

Sedimentation (section of ch. 15 with diffusion etc.)

Ref: Van Holde Ch. 5

SEDNTERP (<http://www.jphilo.mailway.com/>)

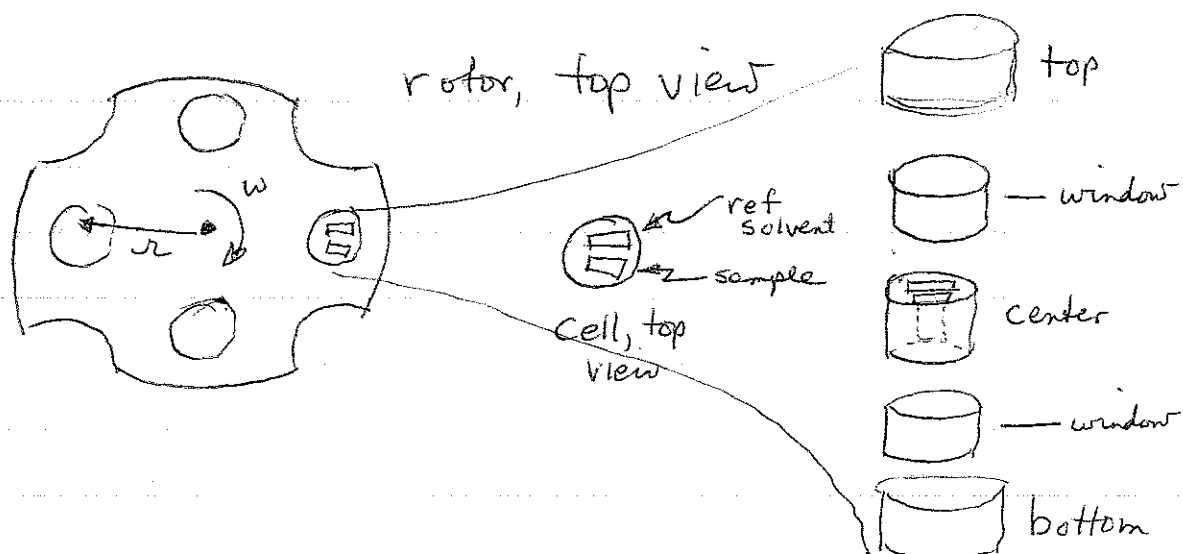
Sedimentation Velocity vs. Sedimentation Equilibrium

Analytical ultracentrifugation has a dual nature comparable to light scattering. In both methods, there is a dynamic variant devoted to getting a transport coefficient and an equilibrium variant that can return a molecular weight.

	Transport	Equilibrium
Light Scattering	DLS	SLS
AUC	Sed. Velocity	Sed. Eq.

In this section, we consider both Sed Vel and Sed Eq. The starting point is a "mechanical" analysis of velocity. It will help first, though, to sketch the rotor & cells,

