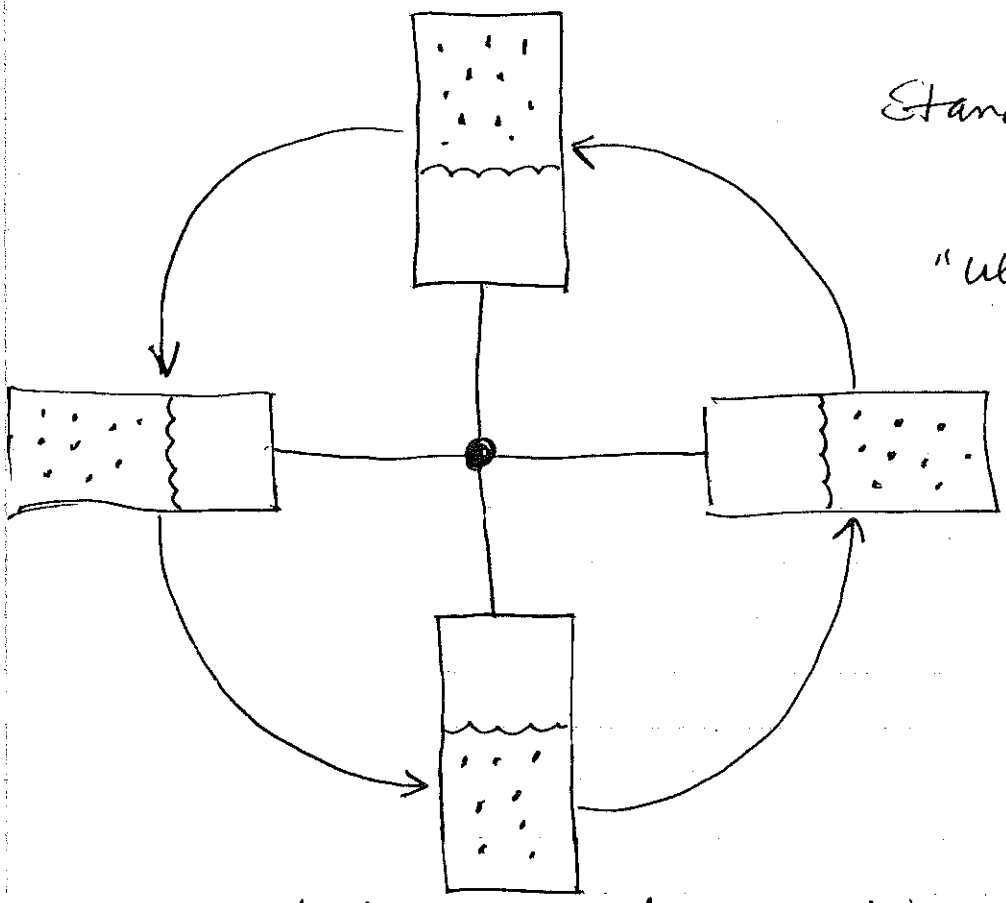
ENCH 630

Centrifugation



Standard centrifuge
< 10,000 rpm

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

centrifugal force
> 200,000 g

1 g = force of gravity on earth

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

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

$$\frac{v_A - v_s}{\lambda_A} = -\nabla \mu_A^{chem} + \overbrace{(MW_A)(1 - \rho \bar{v}_A)\omega^2 r}^{\nabla \mu_A^{centrifugal}}$$

- MW_A: molecular weight of "A", i.e., the mass per mole
- ρ: density of fluid
- $\omega^2 r$: centrifugal acceleration
- $\bar{v}_A$: partial specific volume of A, i.e., volume of A per gram, $\bar{v}_A = \bar{V}_A / (MW_A)$
- $(MW_A)(1 - \rho \bar{v}_A)$: mass of one mole of A corrected for buoyancy by Archimedes principle

DS-9339A

OPTIMA™ L-90 K ULTRACENTRIFUGE

Located in TRC building

Ordering Information

Part No.	Description
365672	60/50 Hz, 220-240 VAC
365670	50 Hz, 220-240 VAC

Specifications

Maximum speed	90,000 rpm
Maximum *g*-force	694,000 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/Hr (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)

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Capable of generating 694,000 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 superb 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.

