

New Posts at the Course Website:

Origin Assignment 3 on Analytical Ultracentrifugation (due 3/2/12)

Problem Set

Data (xls file)

Resource Booklet (R7) on Analytical Ultracentrifugation

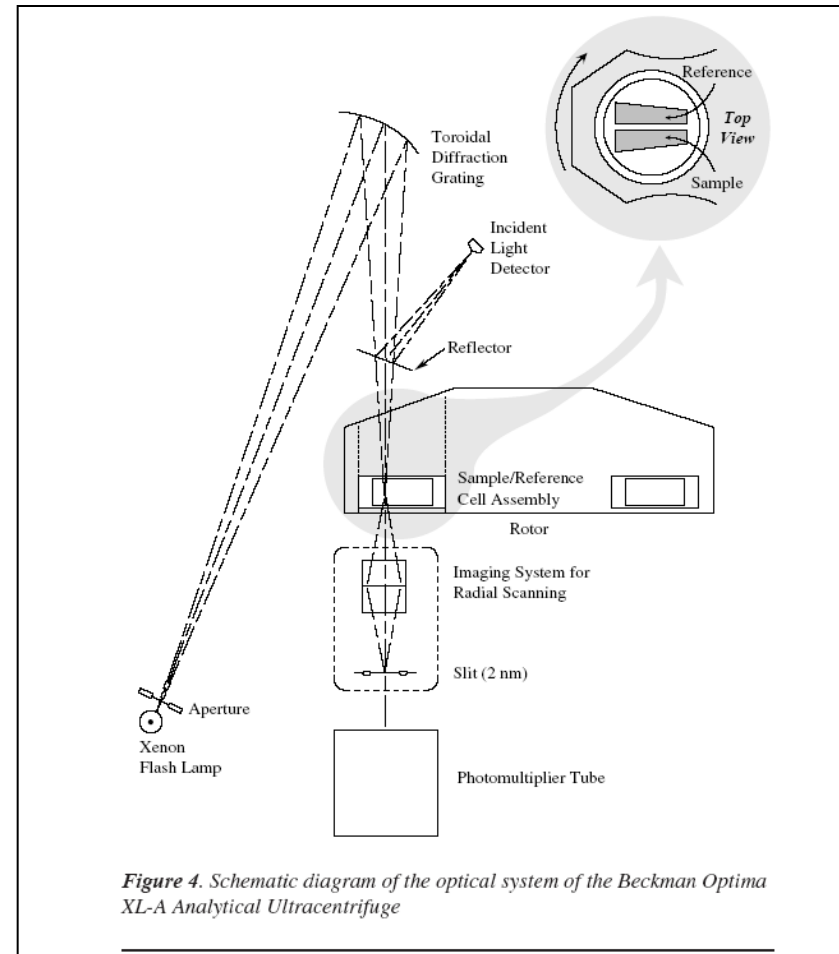
***Equilibrium Sedimentation &
Sedimentation Velocity: Random
Walks in the presence of forces.***

*A Beckman Analytical Ultracentrifuge
– circa 1950*



The Beckman XL1 AUC

– circa 2000



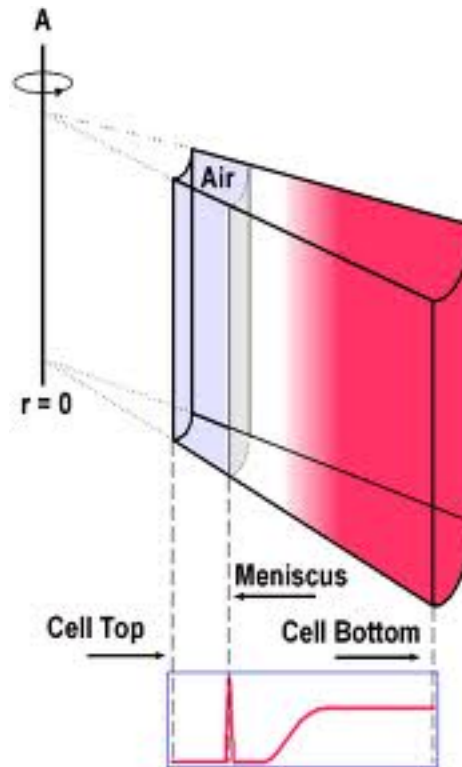
Sedimentation Velocity - History

Theodore Svedberg was awarded the 1926 Nobel Prize in Chemistry for
“for his work on disperse systems”