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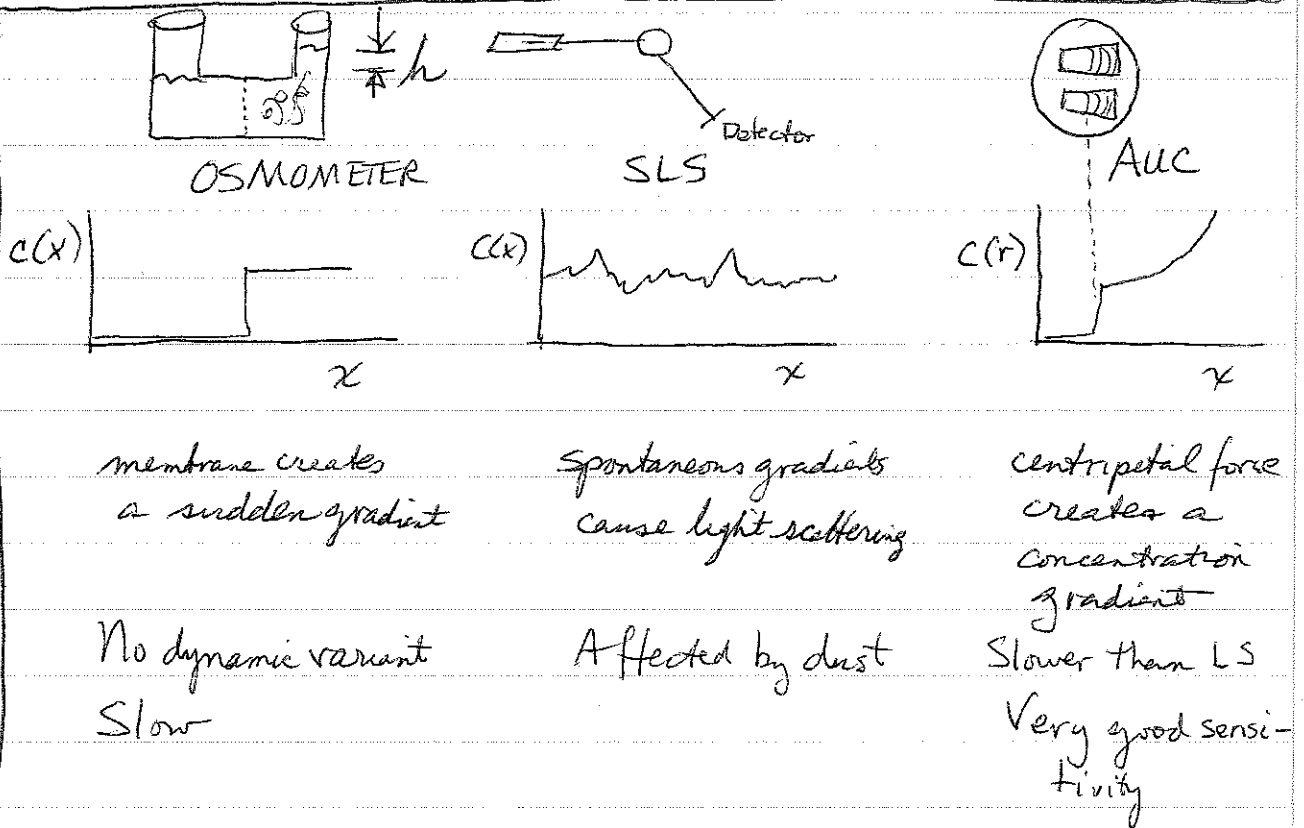
Principle features of The rotor & cell assembly

- rotor is $\sim 8''$ dia, holds 3 cells & balance
- rotor good to $\sim 60,000$ RPM
- cells hold sample & reference solvent for UV-vis detection.
- gaskets (not shown) seal the cells.
- aqueous or non-aqueous solvents are OK.

Principle features of The analysis system

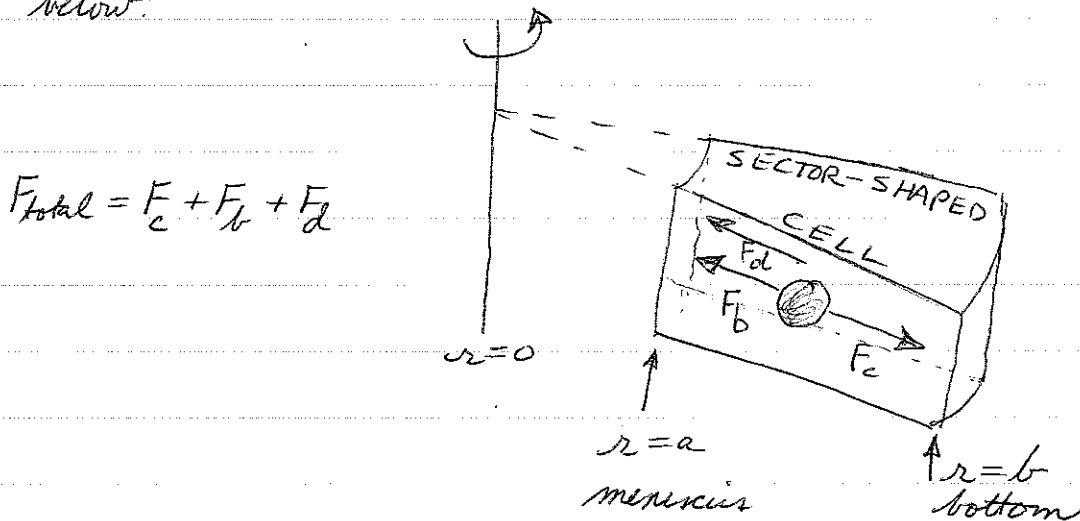
- can determine absorbance at a given radial position
- very good radial resolution
- can tell whether it is measuring sample position or nearby reference cell... at up to 60,000 RPM
- light can be monochromatic
- you can scan wavelength at constant r
- you can scan r at the best wavelength (usually in the UV).