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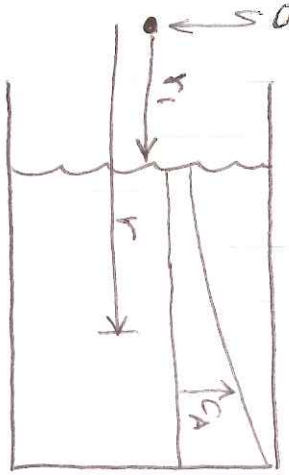
From before we have that

$$\nabla \mu_A^{\text{chem}} = \frac{RT}{C_A}\frac{dC_A}{dr} = RT\frac{d\ln C_A}{dr}$$

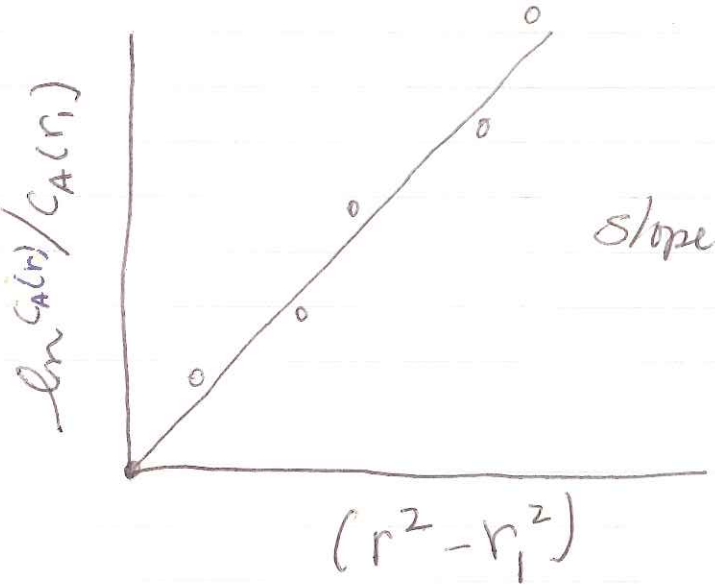
"Sedimentation equilibrium" is the condition where the concentration profile in the centrifuge tube reaches a steady state.



$$RT\frac{d\ln C_A}{dr} = (MW_A)(1-\rho\bar{v}_A)\omega^2 r$$

Integration yields:

$$RT\ln\frac{C_A(r)}{C_A(r_1)} = (MW_A)(1-\rho\bar{v}_A)\cdot\frac{\omega^2(r^2-r_1^2)}{2}$$



Can measure MW_A of a molecule

$$\text{Slope} = \frac{\omega^2(MW_A)(1-\rho\bar{v}_A)}{2RT}$$

The first measurements of the molecular weights of proteins were done this way.

Can also use a dense small molecule such as $CsCl_2$ or sucrose to form a density gradient (used in isopycnic).

Consider next the "motility" of a molecule

v_A ← velocity of molecule A

$$\frac{v_A}{F_A'} = \text{motility}_A$$

F_A' ← force on a single molecule

From Stokes Law for creeping flow past a sphere:

$$\frac{v_A}{F_A'} = \frac{1}{6\pi \mu R_A} = \text{motility}_A$$

μ ← viscosity of fluid; R_A ← radius of molecule A

F_A' ← force on one molecule

What is the force on one molecule caused by the concentration gradient?

$$F_A' = F_A / N_{Avogadro} = \frac{-\nabla \mu_A^{chem\ pot}}{N_{Avogadro}} = -\frac{RT}{C_A N_{Avogadro}} \frac{dC_A}{dr}$$

F_A ← Force per mole

Boltzman constant: $R = k N_{Avogadro}$

$$= -\frac{kT}{C_A} \frac{dC_A}{dr}$$

Substituting:

$$\frac{v_A}{F_A'} = -\frac{v_A C_A}{kT}\left(\frac{dC_A}{dr}\right)^{-1} = \text{motility}_A \Rightarrow \underbrace{v_A C_A}_{J_A} = -kT\ \text{motility}_A * \frac{dC_A}{dr}$$

