

CENTRIFUGATION

Circular motion:

ω = angular velocity (rad sec⁻¹).

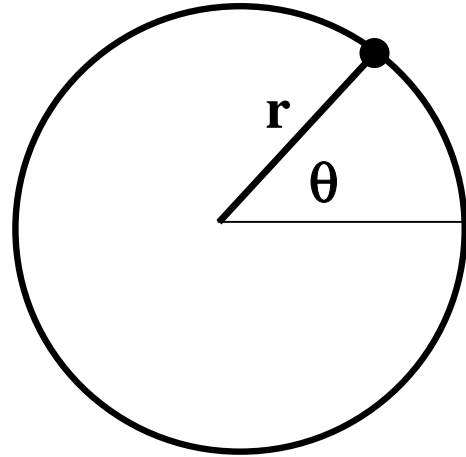
$(x,y) = r (\cos\theta, \sin\theta)$; $\theta = \omega t$

$\therefore \vec{r} = (x,y) = (r \cos(\omega t), r \sin(\omega t))$

$\vec{a} = (d^2x/dt^2, d^2y/dt^2)$

$= -r \omega^2 (\cos(\omega t), \sin(\omega t))$

$\therefore \vec{a} = -r \omega^2 \vec{r}$



$F = m \vec{a}$

\therefore Centripetal force: $m\omega^2 r$; radial; towards centre.

(In the spinning particle's frame of reference, the fictitious force is “centrifugal”.)

Sedimentation Archimedes' Principle

(see .ppt slide)

Partial specific volume

What is the “volume” of a molecule in solution?

1 cup sugar + 1 cup water \ll 2 cups

$$\bar{v} \equiv \frac{dv}{dm}$$

volume increment of solution per unit mass solute

$$\bar{v} \approx \frac{1}{\rho_{\text{solute}}}$$

volume displaced by solute:

$$\Delta v = \Delta m \frac{dv}{dm} = \Delta m \bar{v}$$

Measurement of \bar{v} : pycnometry (solution density)
(or calculation based on a.a. composition)

For proteins, $\bar{v} \approx 0.73 \text{ cm}^3/\text{g}$

For nucleic acids, $\bar{v} \approx 0.5 \text{ cm}^3/\text{g}$

For a molecule in solution:

$$\text{volume} = m_0 \frac{dv}{dm} = m_0 \bar{v}$$

$$\text{buoyancy} = m_0 \bar{v} \rho g$$

$$\text{mass}_{\text{eff.}} = m_0 (1 - \bar{v} \rho)$$

$$\text{weight}_{\text{eff.}} = m_0 (1 - \bar{v} \rho) \omega^2 r$$

$$\text{drag} = f v = m_0 (1 - \bar{v} \rho) \omega^2 r$$

$$v = \frac{m_0 (1 - \bar{v} \rho) \omega^2 r}{f}$$

$$s = \frac{v}{\omega^2 r} = \frac{m_0 (1 - \bar{v} \rho)}{f}$$

Sedimentation coefficient

$$s = \frac{v}{\omega^2 r} = \frac{m_0 (1 - \bar{v} \rho)}{f}$$

dimensions of s are time

Svedberg unit: $1S \equiv 10^{-13}$ sec.

For a “hard” spherical particle:

$$f = 6 \pi \eta r \text{ (Stokes equation)}$$

r = radius

η = viscosity of solution

For proteins, $f \sim 1$ to $1.5 \times$ the Stokes value

(larger for fibrous proteins)

Centrifugation methods:

see cent.ppt slides

Classes of centrifuge:

low speed: to $\sim 5,000 \times g$ (Beckman J6)

high speed: to $\sim 40,000 \times g$ (Beckman J21)

vacuum ultracentrifuge: to $\sim 400,000 \times g$ (Beckman L8-55)

Most lab. centrifuges are *preparative* instruments designed for recovery of samples after the run. An *analytical ultracentrifuge* incorporates an optical system designed to allow the progress of the sedimentation to be monitored continuously *during* the run. This facilitates determinations of sedimentation coefficients and molecular weights.