The underlying idea is familiar: create a concentration gradient to probe the thermodynamics (eg. AUC) or create a gradient & watch it change (vel. AUC). In a sense, it's like osmometry or even LS.



A simple, mechanical picture

A particle in the AUC can be located at any radius between the meniscus ($r=a$) and the "floor" of the rotating cell ($r=b$). The scenario is sketched below.



The forces on a particle at position r ($a < r < b$) are three:

1. Centripetal force: $m\omega^2 r = F_c$
2. Buoyant force: $-m_0\omega^2 r = F_b$
3. Drag force: $-f v = F_d$

Where

m = particle mass

ω = circular frequency (radians/second)

v = velocity of particle

m_0 = mass of displaced solvent

After a short time, the particle no longer accelerates. This implies absence or balance of forces. It's balance in this case, so:

$$\Rightarrow \underline{F} = 0 = \omega^2 r (m - m_0) - f v$$

What is m_0 ?

An approximate form would be to compute:

It is not yet obvious that we should use ρ_{solution} and \bar{v}

$$m_0 = (\text{volume displaced}) (\text{density of solution})$$

$$\approx M \bar{v} \rho ; \bar{v} = \text{partial specific volume of solute}$$

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

$$\text{Now } m \equiv \frac{M}{N}$$

Rearrange:

\Rightarrow

$$\frac{M (1 - \rho \bar{v})}{N f} = \frac{v}{\omega^2 r} = \underline{\underline{S}}$$

define S

= Sedimentation

coefficient

= ratio of velocity to acceleration

Note: if f was known, ρ and \bar{v} is known, then M can be determined from S.

Units of S :
$$\frac{N}{\omega^2 r} = \frac{\text{cm/sec}}{\left(\frac{\text{radians}}{\text{sec}}\right)^2 \text{cm}} = \frac{\text{sec}}{\text{rad}^2}$$

Usually, we suppress the radians \Rightarrow Units of $S = \text{sec}$

Defn: $1 \times 10^{-13} \text{ sec} = 1 \text{ Svedberg} = 1 S.$

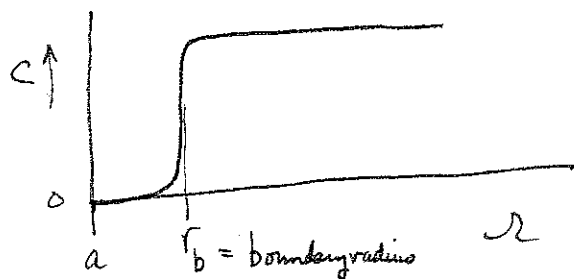
(Theodor Svedberg, 1926 Nobel Prize in Chemistry, 1884-1971)

Often, a protein or nucleic acid or ribosome, etc. is identified by its value of S :

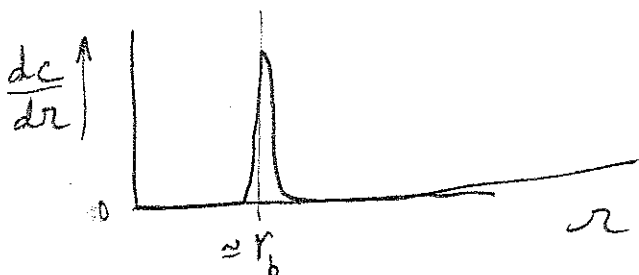
e.g. 4S RNA

Measuring S

As centrifugation proceeds, the molecules ~~to~~ pile up near the bottom; Near the meniscus, they are at $c \approx 0$