J_A ← Fick's Law of diffusion; Diffusion coefficient

$$\frac{D_A}{kT} = \text{motility}_A \leftarrow \text{Nernst-Einstein Eqn.}$$

also

$$D_A = \frac{kT}{6\pi \mu R_A} \leftarrow \text{Stokes-Einstein Eqn.}$$

Define the "Sedimentation Coefficient" as follows:

$$S_A = \left(\frac{\mathcal{D}_A}{RT}\right)\underbrace{(MW_A)(1-\rho\bar{v}_A)}$$

$\frac{\mathcal{D}_A}{RT}$: mobility of one mole of molecules

$(MW_A)(1-\rho\bar{v}_A)$: mass of one mole corrected for buoyancy using Archimedes' principle

Flux of A caused by centrifugal force is given by $C_A S_A \omega^2 r$

$\omega^2 r$: centrifugal acceleration

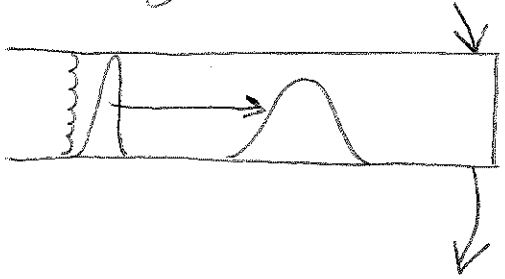
$S_A \omega^2 r$: velocity caused by centrifugal force

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

$$J_A = S_A C_A \omega r^2 - \mathcal{D}_A \, dC_A/dr$$

If $J_A = 0$, 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 $J_A = v_A C_A = S_A C_A \omega^2 r$

or $v_A = S_A \omega^2 r$

For a spherical molecule, since

$$S = \frac{\mathcal{D}_A}{RT}(MW_A)\left(1 - \rho \bar{v}_A\right)$$

(ρ: density of solvent; $\frac{1}{\rho_A}$: density of molecule A)

and

$$\frac{\mathcal{D}_A}{RT} = \frac{1}{N_{av}}\left(\frac{u_A}{F_A}\right)\frac{1}{6\pi\mu R_p}$$

(u_A: motility; N_{av}: Avogadro's number)

and assuming

$$\frac{4}{3}\pi R_p^3 \cdot N_{av} = (MW_A)\,\bar{v}_A$$

($\bar{v}_A$: volume/mass; MW_A: mass/mole)

substituting yields:

$$S_{sphere} = \frac{(MW_A)\left(1 - \rho \bar{v}_A\right)}{N_{av}\, 6\pi\mu \left(\frac{3\, MW_A\, \bar{v}_A}{4\pi N_{av}}\right)^{1/3}}$$

substituting values:

$$S_{sphere} = 0.012\,\frac{(MW_A)^{2/3}\left(1 - \rho \bar{v}_A\right)}{\bar{v}_A^{1/3}}$$

(S_{sphere}: svedbergs ($= 10^{-13}$ s); MW_A: Daltons; ρ: gm/ml; $\bar{v}_A$: ml/gm)

