Centrifugation

Standard centrifuge
< 10,000 rpm

"Ultracentrifuge"
> 20,000 rpm
up to ~100,000 rpm

Centrifugal force
> 200,000 g

\( 1 \text{g} = \text{force of gravity on earth} \)

Invented by The Svedberg at the University of Uppsala, Sweden (Nobel Prize 1926)

Basic relation (note \( \nabla = \frac{d}{dr} \))

\[
\frac{v_A - v_s}{x_A} = -\nabla M_A^{\text{chem}} \bigg[ (M_{WA})(1 - \frac{\rho_{WA}}{\rho_A}) \bigg] \frac{w^2 r}{2} + \nabla M_A^{\text{centrifugal}}
\]

- Molecular weight of "A", i.e., the mass per mole
- Mass of one mole of A corrected for buoyancy by Archimedes principle
- Partial specific volume of A, i.e., volume of A per gram
- Density of fluid
OPTIMA® L-90 K ULTRACENTRIFUGE

Ordering Information
Part No.
365672
365670

Description
60/50 Hz, 220-240 VAC
50 Hz, 220-240 VAC

Specifications
Maximum speed
90,000 rpm
Maximum g-force
69,400 x g (Type 90 Ti)
Speed control
+/- 20 rpm of set speed
Sample imbalance tolerance
+/- 5 mL or 10%, whichever is greater
Drive cooling
Air-cooled
Refrigeration system
Thermoelectric - no CFCs, ODCs
Set temperature
0 to 40 degrees centigrade in 1 degree increments
Temperature control
+/- 0.5 degrees of set
Ambient operating range
15 to 40 degrees centigrade
User-settable programs
9 user programs with delayed start capability
Acceleration/deceleration rates
2 accel/3 decel
Moisture-purging vacuum system
Yes
Heat output
1.0 kW/Hz (3400 BTU/Hr)
Sound level
<57 dBA
Rotor Safety
DRIC (Dynamic Rotor Inertia Check) included
HEPA filter
Available
Dimensions H x W x D inches (cm)
47.5 x 37 x 26.5 (120.7 x 94.0 x 67.3)
Weight
1025 lb (465 kg)

Unmatched versatility, reliability and safety.
Capable of generating 69,400 x g at speeds up to 90,000 rpm, Beckman Coulter's Optima L-90 K Ultracentrifuge enables you to perform more separations in less time. This versatile floor model operates with a broad range of super-rotors, including zonal and continuous flow for large-volume separations. The L-90 K offers the reliability of a vacuum-encased induction drive, the simplicity of user-friendly, microprocessor-based control, and environmentally-friendly cooling systems that eliminate the need for CFCs and other harmful liquid refrigerants. All this makes the L-90 K the right choice for your laboratory's routine separation needs.

Beckman Coulter, Inc. • 4300 N. Fairview Boulevard, Box 3100 • Fullerton, California 92834-3100
Sales: 1-800-742-2345 • Service: 1-800-551-1150 • Telex: 678413 • Fax: 1-800-643-4366 • www.beckmancoulter.com
Worldwide Life Science Research Division Offices:
Australia (61) 2 9844-6000 Canada (905) 819-1234 China (86) 10 6515 6028 Eastern Europe, Middle East, North Africa (41) 22 994 07 07
France 01 49 90 90 00 Germany (0) 358700 Hong Kong (852) 2814 7431 2814 0481 Italy 02-953921 Japan 03-5484-8359
Mexico 525-605-77-70 Netherlands 0237-230630 Singapore (65) 6339 3633 South Africa, Sub-Saharan Africa (27) 11-805-2014/5 Spain (34) 91 3350680
Sweden 08-564 85 900 Switzerland 0800 850 810 Taiwan (886) 2 2378 3456 Turkey 90 216 309 1900 U.K. 01494 441181 U.S.A. 1-800-742-2345
From before we have that
\[
\frac{\eta M_A}{C_A} = \frac{RT}{C_A} \frac{dC_A}{dr} = RT \frac{d \ln C_A}{dr}
\]

"Sedimentation equilibrium" is the condition where the concentration profile in the centrifuge tube reaches a steady state.

Integration yields:
\[
RT \frac{d \ln C_A}{dr} = (MW_A) (1 - \bar{v}_A) w^2 r
\]

\[
\int \ln \frac{C_A(r)}{C_A(r_1)} = (MW_A) (1 - \bar{v}_A) \cdot \frac{w^2 (r^2 - r_1^2)}{2}
\]

Can measure \( MW_A \) of a molecule.

\[
\text{Slope} = \frac{w^2 (MW_A) (1 - \bar{v}_A)}{2RT}
\]

Can also use a dense small molecule such as \( CsCl_2 \) or sucrose to form a density gradient (used in isopycnic).
Consider next the "mobility" of a molecule

velocity of molecule A

\[ \frac{V_A}{F_A'} = \text{mobility}_A \]

force on a single molecule

From Stokes Law for creeping flow past a sphere:

viscosity of fluid

\[ \frac{V_A}{F_A'} = \frac{1}{6 \pi \mu R_A} = \text{mobility}_A \]

radius of molecule A

Force on one molecule

What is the force on a molecule caused by a concentration gradient:

\[ F_A' = \frac{F_A}{N_{\text{Avogadro}}} = -\frac{\nabla N_A}{N_{\text{Avogadro}}} = -\frac{RT}{CA N_{\text{Avogadro}}} \frac{dCA}{dr} \]