Could get c from
Absorbance A



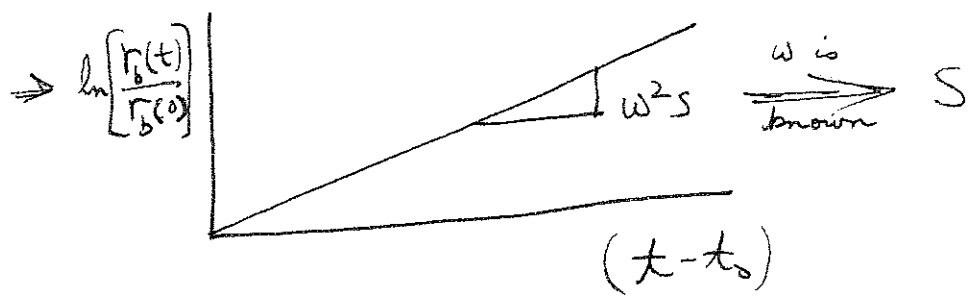
In older machines
~~the~~ the optics
often used for detection
give ~~the~~ a photographic
trace of dc/dr .

They are called Schlieren Optics

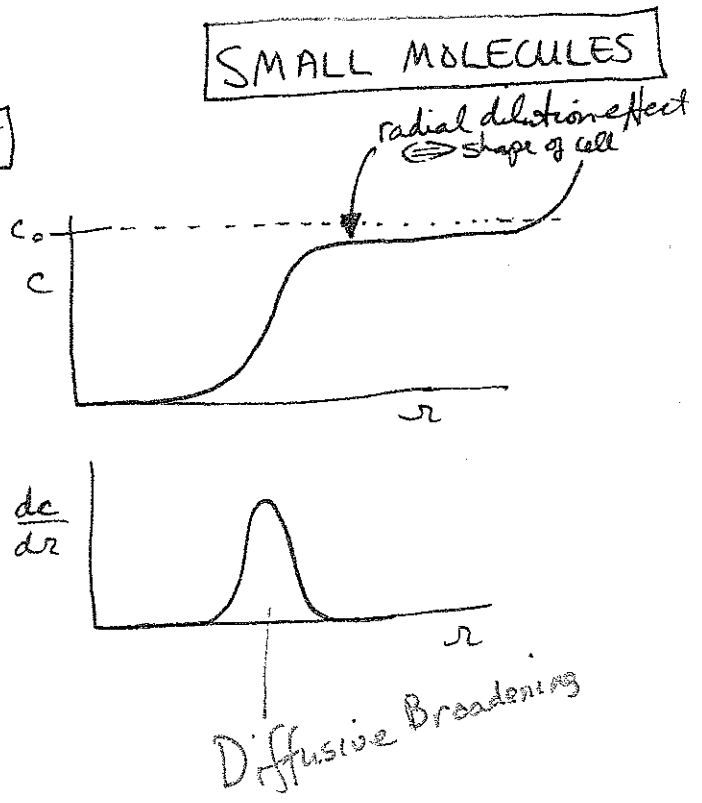
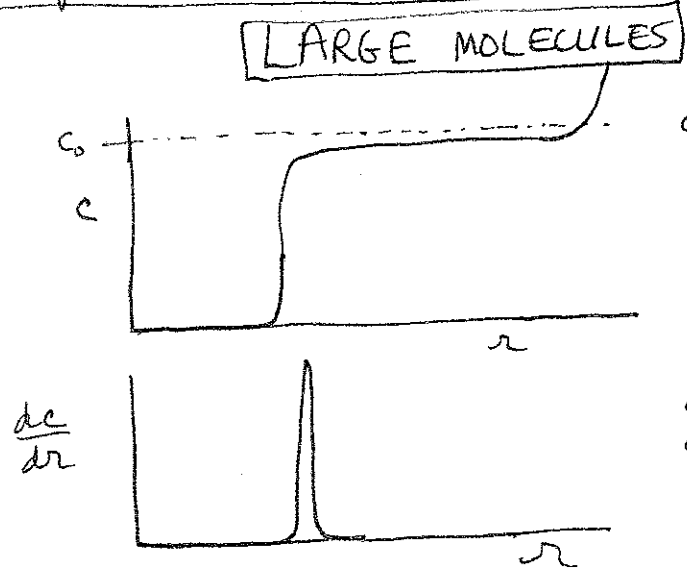
If we monitor the peak position vs. time, this would give us the velocity. (This is an approximation; see Van Holde).

