium more rapidly than swinging-bucket rotors because of the decreased path length, resulting in a much shorter run.

Swinging-bucket rotors are most commonly used for density-gradient centrifugation. In accelerating swinging-bucket rotors, the buckets containing the samples swing outward until horizontal, where they remain for the entire run. They return to their original position on deceleration. Thus the tube is always oriented in the direction of centrifugal force. This effectively reduces artifactual problems introduced by wall effects. Swinging buckets also usually have a relatively long path length, which allows increased spatial separation of the contents. However, this increased resolution of the contents comes at a slight cost in terms of separation time. The volume of the sample which can be applied to the gradient in a swinging-bucket rotor is a function of the cross-sectional area of the tube. If too large a sample volume is applied, there is insufficient radial distance to allow effective separation of the subcellular particles. Similarly, there is a limit on the sample concentration that is effectively applied to a tube; if the concentration is too high, 'streaming' of the sample may result.

Zonal rotors can handle sample sizes of 50 to 100 times that of swinging-bucket rotors and are highly useful for large-scale purification of a variety of different types of particles. These rotors have an internal large cylindrical chamber that is divided into sectors by vanes attached to the central core. There is additionally a rotating seal assembly which allows fluid to be pumped in or out of the cavity while the rotor is spinning. Particles are purified on the basis of their rate-zonal separation. Buffer of known density is pumped in either the central core or at the periphery and fractions are collected exiting through the edge port or at the rotor centre.

Examples of Media Used for Fractionation

Most membrane-bound organelles have a density that is not widely variant from the buffer itself. In addition, the biological nature of most organelles requires that they be maintained in medium that will not alter their biological properties in a way that masks function. This has necessitated the development of media that are consistent with maintaining biological function yet can differentiate buoyant densities that are only marginally different from each