Force per mole

Boltzmann constant

\[ = \frac{kT}{CA} \frac{dCA}{dr} \]

Substituting:

\[ \frac{V_A}{F_A'} = \frac{V_A CA (dCA)^{-1}}{RT} \text{ mobility}_A \Rightarrow V_A CA = kT \text{ mobility}_A \]

Fick's Law of diffusion

\[ \frac{D_A}{kT} \]

Also

\[ D_A = \frac{kT}{6 \pi \mu R_A} \]

\[ \text{mobility}_A \leftarrow \text{Nernst - Einstein Eqn.} \]

\[ \text{mobility}_A \leftarrow \text{Stokes - Einstein Eqn.} \]
Define the "Sedimentation Coefficient" as follows:

\[ S_A = \left( \frac{\partial A}{RT} \right) (MW_A) \left( 1 - \rho \bar{v}_A \right) \]

mobility of one mole

molecular weight of one mole

corrected for buoyancy

using Archimedes principle


A flux \( \Phi \) caused by centrifugal force is given by \( \Phi = CA_i S_A \omega^2 r \)

centrifugal acceleration

velocity caused by centrifugal force

The flux caused by both diffusion (or Brownian motion) and centrifugal force is:

\[ \Phi = S_A C_A \omega r^2 - \frac{\partial}{\partial r} \frac{dC_A}{dr} \]

If \( \Phi = 0 \), the above result reduces to the result for sedimentation equilibrium developed earlier.

Consider "Zonal" centrifugation:

At band maximum \( \frac{dC_A}{dr} = 0 \)

so that \( \Phi = \bar{V}_A C_A = S_A C_A \omega r^2 \)

\( \bar{V}_A = S_A \omega^2 r \)
For a spherical molecule, since

\[ S = \frac{\rho_A}{RT} (MW_A) \left( 1 - \rho \overline{V}_A \right) \]

and

\[ \frac{\bar{e}_A}{RT} = \frac{1}{N_{av}} \left( \frac{U_A}{F_A} \right) \]

and assuming

\[ \frac{4}{3} \pi R^3 \cdot N_{av} = (MW_A) \frac{\overline{V}_A}{\rho} \]

Substituting yields:

\[ S_{\text{sphere}} = \frac{(MW_A) \left( 1 - \rho \overline{V}_A \right)}{N_{av} 6 \pi \mu \left( \frac{3 MW_A \overline{V}_A}{4 \pi N_{av}} \right)^{\frac{1}{3}}} \]

Substituting values:

\[ S_{\text{sphere}} = 0.012 \frac{(MW_A)^{\frac{2}{3}} \left( 1 - \rho \overline{V}_A \right)}{\overline{V}_A^{\frac{1}{3}}} \]

Dallons

\[ \sqrt[2/3]{\text{Svedberg}} \left( = 10^{-13} \text{s} \right) \]

\[ \text{gm/ml} \]

\[ \text{ml/gm} \]
This page is maintained by the Natural Toxins Research Center at Texas A&M University - Kingsville.
Methods of centrifugation:

"Sedimentation equilibrium"

Moving boundary sedimentation

Zonal centrifugation

Isopycnic sedimentation:

Solute "A": $J_A^* = 0 \Rightarrow S_A = 0$

$0 = (1 - \rho \overline{V}_A) v^2 \Rightarrow \overline{V}_A = \frac{1}{g}$

Assume Gaussian peak
Figure 5.2 Common types of production centrifuge: (a) tubular bowl, (b) multichamber, (c) disk, nozzle, (d) disk, intermittent discharge, (e) scroll, and (f) basket. Arrows indicate the path of the liquid phase; dashed lines show where the solids accumulate.