$$v \approx \frac{dr_b}{dt} \approx r_b \omega^2 s \left\{ \begin{array}{l} v \approx \frac{dr_b}{dt} \\ \text{also } v = s \omega^2 R \\ \Rightarrow \frac{dr_b}{dt} = s \omega^2 r_b \end{array} \right. \begin{array}{l} \text{can evaluate at } r_b \\ \text{but } v \text{ is really} \\ \text{dependent on } r \\ \text{generally.} \end{array}$$

$$\Rightarrow \ln \left[\frac{r_b(t)}{r_b(0)} \right] = \omega^2 s (t - t_0)$$



Sharpness of Boundary



Molecular Weights by Sedimentation & Diffusion:

- 1) Assume D° can be easily measured by dynamic light scattering
- 2) Note that both D° and S° are reciprocal in f

$$D^\circ = \frac{kT}{f^\circ} = \frac{RT}{Nf^\circ}$$

$$S^\circ = \frac{M(1-\bar{v}\rho)}{Nf^\circ}$$

$$\therefore \frac{S^\circ}{D^\circ} = \frac{M(1-\bar{v}\rho)}{RT}$$

or...

$$M = \frac{RT}{(1-\bar{v}\rho)} \left(\frac{S^\circ}{D^\circ} \right)$$

Note: effect of polydispersity depends on avg. measured for D and also on avg. measured for S .

Note: M is exact; combining the 2 transport experiments gives an exact MWT if \bar{v}_2 and ρ are known.

Corrections of S and D to 20°C; water

Note: $D^0 = \frac{RT}{Nf} = \frac{RT}{N6\pi R \eta_0}$

for spheres

i.e. $D^0 \propto \frac{T}{\eta_0}$ [Result is true for Any Shape]

Similarly, $S^0 = \frac{M(1-\rho v_2)}{Nf} = (\text{Constants}) \left(\frac{1}{\eta_0} \right)$

i.e. $S^0 \propto \frac{1}{\eta_0}$ ————— There should be a density term here, too!

Thus, often "correct" results for these 2 trivial effects;
Enables more direct comparison between molecules:

$$D_{20,w}^0 = D_{T,\eta}^0 \left(\frac{\eta}{\eta_{w,20^\circ\text{C}}} \right) \left(\frac{293.15}{T(\text{K})} \right)$$

$$S_{20,w}^0 = S_{\eta}^0 \left(\frac{\eta}{\eta_{w,20^\circ\text{C}}} \right) \left(\frac{(1-\tilde{v}^0 \rho)_{20,w}}{(1-\tilde{v}^0 \rho)} \right)$$

Note: $\eta_{w,20^\circ\text{C}} = 0.01 \text{ poise} = 1 \text{ cp}$

Concentration Dependence of Sedimentation

Generally $S \downarrow$ as $c \uparrow$

This is just the enhanced friction effect.

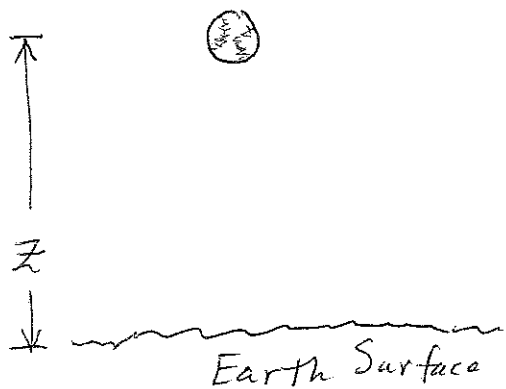
Thermodynamic effects which cause $D \uparrow$ as $C \uparrow$ are relatively weak compared to the g -fields, which is why we can overcome diffusion \neq concentrate molecules in the centrifuge.

$$S = \frac{S^0}{1 + k_f c} ; k_f \text{ is a friction-concentration coefficient for mutual processes.}$$

? Should be the same k_f one gets from diffusion.