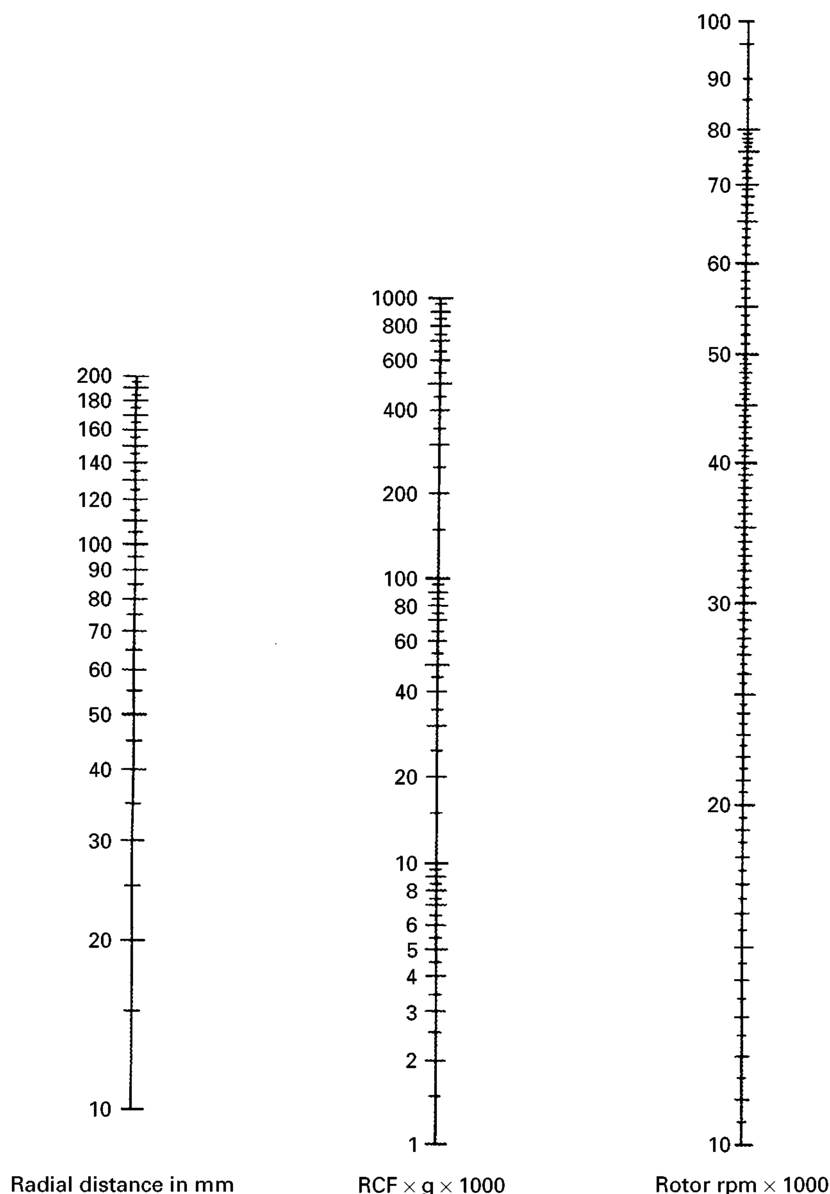

Figure 5 Nomogram. A nomogram is an invaluable diagram which allows one to compute the rpm necessary in order to achieve the same RCF in two rotors with different *r* values. Use a straight edge to connect the values known in two of the columns, and the appropriate value for the third column can be read where the straight edge intersects it. (Data compiled from *Beckman Rotors and Tubes for Preparative Ultracentrifuges, a User's Manual*, Spinco Business Unit Technical Publications, Palo Alto, CA, USA, 1993.)

other. These media are usually dissolved in neutral, aqueous buffers (phosphate, Tris, etc.) of physiological osmolarity.

A good separation medium (a) can establish a gradient over the appropriate density range, (b) does not affect biological activities of interest, (c) is isosmotic in the presence of sensitive organelles, and (d) does not interfere with assays that may be necessary for the characterization of particular bands. The medium should be easily removable from the sample either by dialysis or by dilution and pelleting of the band, and the medium material should not bind irreversibly to biological samples. It is further helpful if the medium does not absorb light in the UV or visible range because fractionation of gradients may require monitoring. A last consideration is cost and availability.

Sucrose, sorbitol and, to some extent, cesium chloride are the three media most commonly used for subcellular fractionation. Sucrose and sorbitol are often used because they are fairly neutral, they can be easily removed from the sample of interest by dialysis