The (Theodor) Svedberg

The Nobel Prize in Chemistry 1926.
Nobelprize.org. 26 Feb 2012 http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1926/



UltraScan Website
Borris Demeler
<http://ultrascan.uthscsa.edu/index.php>



The Svedburg (by Isaac Grunewald)
Uppsala University Art Collection.
2010 Minnesskrift. Royal Swedish
Academy of Engineering Sciences (IVA)

Sedimentation Velocity

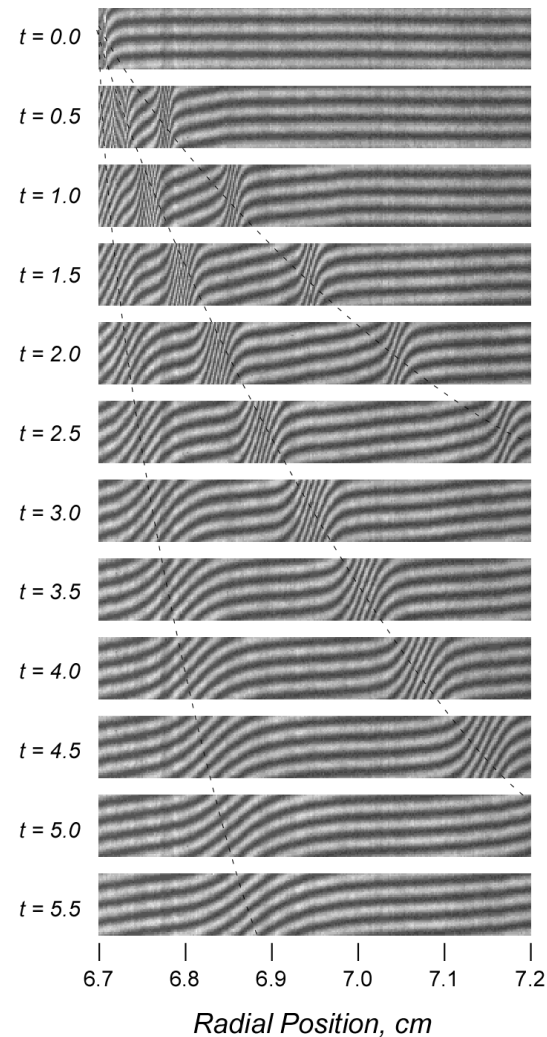
Interference fringes vs. time & radial position

'Fringe number' (the number of fringes that cross a horizontal axis) is proportional to the solute (macromolecule or particle) concentration.

When the fringes are horizontal, the concentration is steady (~constant). When the fringes turn vertical, the macromolecule concentration is changing rapidly. These are boundaries between regions that contain particles and are depleted of particles.

In the series of interference patterns shown at right, the sample chamber has a uniform distribution (concentration) of particles at the beginning of the experiment ($t = 0.0$). As a function of time, three boundaries move away from the top of the sample column (at left, $r \sim 6.7$ cm) at different rates. The fastest moving boundary corresponds to a component in the sample with the largest sedimentation coefficient, s , the slowest moving boundary is characterized by the smallest value of s .