Fixed-angle rotors

KA 23.50

KOMPspin® Fixed Angle Carbon Fiber Rotor

For use with most high/super and ultra speed centrifuges



Product Features:

- ◆ Versatile—utilizes 50 mL open top, hand sealable™ or conical bottom tube holders for 50 mL round bottom or conical culture tubes
- ◆ Carbon fiber—not corroded by laboratory chemicals, less frequent need for replacement
- ◆ Saves time—faster acceleration and deceleration, shorter run times
- ◆ Lightweight—less wear on centrifuge drive, easier to lift than comparable metal rotors
- ◆ Seven Year Warranty—with no deration
- ◆ Autoclavable
- ◆ Biocontainment—Individually sealed tube holder option
- ◆ Flexible—Adapters permit the use of 38.5 mL, 13.5 mL centrifuge and 1.5 mL microcentrifuge tubes

Product Specifications:

Rotor

Maximum Speed‡	23,000 rpm
Maximum Sample Density	1.3 g/mL
Relative Centrifugal Force (RCF) at maximum rpm:	
At R_{max} (131.9 mm)	78,200 x G
At R_{avg} (101.2 mm)	59,900 x G
At R_{min} (70.6 mm)	41,800 x G
K-factor at maximum rpm	299
Number of tubes‡	12
Nominal dimensions of largest tubes	29 x 108 mm
Nominal tube capacity	50 mL
Nominal rotor capacity	600 mL
Weight (empty)	6.3 kg (14 lbs)
Angle	23°

Swinging-bucket rotors



Density gradient centrifugation of cells and organelles

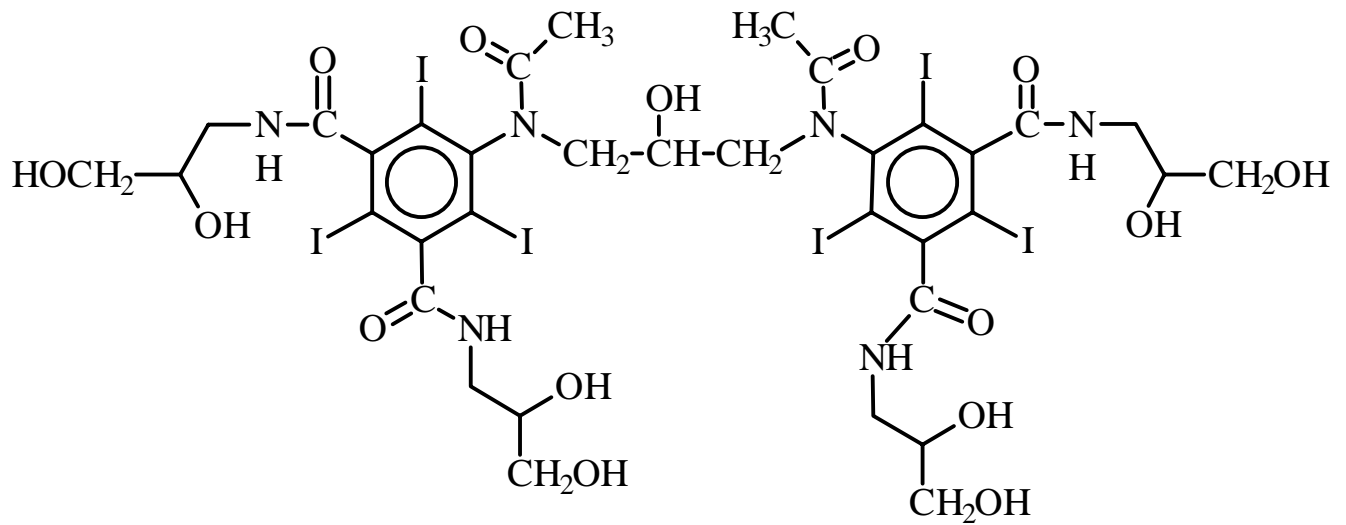
s high (10^6 S to 10^7 S)