If $S \uparrow$ with $c \uparrow$ it almost always means aggregation. See Van Holde.

In the next few paragraphs, we are going to consider how regular gravity (Earth gravity) differs from centripetal acceleration. We will use this to get an expression for pressure in the analytical ultracentrifuge, which is substantial. It may also help us understand potential energy in the AUC.



Consider a baseball at distance z above the surface of the Earth. Its potential energy U is the force acting along the distance to raise the ball. The force is mg and (here's the important

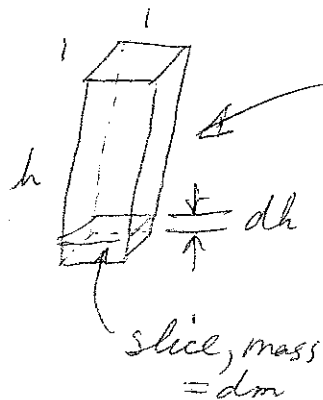
part) g is almost constant for a baseball at any reasonable height:

$$U = mgh$$

The farther the object is from the source of gravity (center of the Earth) the greater the potential energy.

A look at pressure in the centrifuge (it is a different kind of gravity).

Recall pressure in a normal Earth gravity field



column of water, $1 \times 1 \times h$ cm

slice, mass = dm

$p = \frac{\text{force}}{\text{slice area}}$

$= \frac{dm \cdot g}{\text{area}} \xrightarrow{\text{area}=1} \frac{dm \cdot g}{(1)}$

where $dm = \text{mass of slice of water}$

$$P_{\text{total}} = \sum P_{\text{slice}}$$

$$\text{or... } P_{\text{total}} = \int_0^h \frac{dm \cdot g}{(1)}$$

$$= \int_0^h \frac{\rho (1) dh g}{(1)}$$

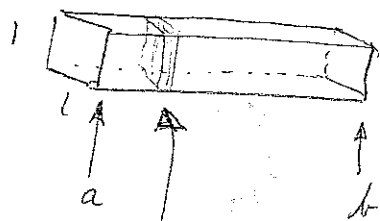
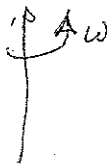
if $g = \text{constant}^*$ $\Rightarrow \rho g h$

$\rho = \text{constant}$

neither is strictly true; g varies a little bit with distance from center of Earth, and fluids are slightly compressible.

* Actually not! e.g. earth radius = $\frac{25,000 \text{ miles}}{2\pi} \approx 3800 \text{ mi}$. Your weight in Denver $\approx \frac{1}{3800}$ less than in Baton Rouge.

Now do pressure in centrifugal field at arbitrary
"depth" r



mass dm at depth r

$$P = \int_a^r dm \cdot g$$

$$= \int_a^r (\rho(r) dr) \cdot \omega^2 r$$

We can assume $\rho(r) \approx$ constant (incompressible fluid), but

we cannot ignore that g now depends on r seriously

$$P \Rightarrow \rho \int_a^r \omega^2 r = \frac{\rho \omega^2 (r^2 - a^2)}{2}$$

A typical pressure: $\rho = 1 \text{ g/cm}^3$
 $r = 6 \text{ cm}$
 $a = 5 \text{ cm}$

40000 RPM

$$\omega = \left(\frac{2\pi \text{ rad}}{\text{rotation}} \right) \left(\frac{40000 \text{ rotations}}{60 \text{ s}} \right) = 4200 \frac{\text{rad}}{\text{s}}$$

do w/o calculator

$$\frac{\left(\frac{1 \text{ g}}{\text{cm}^3} \right) (4200)^2 \frac{\text{rad}^2}{\text{s}^2}}{2} \left(9 \text{ cm}^2 \right) = 8 \times 10^7 \frac{\text{g}}{\text{cm} \cdot \text{s}^2} \times \frac{1 \text{ kg}}{1000 \text{ g}} \times \frac{100 \text{ cm}}{\text{m}} = 8 \times 10^6 \text{ Pa}$$

$\approx 80 \text{ atm}$

$= 80 \text{ atm}$

Now, we will take a more careful look at sedimentation velocity and begin to develop a view based on thermodynamic potential and how it couples to flow. This is to solve some problems in the foregoing, such as:

how should one define r_0 ?

why did we use the solution density instead of the solvent density?