Note that rate of movement of the boundaries increases as the radial position of the boundary becomes larger.



downward velocity, v

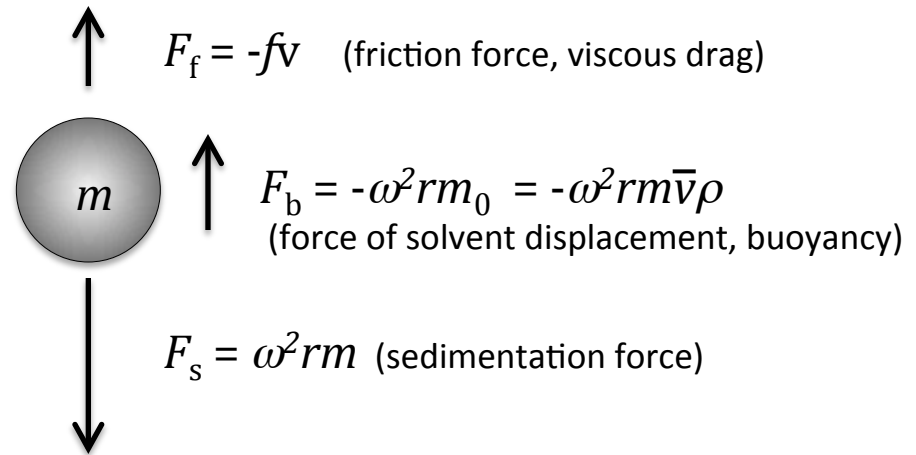


In the sedimentation velocity experiment, the forces acting on a particle add to zero, due to the fact that viscous drag (F_f) grows in proportion to the particle velocity.

- m = mass (grams)
- r = radial position (cm)
- ω = angular velocity (radians/s)
- \bar{v} = partial specific volume of particle (cm^3/g)
- ρ = solvent density (g/cm^3)
- f = friction coefficient (g/s)

Collecting the terms that relate to the particle on the left hand side and those that relate to the experimental conditions on the other, leads to the definition of the sedimentation coefficient, s . s equals the velocity divided by the centrifugal acceleration.

- M = molecular weight (grams/mole)
- N = Avogadro's number (particles/mole)
- v = sedimentation velocity (cm/s)
- s = sedimentation coefficient (s)



$$F_f = -fv \quad (\text{friction force, viscous drag})$$

$$F_b = -\omega^2 r m_0 = -\omega^2 r m \bar{v} \rho$$

(force of solvent displacement, buoyancy)

$$F_s = \omega^2 r m \quad (\text{sedimentation force})$$

$$F_s + F_b + F_f = 0$$

$$m\omega^2 r - m\omega^2 r \bar{v} \rho - fv = 0$$

$$m(1 - \bar{v} \rho) \omega^2 r - fv = 0$$

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

$$\frac{M(1 - \bar{v} \rho)}{Nf} = \frac{v}{\omega^2 r} \equiv s$$

The simplest way to retrieve estimates of the sedimentation coefficient from velocity data, is to determine the rate of movement of the solvent/solute boundary, dr_b/dt .

- r_b = radial at solute boundary
- ω = angular velocity (radians/s)
- s = sedimentation coefficient (s)

A plot of the $\ln(r_b)$ as a function of time gives $\omega^2 s$ as the slope.

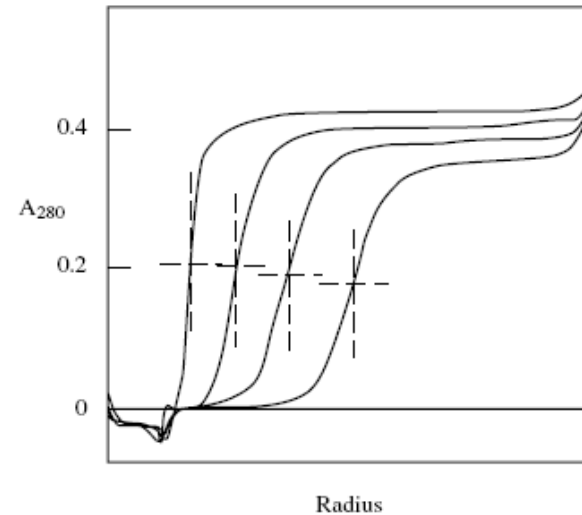
$$v = \frac{dr_b}{dt} = r_b \omega^2 s$$