ρ low (1 to 1.2)

Media:

- appropriate density
- high water solubility
- low viscosity
- sterile
- biocompatible
- membrane-impermeant
- iso-osmolar: high MW

Ficoll, albumin, metrizamide, etc. - *not* sucrose or salts

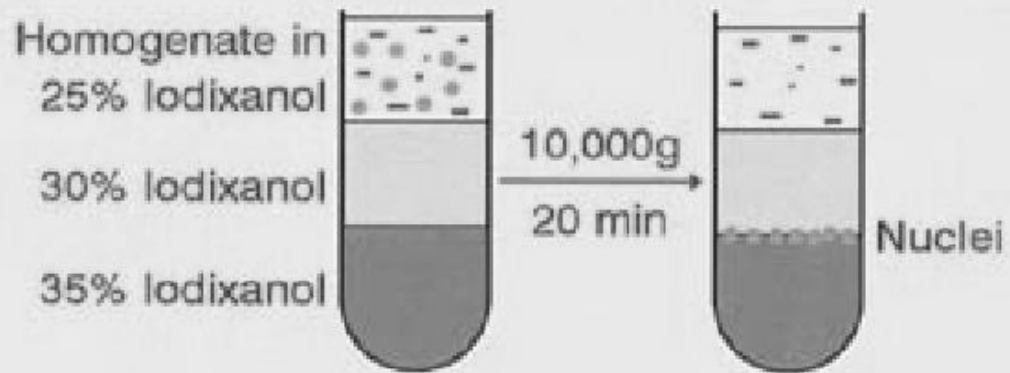


Iodixanol MW = 1550

Smith *et al.*, The use of iodixanol as a density gradient material for separating human sperm from semen, *Arch. Androl.* 38: 223-230, 1997.

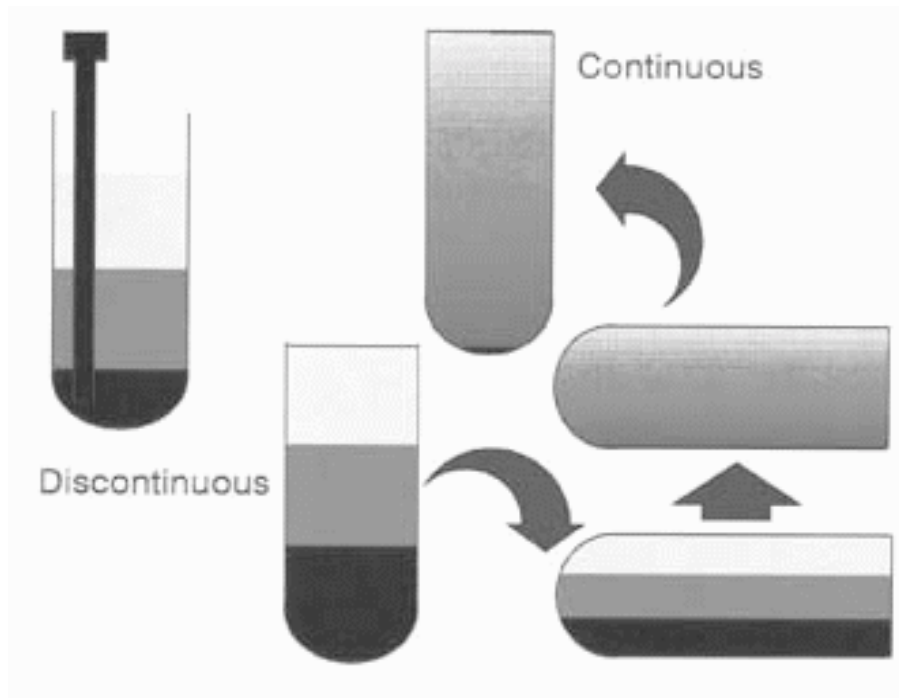
Iodixanol, a new nonionic density gradient material with relatively low osmolality and high density, was evaluated ... for the separation of human sperm from semen.... Using a three-layer iodixanol gradient (approximately 1.17/1.15/1.05 g/mL), sperm were centrifuged at 1,000×g for 30 min and collected from the 1.05/1.15 interface. ... Sperm survived iodixanol density gradient centrifugation well, showing only modest declines in motility and velocity during a subsequent 24h incubation period. ...