The general plan will be to add a centripetal potential to the chemical potential. This new total potential is

$$\tilde{\mu} = \mu_2 + U_c$$

The centripetal potential has characteristics shown in this box

Normal Gravity (Earth)

Z axis positive as one rises from surface of Earth

$$\text{force} = -mg$$

$$U_{\text{potential}} = +mgh$$

$$= 0 @ Z=0$$

$$= \infty @ Z=\infty$$

Centripetal Acceleration

Z axis positive as one moves away from center of the rotor.

$$\text{force} = +\omega^2 r m$$

$$U_{\text{potential}} = -m \int_0^r \omega^2 r dr$$

$$= 0 \text{ at rotor center}$$

$$= -\infty \text{ at } r=\infty$$

$$= -\frac{m\omega^2 r^2}{2}$$

$$0 < r < \infty$$

It has seemed to me that U_2 should be $+\infty$ at $r=0$ and 0 at $r=\infty$. The convention chosen here is correct in the sense that we want to reduce the escaping potential (i.e. chemical potential).

$$\text{So... } \tilde{\mu} = \mu_2 - \frac{m \omega^2 r^2}{2}$$

As we saw for diffusion, a potential sets up a flux. We now have a two-term potential, so it will not be surprising if pretty soon we see a two-term flux equation. For now, let's just write a formal expression for the gradient in total potential

$$\frac{\partial \tilde{\mu}}{\partial r} = \frac{\partial \mu_2}{\partial r} - m \omega^2 r$$

The flux is given by $J = -L \frac{d\tilde{\mu}}{dr}$ where L is the mobility

$$J = -L \left(\frac{\partial \tilde{\mu}}{\partial r} \right) = -L \left(\frac{\partial \mu_2}{\partial r} \right) + L M \omega^2 r$$

We can anticipate the first term will be connected to diffusion and the second to sedimentation, but let's slog through the whole thing properly and see, beginning with the lead term:

$$\mu_2 = \mu_2(T, p, c) \quad \text{so}$$

$$d\mu_2 = \left(\frac{\partial \mu_2}{\partial T} \right)_{p,c} dT + \left(\frac{\partial \mu_2}{\partial p} \right)_{T,c} dp + \left(\frac{\partial \mu_2}{\partial c} \right)_{T,p} dc$$

$$\text{Thus } \frac{\partial \mu_2}{\partial r} = \underbrace{\left(\frac{\partial \mu_2}{\partial T}\right) \left(\frac{\partial T}{\partial r}\right)}_{\text{I}} + \underbrace{\left(\frac{\partial \mu_2}{\partial P}\right) \left(\frac{\partial P}{\partial r}\right)}_{\text{II}} + \underbrace{\left(\frac{\partial \mu_2}{\partial c}\right) \left(\frac{\partial c}{\partial r}\right)}_{\text{III}}$$

Term I: If T is uniform anywhere in the cell, this term disappears.