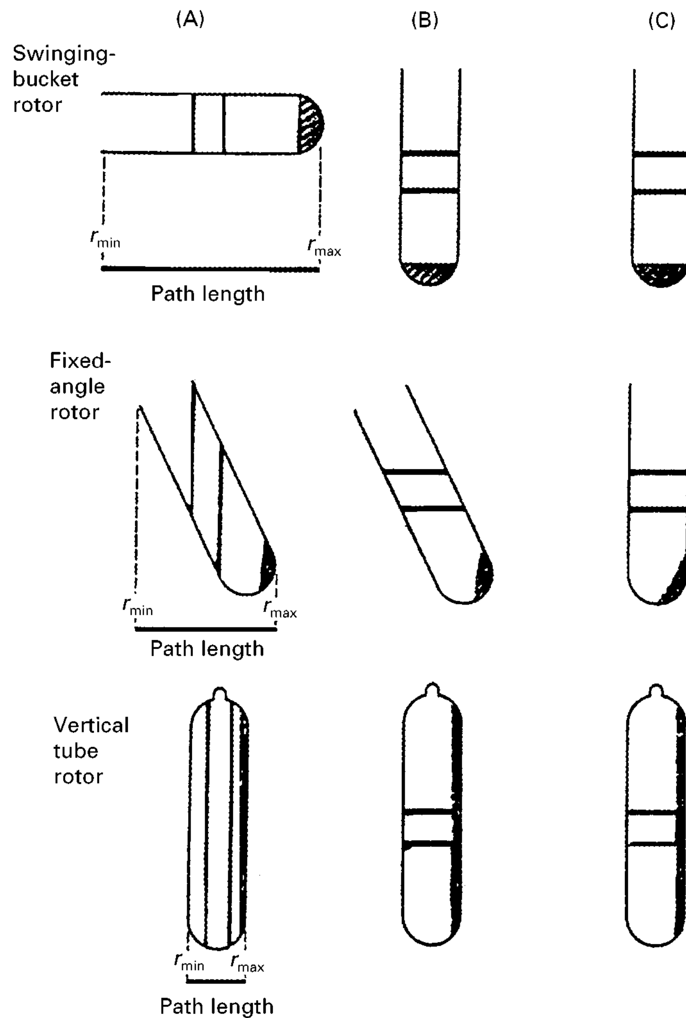

Figure 6 A comparison of gradient samples run in swinging-bucket, fixed-angle, and vertical rotors. This diagram shows the location of sample bands in centrifuge tubes during the centrifugation (A), at rest in the rotor (B), and at rest outside the rotor (C) for each type of rotor commonly used to separate subcellular organelles. Swinging-bucket rotors have the longest path length, can achieve the widest separation of sample bands, and the samples are always oriented in the direction of centrifugal force. Thus, there is little, if any, disruption of the bands. Run lengths, however, are longer because of the increased pathlength. Fixed-angle rotors allow for efficient pelleting within a short time over a shorter path length. Vertical tubes require shortest time for separation as they have a very short path length, and achieve good separation because of the large cross-sectional area of the sample within the tube. However, sample can mix with materials pelleted against the outside wall. (Reprinted as modified with permission from *Beckman Rotors and Tubes for Preparative Ultracentrifuges: a User's Manual,* Spinco Business Unit Technical Publications, Palo Alto, CA, USA, 1993.)

or by dilution and re-centrifugation, and are relatively inexpensive. Cesium chloride is more often used for isolation of macromolecules than organelles, but has the added advantage that it self-generates gradients in a centrifugal field. The refractive index of both sucrose and cesium chloride can be measured with a refractometer, which allows one to determine the actual density of each fraction recovered from a gradient (**Tables 2** and **3**). Alternatively, use of markers of known buoyant density in control gradients (Pharmacia Density Marker Beads) or oscillating densitometers can be used to determine density.

If osmolarity is a factor, in that the organelle to be separated can be made to swell or shrink in hypo- or hyperosmotic media respectively, a medium that is high in density but composed of extremely large molecules should be chosen, thus increasing density but decreasing osmolarity. Examples of such media are Ficoll® (Pharmacia), a synthetic polymer of copolymerized sucrose and epichlorohydrin of average molecular weight 400 000 daltons, Percoll® (Pharmacia), a colloidal suspension of polyvinylpyrrolidone-coated silica, and Nycodenz® (Nycomed), a non-ionic, tri-iodinated derivative of benzoic acid. All of these media are higher in molecular weight

Table 2 Sucrose density, refractive index and concentration[a]