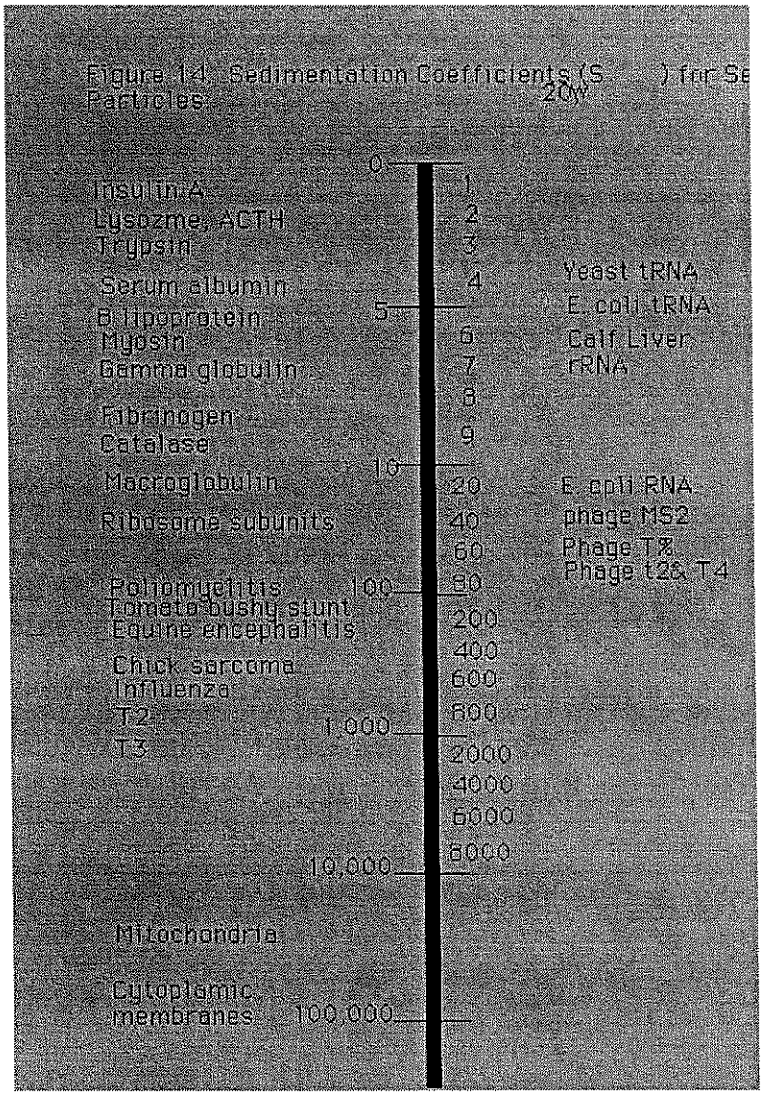
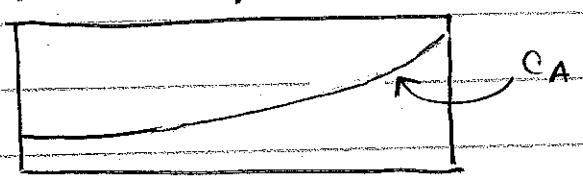


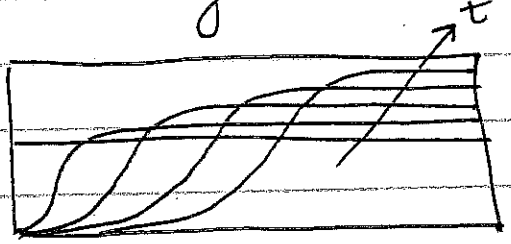
Figure 14 Sedimentation Coefficients (S) for Se
Particles
20w
Insulin A
Lysozme, ACTH
Trypsin
Serum albumin
Bilipoprotein
Myosin
Gamma globulin
Fibrinogen
Catalase
Macroglobulin
Ribosome subunits
Poliomyelitis
Tomato bushy stunt
Equine encephalitis
Chick sarcoma
Influenza
T2
T3
Mitochondria
Cytoplasmic membranes
Yeast tRNA
E. coli tRNA
Calf Liver tRNA
E. coli RNA
phage MS2
Phage T%
Phage t2& T4
0
1
2
3
4
5
6
7
8
9
10
20
40
60
80
100
200
400
600
800
1,000
2000
4000
6000
8000
10,000
100,000

Methods of centrifugation:

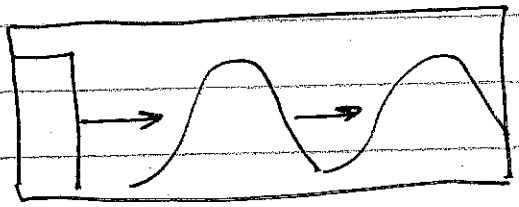
"Sedimentation equilibrium"