Purification of nuclei



Rate zonal (band) separation of macromolecules on preformed gradients (sucrose or glycerol).

The gradient minimizes mixing due to mechanical disturbances or convection currents.



Bands are collected in fractions; detected by UV-vis., fluorescence, radioactivity, enzyme assay, etc.

Applications:

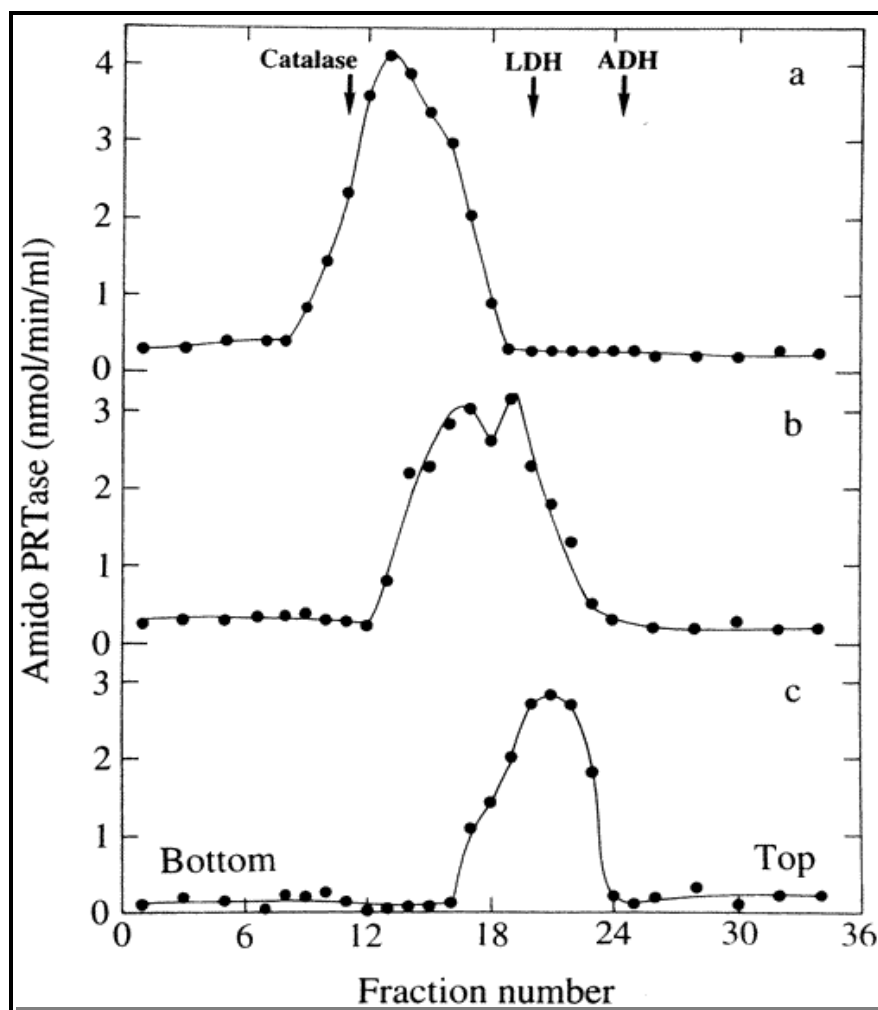
Analytical: determination of s value

Preparative: separation of proteins, nucleic acids

Schoettle *et al.*, Mechanisms of inhibition of amido phosphoribosyltransferase from mouse L1210 leukemia cells, *Biochemistry* 36: 6377-6383, 1997.