Percentage concentration (w/v)	*Density (g mL⁻¹)*	*Refractive index*	*Percentage concentration (w/v)*	*Density (g mL⁻¹)*	*Refractive index*
0	0.9982	1.3330	34	1.1463	1.3883
1	1.0021	1.3344	35	1.1513	1.3902
2	1.0060	1.3359	36	1.1562	1.3920
3	1.0099	1.3374	37	1.1612	1.3939
4	1.0139	1.3388	38	1.1663	1.3958
5	1.0179	1.3403	39	1.1713	1.3978
6	1.0219	1.3418	40	1.1764	1.3997
7	1.0259	1.3433	41	1.1816	1.4016
8	1.0299	1.3448	42	1.1868	1.4036
9	1.0340	1.3464	43	1.1920	1.4056
10	1.0381	1.3479	44	1.1972	1.4076
11	1.0423	1.3494	45	1.2025	1.4096
12	1.0465	1.3510	46	1.2079	1.4117
13	1.0507	1.3526	47	1.2132	1.4137
14	1.0549	1.3541	48	1.2186	1.4158
15	1.0592	1.3557	49	1.2241	1.4179
16	1.0635	1.3573	50	1.2296	1.4200
17	1.0678	1.3590	51	1.2351	1.4221
18	1.0721	1.3606	52	1.2406	1.4242
19	1.0765	1.3622	53	1.2462	1.4264
20	1.0810	1.3639	54	1.2519	1.4285
21	1.0854	1.3655	55	1.2575	1.4307
22	1.0899	1.3672	56	1.2632	1.4329
23	1.0944	1.3689	57	1.2690	1.4351
24	1.0990	1.3706	58	1.2748	1.4373
25	1.1036	1.3723	59	1.2806	1.4396
26	1.1082	1.3740	60	1.2865	1.4418
27	1.1128	1.3758	61	1.2924	1.4441
28	1.1175	1.3775	62	1.2983	1.4464
29	1.1222	1.3793	63	1.3043	1.4486
30	1.1270	1.3811	64	1.3103	1.4509
31	1.1318	1.3829	65	1.3163	1.4532
32	1.1366	1.3847	66	1.3224	1.4558
33	1.1415	1.3865	67	1.3286	1.4581

[a]Data were compiled from the US National Research Council (1933) In Washburn EW (ed.) *International Critical Tables of Numerical Data, Physics, Chemistry and Technology.* New York: McGraw-Hill.

than sucrose, and each has its own intrinsic advantages. Ficoll® is neutral, can achieve concentrations up to 50% covering a density range up to 1.2 g mL^{-1}, and does not penetrate biological membranes. Ficoll® is, however, difficult to remove from the sample by dialysis because of its large molecular weight. Percoll® is non-toxic and can be used over wider density ranges (up to 1.3 g mL^{-1}). Percoll® has the added advantage of being a medium that self-generates gradients in a centrifugal field, and the gradients formed are isosmotic throughout. However, Percoll® has the disadvantage that it is difficult to remove from the sample by dilution and recentrifugation, or by dialysis. In addition, Percoll®, Ficoll® and metrizamide (similar to Nycodenz®) are precipitated at low pH, eliminating the possibility of purification of protein from samples by acid precipitation. Nycodenz®, with a density range up to 1.4, can be used to effectively fractionate subcellular particles (particularly small ones). Its advantages include its solubility at low pH, its self-forming gradients, and its lack of interference with enzyme assays. Its relatively low molecular weight (821) allows removal by dialysis. All of these media are significantly more expensive than sucrose or sorbitol.

Tables 2–5 provide data concerning density, refractive index, and concentration of different commonly used media.

Table 3 Cesium chloride density, refractive index and concentration[a]

Percentage concentration (w/v)	*Density (g mL $^{-1}$)*	*Refractive index*	*Percentage concentration (w/v)*	*Density (g mL $^{-1}$)*	*Refractive index*
1	1.0047	1.3333	34	1.336	1.3657
2	1.0125	1.3340	35	1.350	1.3670
3	1.0204	1.3348	36	1.363	1.3683
4	1.0284	1.3356	37	1.377	1.3696
5	1.0365	1.3364	38	1.391	1.3709
6	1.0447	1.3372	39	1.406	1.3722
7	1.0531	1.3380	40	1.420	1.3735
8	1.0615	1.3388	41	1.435	1.3750
9	1.0700	1.3397	42	1.450	1.3764
10	1.0788	1.3405	43	1.465	1.3778
11	1.0877	1.3414	44	1.481	1.3792
12	1.0967	1.3423	45	1.4969	1.3807
13	1.1059	1.3432	46	1.513	1.3822
14	1.1151	1.3441	47	1.529	1.3837
15	1.1245	1.3450	48	1.546	1.3852
16	1.1340	1.3459	49	1.564	1.3868
17	1.1437	1.3468	50	1.583	1.3885
18	1.1536	1.3478	51	1.601	1.3903
19	1.1637	1.3488	52	1.619	1.3920
20	1.1739	1.3498	53	1.638	1.3937
21	1.1843	1.3508	54	1.658	1.3955
22	1.1948	1.3518	55	1.6778	1.3973
23	1.2055	1.3529	56	1.699	1.3992
24	1.2164	1.3539	57	1.720	1.4012
25	1.2275	1.3550	58	1.741	1.4032
26	1.2387	1.3561	59	1.763	1.4052
27	1.2502	1.3572	60	1.7846	1.4072
28	1.2619	1.3584	61	1.808	1.4093
29	1.2738	1.3596	62	1.831	1.4115
30	1.2858	1.3607	63	1.856	1.4137
31	1.298	1.3619	64	1.880	1.4160
32	1.311	1.3631	65	1.905	1.4183
33	1.324	1.3644			