$$\frac{dr_b}{r_b} = \omega^2 s dt$$

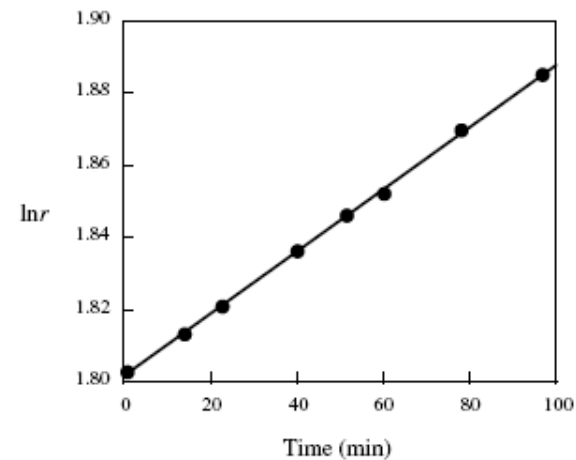
$$d(\ln r_b) = \omega^2 s dt$$

$$\ln r_b = \omega^2 s t + c$$

Absorbance profile vs. time



$\ln(r_b)$ position vs. time



comparing values of s

Since the sedimentation coefficient depends on solvent density and viscosity, standard conditions are necessary to compare the sedimentation coefficients determined for the same molecule by different investigators (under different conditions), or to make comparisons among different particles, where it is probable that s was determined under different conditions.

These standard conditions are 20°C in water as the solvent.

$S_{20,w}$ = sedimentation coefficient in water at 20°C

$S_{T,b}$ = sedimentation coefficient in buffer at T°C

$\eta_{20,w}$ = viscosity of water at 20°C

$\eta_{T,b}$ = viscosity of buffer at T°C

$\rho_{20,w}$ = density of water at 20°C

$\rho_{T,b}$ = density of buffer at T°C

\bar{v} = partial specific volume of particle (cm³/g)

$$S_{20,w} = S_{T,b} \frac{(1 - \bar{v}\rho)_{20,w}}{(1 - \bar{v}\rho)_{T,b}} \frac{(\eta_{T,b})}{(\eta_{20,w})}$$

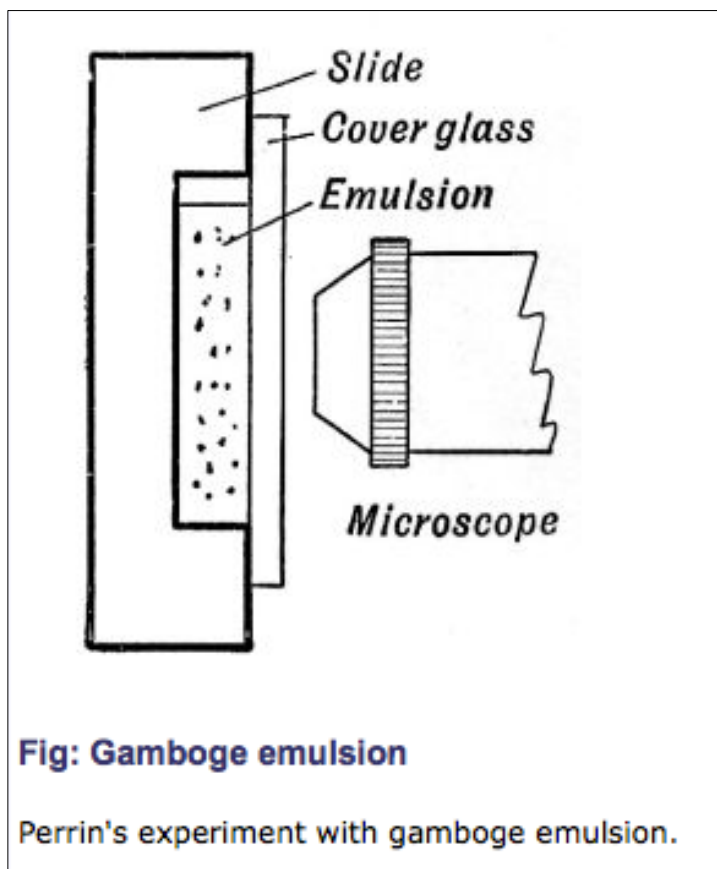
$$S_{20,w} = S_{T,b} \frac{(1 - \bar{v}\rho_{20,w})}{(1 - \bar{v}\rho_{T,b})} \frac{(\eta_{T,b})}{(\eta_{20,w})}$$