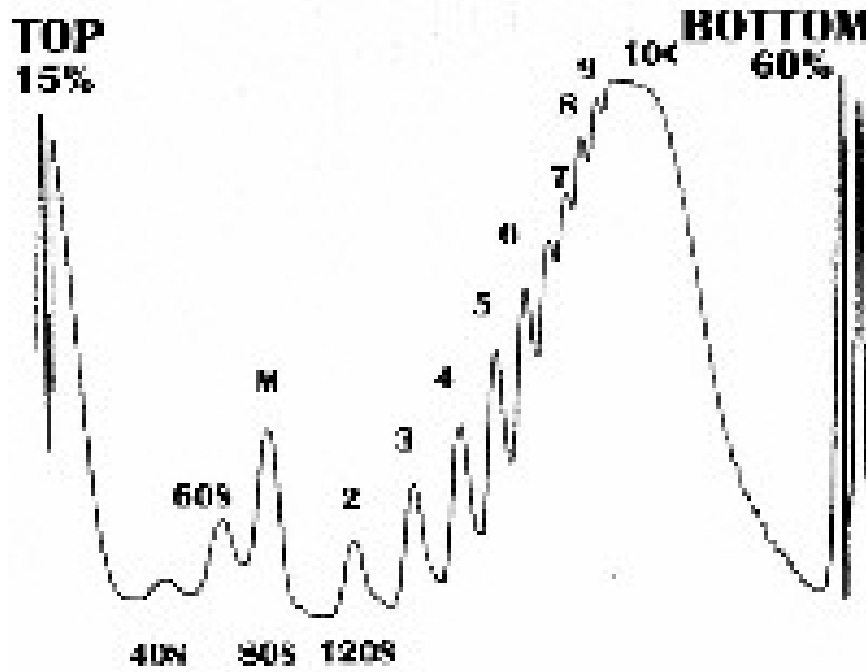
Interconversion between the large and small forms of amido PRTase induced by AMP and piritrexim:

Amido PRTase (0.5 mg protein) was sedimented through sucrose gradients containing (a) 5.0 mM AMP, (b) 2.5 mM AMP and 50 μ M piritrexim, and (c) 100 μ M piritrexim.



Methods: Linear sucrose gradients from 10 to 25% (w/v) were prepared using a ... gradient maker. The sucrose gradients also contained ... 20 mM K·Hepes (pH 7.3), 1.0 mM MgCl₂, 1.0 mM DTT, and other components (AMP, piritrexim, or P-Rib-PP) as required. Samples for centrifugation contained in a volume of 200 μL, partially purified murine amido PRTase (0.5 mg of protein), three marker proteins [alcohol dehydrogenase (1 mg), lactate dehydrogenase (150 μg), and catalase (500 μg)] with other components as indicated. Sedimentation profiles for amido PRTase were developed by centrifugation at 40,000 rpm in a Beckman SW41Ti rotor for 24h at 4C using a Beckman L8-80M ultracentrifuge. Gradients were fractionated by puncturing the bottom of the tube; 320 μL fractions were collected and assayed immediately for amido PRTase.

Application: Separation of polysomes



Polysome profile of pea stem tissue using a 15 to 60% sucrose gradient spun at $300,000 \times g$ for 45 min. Polysomes with various size classes were analyzed using gradient fractionator with absorbance at 260 nm.