[a]Data were compiled from the US National Research Council (1933) In Washburn EW (ed.) *International Critical Tables of Numerical Data, Physics, Chemistry and Technology.* New York: McGraw-Hill.

Table 4 Density and refractive index of Ficoll® soultions[a]

Percentage concentration (w/w)	*Density (g mL $^{-1}$)*
0	1.000
10	1.035
20	1.073
30	1.115
40	1.160
50	1.203

[a]Data computed from manufacturer's literature (Amershan Pharmacia Biotech, Uppsala, Sweden).

Table 5 Properties of Nycodenz® solutions[a]

Percentage concentration (w/v)	*Molar concentration*	*Refractive index (20°C)*	*Density (g mL $^{-1}$) (20°C)*
0	0	1.3330	0.999
10	0.122	1.3494	1.052
20	0.244	1.3659	1.105
30	0.365	1.3824	1.159
40	0.487	1.3988	1.212
50	0.609	1.4153	1.265
60	0.731	1.4318	1.319
70	0.853	1.4482	1.372
80	0.974	1.4647	1.426

[a]Data modified from Rickwood D, Ford T and Graham J (1982) *Analytical Biochemistry* 123: 23–31.

Summary

In this review, the principles behind, and the utility of, fractionation of subcellular organelles by centrifugal techniques have been explored. Common methods used, and a review of the advantages and disadvantages of certain experimental tools (centrifuges, rotors, and aqueous media used for fractionation) were also reviewed and tables and graphs useful for designing protocols were also provided.

See also: **II/Centrifugation:** Large-Scale Centrifugation; Theory of Centrifugation.

Further Reading

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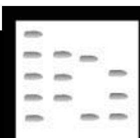
Field Flow Fractionation

See **III/CELLS AND CELL ORGANELLES: FIELD FLOW FRACTIONATION**

PAINTS AND COATINGS: PYROLYSIS: GAS CHROMATOGRAPHY



T. P. Wampler, CDS Analytical, Inc., Oxford, PA, USA

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Because they are complex polymeric materials, usually compounded with coloured or opaque fillers, paints and coatings, especially when dry, pose a difficult analytical problem. Gas chromatography in particular, and mass spectrometry, may seem unlikely tools for the analysis of such materials, but when combined with pyrolysis as a sample introduction technique, they may be used routinely. Via pyrolysis, the polymers used in paints and coatings are fragmented to produce molecules small enough to be compatible with gas chromatography–mass spectrometry (GC-MS), but still characteristic of the original material. Natural polymers, including plant resins and drying oils, as well as synthetic polymers like polyesters, acrylics and polyurethanes, have been studied extensively, and may be easily differentiated using this technique.

Pyrolysis

The general purpose of paints and coatings is to apply a protective or decorative film of material on to a substrate. The range of materials which may be used to form such a film is extremely wide, as is the range of techniques used to apply it. Soluble materials may be dissolved in a suitable solvent and applied as a thin