$$S_{20,w} = S_{T,b} \frac{(1 - \bar{v}\rho_{20,w})}{(1 - \bar{v}\rho_{T,b})} \frac{(\eta_{T,b})}{(\eta_{T,w})} \frac{(\eta_{T,w})}{(\eta_{20,w})}$$

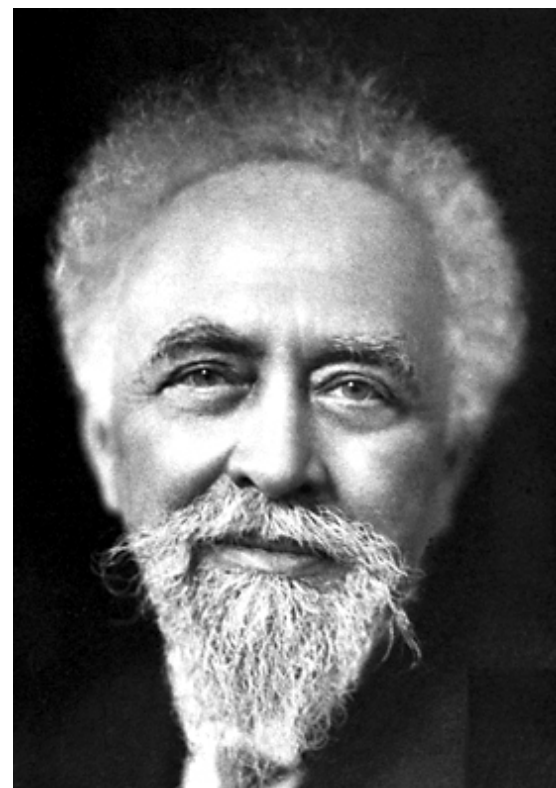
The partial specific volume of the particle does not change much with temperature or buffer; also these dependences usually aren't known. The usual corrections are made to ρ and η , which are measured (or looked up in tables) as a function of temperature and solvent composition.

Sedimentation Equilibrium - History

Jean Baptiste Perrin was awarded the 1926 Nobel Prize in Physics for “for his work on the discontinuous structure of matter, and especially for his discovery of sedimentation equilibrium”