Moving boundary sedimentation



Zonal centrifugation



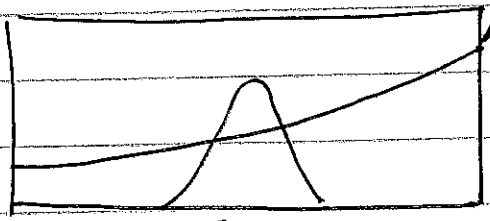
velocity of band: $v_A = S_A \omega^2 r$

width of band: $2 D_A t = \sigma_r^2$

assume Gaussian peak

Isopycnic sedimentation:

density gradient can also be "preformed" in which case it can be either gradual or stepwise



A component which is present in large amounts comes to sedimentation equilibrium so density gradient is formed

solute "A": $J_A = 0 \Rightarrow S_A = 0$

$(1 - \rho \bar{v}_A) = 0$

$\bar{v}_A = 1/\rho$

From "Bioseparations Science and Engineering" by Harrison, Todd, and Rudge.

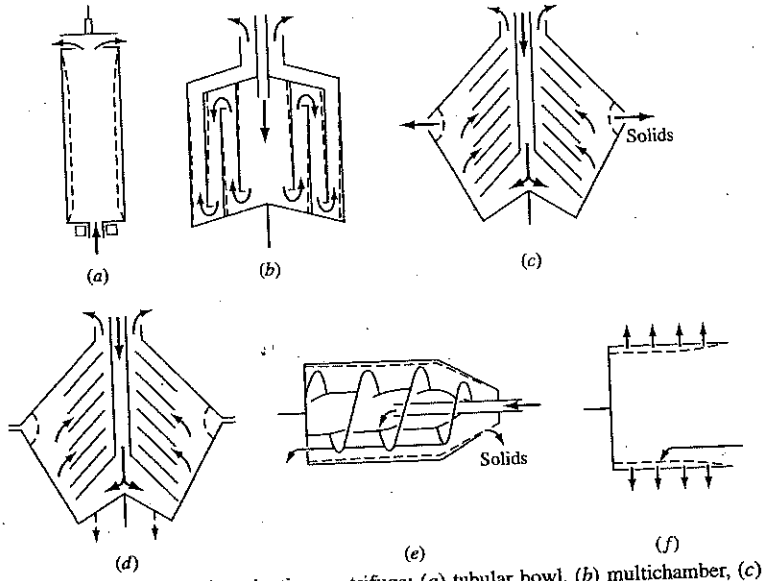


Figure 5.2 Common types of production centrifuge: (*a*) tubular bowl, (*b*) multichamber, (*c*) disk, nozzle, (*d*) disk, intermittent discharge, (*e*) scroll, and (*f*) basket. Arrows indicate the path of the liquid phase; dashed lines show where the solids accumulate.

TABLE 5.4
Comparison of Production Centrifuges[a]

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

[a] See reference 11.

TABLE 5.5
Capabilities of Tubular and Disk Centrifuges[a]

Type	Bowl diameter (mm)	Speed (rpm)	Maximum dimensionless acceleration (G), $\omega^2 R/g$	Throughput (liters/min)
Tubular bowl	44	50,000	61,400	0.2–1.0
	105	15,000	13,200	0.4–38
	127	15,000	16,000	0.8–75
Disk with nozzle discharge	254	10,000	14,200	40–150
	406	6,250	8,850	100–570
	686	4,200	6,760	150–1500
	762	3,300	4,630	150–1500

[a] See reference 12.

Apheresis

Return to the Blood Bank tutorial menu.

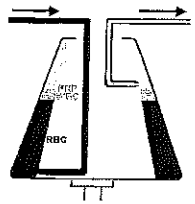
What is Apheresis?

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 retransfused 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.



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 Syndromes
  - Paraproteinemia
  - Cryoglobulinemia

  - Goodpasture's syndrome
- Plateletpheresis: rarely, in myeloproliferative disorders, the platelet count can be very high (thrombocytosis). Removal of platelets can help to avoid complications of thrombosis and bleeding.
- Leukapheresis: in some cases of leukemia with very high white blood cell counts, removal of the excess leukocytes may help to prevent complications of thrombosis.
- Stem Cell Harvesting: the small number of circulating bone marrow stem cells can be harvested to use in transplantation procedures.

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:

- Plateletpheresis: 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.