<http://133.71.125.239/english/methods/gradient.htm>

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**Determination of s
(using analytical ultracentrifuge):**

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

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

$$\therefore \int_{r_1}^{r_2} \frac{dr}{r} = \omega^2 s \int_0^t dt$$

$$\therefore \ln r_t - \ln r_0 = \omega^2 s t$$

$$\therefore \ln r_t = \omega^2 s t - \ln r_0$$

So, if we plot $\ln r_t$ vs. t , slope = $\omega^2 s$.

Determination of MW from s

$$s = \frac{m_0 (1 - \bar{v} \rho)}{f}$$

$$f = \frac{RT}{ND}$$

R = gas constant; **T** = temperature;
D = diffusion coefficient;
N = Avogadro's number.

(G.M. Barrow, *Physical Chemistry*, 5th ed., 1988, §20.5)

$$s = \frac{m_0 (1 - \bar{v} \rho) ND}{RT}$$

$$m_0 N = M$$

$$M = \frac{RTs}{D (1 - \bar{v} \rho)}$$

Svedberg equation

But, accurate measurement of D is not easy.

Sedimentation equilibrium

(G.M. Barrow, *Physical Chemistry*, 5th ed., 1988, §20.7)

- One of the best methods for determining protein molecular weights, under native conditions (Laue, T.M., *Advances in sedimentation velocity analysis*, *Biophys. J.* 72: 395-396, 1997).
- Relatively low speed (about 20,000 rpm). Sedimentation tends to pull the protein to the bottom of the tube, but speed is insufficient to pellet out the protein.
- Protein concentration gradient develops, but the concentration difference (*i.e.*, the gradient of chemical potential) causes net diffusion of protein molecules back up the tube.
- Centrifugation and diffusion processes reach *sedimentation equilibrium*: rate of sedimentation = rate of counter-diffusion.

Difference in energy due to concentration
(chemical potential energy difference):

$$\frac{C_1}{C_2} = e^{\frac{-(E_1 - E_2)}{kT}} \quad \text{or} \quad \ln \left[\frac{C_1}{C_2} \right] = \frac{(E_2 - E_1)}{kT}$$

($k = R/N = \text{Boltzmann's constant}$)

At equilibrium:

$$\begin{aligned} E_1 - E_2 &= - \int_{r_2}^{r_1} F(r) dr = - \int_{r_2}^{r_1} m_0 (1 - \bar{v}\rho) \omega^2 r dr \\ &= \frac{1}{2} \omega^2 (r_2^2 - r_1^2) m_0 (1 - \bar{v}\rho) \\ \therefore \ln \left(\frac{C_2}{C_1} \right) &= \frac{\omega^2 (r_2^2 - r_1^2) m_0 (1 - \bar{v}\rho)}{2kT} \end{aligned}$$

M = m₀ N and R = k N, so m₀/k = M/R.

$$\therefore \ln \left(\frac{C_2}{C_1} \right) = \frac{\omega^2 (r_2^2 - r_1^2) M (1 - \bar{v}\rho)}{2RT}$$

plot ln c vs r²

$$\therefore \text{slope} = \frac{(1 - \bar{v}\rho) \omega^2 M}{2RT}$$

$$\therefore M = \frac{2RT}{(1 - \bar{v}\rho) \omega^2} \frac{d(\ln c)}{d(r^2)}$$

Application of sedimentation equilibrium method:

Pauls, J.E.D., *et al.*, Self-association of human protein S, *Biochemistry* 39, 5468-5473, 2000.

Isopycnic buoyant density centrifugation of nucleic acids

Cs⁺ salts of nucleic acids are very dense ($\rho \sim 1.8$).

CsCl (7M) spun to sedimentation equilibrium at very high speeds, forming a narrow [CsCl] gradient.

Applications:

- **Analytical: Separate ¹⁴N-DNA from ¹⁵N-DNA:
Meselson-Stahl experiment, 1958;
semi-conservative replication of DNA.
(method was developed for this experiment)**
- **Preparative:
Plasmid purification.**