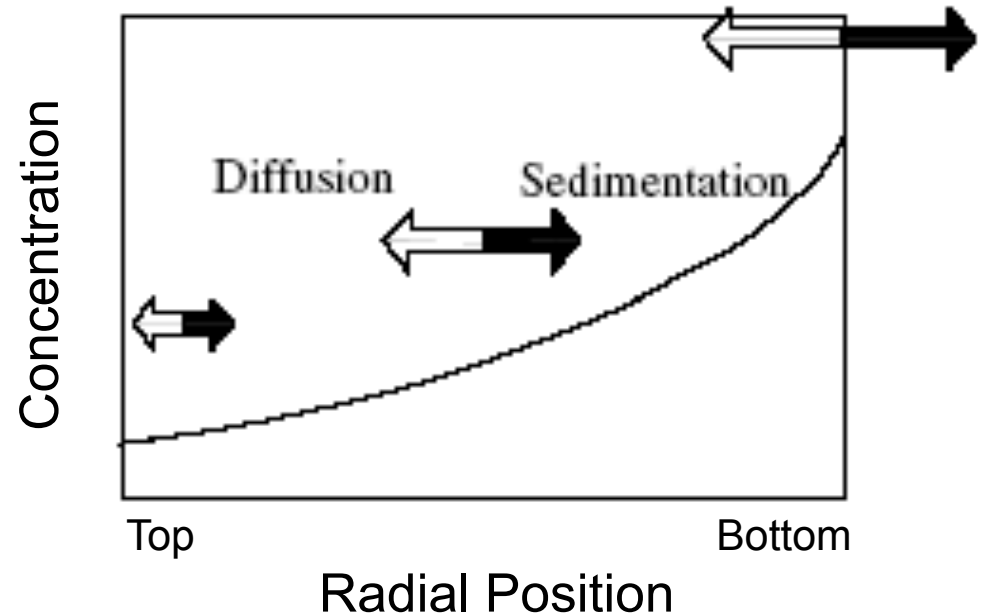
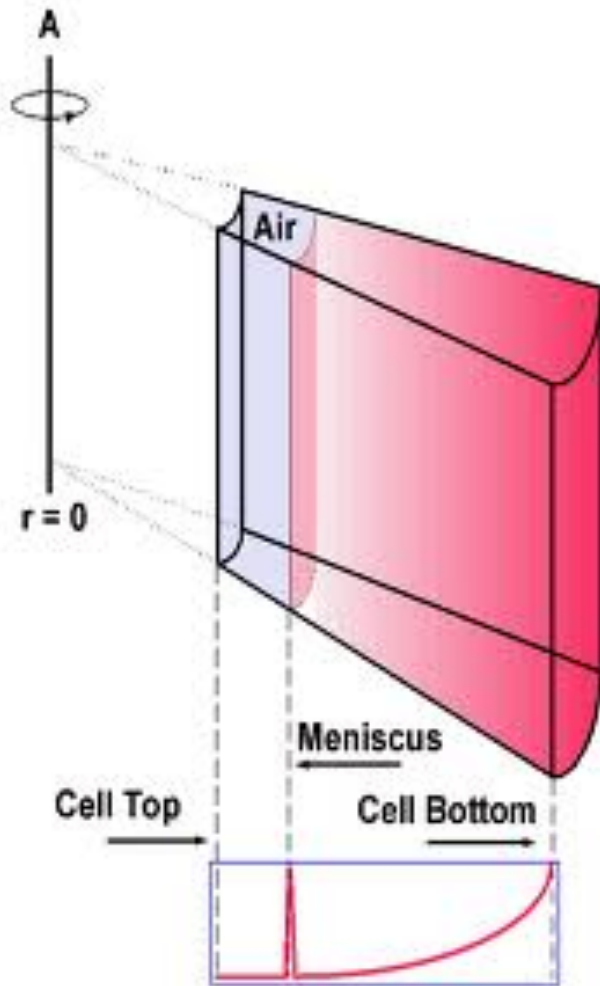
<http://chemistry.proteincrystallography.org/article123.html>



Jean Baptiste Perrin

"Jean Baptiste Perrin - Biography".
Nobelprize.org. 26 Feb 2012 http://www.nobelprize.org/nobel_prizes/physics/laureates/1926/perrin.html

Sedimentation Equilibrium



The system is at equilibrium at every point in the centrifuge cell.

The decrease in potential energy of the particle in a centrifugal field, by movement to the right (rightward filled arrows), is matched a decrease in chemical potential energy that results from the concentration gradient (leftward pointing open arrows), which acts in opposition to sedimentation.