Term II can be split (factorized).

$$\frac{\partial \mu_2}{\partial P} \xrightarrow[dT=0]{dG = v dP - S dT} \bar{V}_2 = M \tilde{V}_2 \quad \text{IIa}$$

$\frac{\partial P}{\partial r}$ we will get from our pressure relation:

$$P = P_a + \frac{\omega^2}{2} (r^2 - a^2) \rho$$

$$\frac{\partial P}{\partial r} = \omega^2 r \rho \quad \text{IIb}$$

$$\text{Term II} = \text{IIa IIb} = M \omega^2 \rho r \tilde{V}_2$$

Term III is evaluated from $\mu_2 = \mu_2^\circ + RT \ln x_2$.
 We have done this in great detail earlier. This time,
 we permit the μ_2° term to absorb changes incurred
 as we switch concentration units, and immediately write:

$$\mu_2 = \mu_2^\circ + RT \ln c_2$$

$$\frac{\partial \mu_2}{\partial c} = \frac{RT}{c_2}$$

Putting all our terms back together gives

$$\frac{\partial \mu_2}{\partial r} = \underbrace{0}_I + \underbrace{\tilde{v}_2 M \omega^2 r \rho}_{II} + \underbrace{\frac{RT}{c} \frac{\partial c}{\partial r}}_{III}$$

Earlier we had (Eq. —)

$$J = -L \left[\frac{\partial \mu_2}{\partial r} - M \omega^2 r \right] = +L \left(M \omega^2 r - \frac{\partial \mu_2}{\partial r} \right)$$

From our discussion on diffusion (Eq. —) $L = \frac{c}{Nf}$

Thus,

$$J = \frac{M(1 - \rho \tilde{v}_2)}{Nf} \omega^2 r c - \frac{RT}{Nf} \left(\frac{\partial c}{\partial r} \right)$$

Recalling our definition of sedimentation & diffusion coefficients, this becomes

$$J = S \omega^2 r c - D \frac{\partial c}{\partial r}$$

One term for sedimentation, opposed by the spreading out term for diffusion.

Sedimentation Equilibrium

At equilibrium $\frac{\partial \tilde{\mu}}{\partial r} = 0$ and $J = 0$

$$\text{i.e. } J = L \left\{ \omega^2 r M (1 - \rho \tilde{v}_2) - \frac{RT}{c} \frac{\partial c}{\partial r} \right\} = 0$$

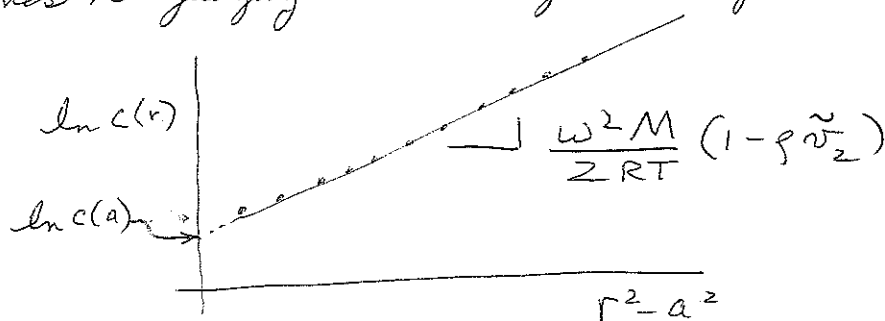
This rearranges easily to

$$\frac{1}{c} \frac{dc}{dr} = \frac{\omega^2 r M (1 - \rho \tilde{v}_2)}{RT}$$

which is easily integrated

$$\ln \frac{c(r)}{c(a)} = \frac{\omega^2 M}{2RT} (1 - \rho \tilde{v}_2) (r^2 - a^2)$$

This linearized expression appears in plotted form in older work and should always be considered in new works, too: The mind's eye has great abilities when it comes to judging the straightness of a line.



In modern literature, the exponential "growth" form of Eq— is often the only one shown (sadly):

$$c(r) = c(a) e^{\frac{\omega^2 r M (1 - \rho \tilde{v}_2)}{2RT}}$$

This nonlinear form is often fit by Excel Solver, a dedicated Levenberg Marquardt algorithm, etc.

Summary

There are 2 varieties of sedimentation

- velocity, use to get S
- equilibrium, use to get M

You must know \tilde{v}_z

You can combine S & D to get M.

D can come from DLS, FPR, NMR or even from AUC itself (modern programs can fit the profile of the set of curves).

The AUC community (especially the National Center for Centrifugation at U. Connecticut) has been especially active providing more information and useful algorithms to get quite a lot of information out of the "ancient" AUC method.

More information

See our How To Guide

See The Appendix for This Chapter.