### TABLE 5.4

<table>
<thead>
<tr>
<th>System</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular bowl</td>
<td>(a) High centrifugal force</td>
<td>(a) Limited solids capacity</td>
</tr>
<tr>
<td></td>
<td>(b) Good dewatering</td>
<td>(b) Foaming unless special skimming</td>
</tr>
<tr>
<td></td>
<td>(c) Easy to clean</td>
<td>or centrifugal pump used</td>
</tr>
<tr>
<td></td>
<td>(d) Simple dismantling of bowl</td>
<td>(c) Recovery of solids difficult</td>
</tr>
<tr>
<td>Chamber bowl</td>
<td>(a) Clarification efficiency remains constant</td>
<td>(a) No solids discharge</td>
</tr>
<tr>
<td></td>
<td>until sludge space full</td>
<td>(b) Cleaning more difficult than tubular bowl</td>
</tr>
<tr>
<td></td>
<td>(b) Large solids holding capacity</td>
<td>(c) Solids recovery difficult</td>
</tr>
<tr>
<td></td>
<td>(c) Good dewatering</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(d) Bowl cooling possible</td>
<td></td>
</tr>
<tr>
<td>Disk centrifuge</td>
<td>(a) Solids discharge possible</td>
<td>(a) Poor dewatering</td>
</tr>
<tr>
<td></td>
<td>(b) Liquid discharge under pressure</td>
<td>(b) Difficult to clean</td>
</tr>
<tr>
<td></td>
<td>eliminates foaming</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(c) Bowl cooling possible</td>
<td></td>
</tr>
<tr>
<td>Scroll or decanter centrifuge</td>
<td>(a) Continuous solids discharge</td>
<td>(a) Low centrifugal force</td>
</tr>
<tr>
<td></td>
<td>(b) High feed solids concentration</td>
<td>(b) Turbulence created by scroll</td>
</tr>
<tr>
<td>Basket centrifuge</td>
<td>(a) Solids can be washed well</td>
<td>(a) Not suitable for soft biological solids</td>
</tr>
<tr>
<td></td>
<td>(b) Good dewatering</td>
<td>(b) No solids discharge</td>
</tr>
<tr>
<td></td>
<td>(c) Large solids holding capacity</td>
<td>(c) Recovery of solids difficult</td>
</tr>
</tbody>
</table>

*See reference 11.

### TABLE 5.5

<table>
<thead>
<tr>
<th>Type</th>
<th>Bowl diameter (mm)</th>
<th>Speed (rpm)</th>
<th>Maximum dimensionless acceleration (G, gR/g)</th>
<th>Throughput (liters/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular bowl</td>
<td>44</td>
<td>105</td>
<td>127</td>
<td>61,400</td>
</tr>
<tr>
<td>50,000</td>
<td>15,000</td>
<td>16,000</td>
<td>13,200</td>
<td>61,400</td>
</tr>
<tr>
<td>105</td>
<td>15,000</td>
<td>16,000</td>
<td>13,200</td>
<td>61,400</td>
</tr>
<tr>
<td>127</td>
<td>10,000</td>
<td>8,850</td>
<td>8,850</td>
<td>40–150</td>
</tr>
<tr>
<td>Disk with nozzle</td>
<td>254</td>
<td>406</td>
<td>686</td>
<td>6760</td>
</tr>
<tr>
<td>discharge</td>
<td>10,000</td>
<td>3,300</td>
<td>4,630</td>
<td>40–150</td>
</tr>
<tr>
<td>406</td>
<td>6,250</td>
<td>8,850</td>
<td>8,850</td>
<td>100–570</td>
</tr>
<tr>
<td>686</td>
<td>4,200</td>
<td>6,760</td>
<td>6,760</td>
<td>150–1500</td>
</tr>
<tr>
<td>762</td>
<td>3,300</td>
<td>4,630</td>
<td>4,630</td>
<td>150–1500</td>
</tr>
</tbody>
</table>

*See reference 12.
**Apheresis**

Return to the Blood Bank tutorial menu.

**What is Apheresis?**

The process of apheresis involves removal of whole blood from a patient or donor. Within an instrument that is essentially designed as a centrifuge, the components of whole blood are separated. One of the separated portions is then withdrawn and the remaining components are returned back into the patient or donor.

The components which are separated and withdrawn include:

- Plasma (plasmapheresis)
- Platelets (plateletpheresis)
- Leukocytes (leukapheresis)

In the diagram below, the process is illustrated. Whole blood is introduced into a chamber that is spinning, and the blood separates into components (P = plasma, PRP = platelet-rich plasma; WBC = leukocytes, RBC = red blood cells) by gravity along the wall of the chamber. The component to be removed can be selected by moving the level of the aspiration device at the right. In this example, plasma is being removed.

**Donation by Apheresis**

The process of apheresis has become essential in providing blood components for therapy. A volunteer donor will undergo apheresis to supply specific components. The process takes a couple of hours. Examples include:

- Platelepheresis: this is the most common means for supplying HLA matched platelets to patients who have become HLA sensitized and require platelets from a single donor whose HLA type matches theirs.
- Plasmapheresis: the plasma can be removed to supply blood components such as clotting factors. Donors can give plasma via this mechanism more often than they can donate whole blood.
- Leukapheresis: the leukocytes (specifically the granulocytes) can be harvested from a donor to supply granulocytes to help fight infection in patients such as neonates.

Return to the Blood Bank tutorial menu.

**Therapeutic Apheresis**

The purpose of therapeutic apheresis is to remove a component of the blood which contributes to a disease state. Examples include:

- Plasmapheresis: within the plasma are contained antibodies and antigen-antibody complexes that may contribute to the deleterious effects of autoimmune diseases. Removal of the plasma (and replacement with saline solution) will help to reduce circulating antibodies and immune complexes. In rare circumstances, excess blood proteins are present that may cause circulatory problems. Examples of these diseases include:
  - Waldenstrom's macroglobulinemia
  - Myasthenia gravis
  - Guillain-Barré syndrome
  - Hyperviscosity Syndrome
  - Paraproteinemia
  - Cryoglobulinemia