Total Chemical Potential Energy in the Sedimentation Equilibrium Experiment

At equilibrium, the total chemical potential energy of a particle in a centrifugal field is the sum of the chemical potential energy and the potential energy contributed by the centrifugal field. As a condition of equilibrium, the total chemical potential energy is constant throughout the cell, a minimum, which means that the derivative of the total potential, with respect to r , is zero.

$$\tilde{\mu}_i = \mu_i - \frac{1}{2}M_i\omega^2r^2$$

$$\frac{d\tilde{\mu}_i}{dr} = \frac{d\mu_i}{dr} - M_i\omega^2r = 0$$

$$\frac{d\mu_i}{dr} = \left(\frac{\partial\mu_i}{\partial T}\right)_{P,C_i} \frac{dT}{dr} + \left(\frac{\partial\mu_i}{\partial P}\right)_{T,C_i} \frac{dP}{dr} + \left(\frac{\partial\mu_i}{\partial C_i}\right)_{P,C_i} \frac{dC_i}{dr}$$

1st term = 0
(isothermal conditions)

$$\frac{dT}{dr} = 0$$

2nd term: $\left(\frac{\partial\mu_i}{\partial P}\right)_{T,C_i} \frac{dP}{dr} = (\bar{V}_i)\rho\omega^2r = \bar{M}_i\bar{v}_i\omega^2\rho r$

$\tilde{\mu}_i$ = total potential energy of the solute (or $\tilde{\mu}_2$)
 μ_i = chemical potential energy of the solute (or μ_2)
 M_i = molecular weight of solute (M_2)
 ω = angular velocity (radians/s)
 r = radial position (cm)
 C_i = solute concentration (molarity)

3rd term, assume ideal solution

$$\mu_i = \mu_i^o + RT\ln C_i$$

$$\left(\frac{\partial\mu_i}{\partial C_i}\right)_{P,T} = \frac{RT}{C_i}$$

Total Chemical Potential Energy in the Sedimentation Equilibrium Experiment

$$\frac{d\tilde{\mu}_i}{dr} = \frac{d\mu_i}{dr} - M_i\omega^2r = 0$$

$$\frac{RT}{C_i} \frac{dC_i}{dr} + \bar{M}_i\bar{v}_i\rho\omega^2r - M_i\omega^2r = 0$$

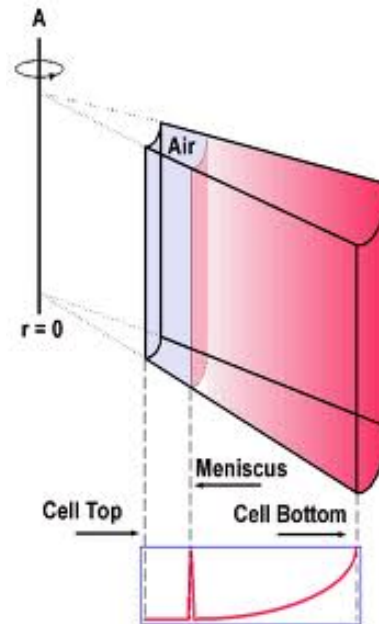
$$\bar{M}_i(1 - \bar{v}_i\rho)\omega^2r - \frac{RT}{C_i} \frac{dC_i}{dr} = 0$$

$$\frac{dC_i}{C_i} = \frac{\bar{M}_i(1 - \bar{v}_i\rho)\omega^2r}{RT} dr$$

$$\int_{C_i(0)}^{C_i(r)} d(\ln C_i) = \int_{r_0}^r \frac{\bar{M}_i(1 - \bar{v}_i\rho)\omega^2r}{RT} dr$$

$$\ln\left(\frac{C_i(r)}{C_i(0)}\right) = \frac{\bar{M}_i(1 - \bar{v}_i\rho)\omega^2}{2RT}(r^2 - r_0^2)$$

$$C_i(r) = C_i(0)\exp\left[\frac{\bar{M}_i(1 - \bar{v}_i\rho)\omega^2}{2RT}(r^2 - r_0^2)\right]$$



The equilibrium distribution of the particle concentration in the centrifuge cell varies exponentially on the radius.

The molecular weight of the particle is determined in a shape-independent manner.