Centrifugation Appendix

Really rough.
See Van Holde
This is just a placeholder.

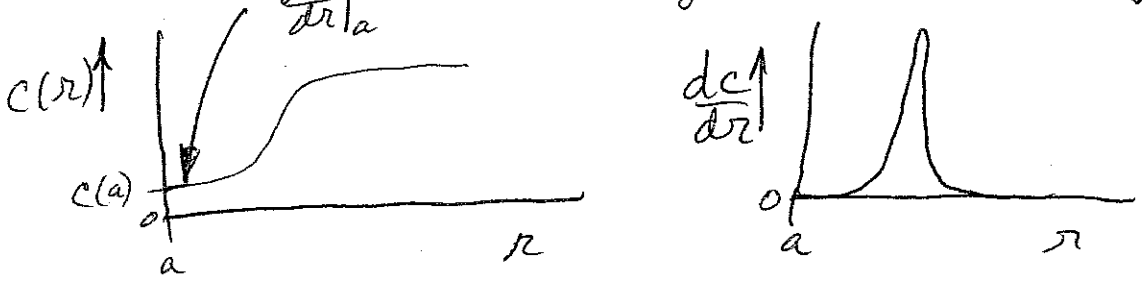
Unfortunately, we don't know ρ vs. r ! } in schlieren optics

We only know $\frac{d\rho}{dr}$ vs. r.

$$\therefore \quad \rho(r) = \underbrace{\rho(a)}_{\text{unknown (so far)}} + \int_a^r \left(\frac{d\rho}{dr}\right) dr$$

$\frac{d\rho}{dr}\Big|_a \simeq 0 \Rightarrow$ Boundary Condition

↖ This much we can get



How do we know $\rho(a)$?

2 methods

A) High Speed (or Yphantis) method.

$\Rightarrow \rho(a) = 0$

B) "Low Speed"

$\Rightarrow$ Use conservation of mass to ~~estimate~~ determine $\rho(a)$

How this works: $\Longrightarrow$

$$c(r) - c(a) = \int_a^r \left(\frac{dc}{dr}\right) dr = \text{a determinable quantity by graphical integration over } \frac{dc}{dr}.$$

define $\Rightarrow \Delta(r)$

Now, conservation of mass says:

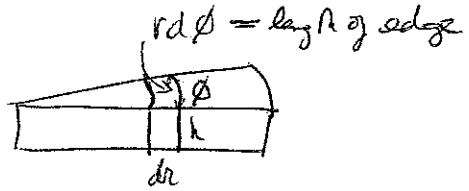
Mass of polymer before centrifugation = Mass After

$\Downarrow$

$$(c^0)(\text{Volume}) = \int_a^b c(r)\, dV(r)$$

where $dV(r)$ = the total volume element of the cell at distance r

$$dV(r) = h r \phi dr$$



$$\Rightarrow \quad c^0 \int_a^b dV(r) = \int_a^b c(r)\, dV(r)$$

$$\Rightarrow \quad c^0 \int_a^b dV(r) = \int_a^b \left[c(a) + \int_a^b \left(\frac{dc}{dr}\right) dr \right] dV(r)$$

$$\Rightarrow \quad \left[c^0 - c(a)\right]\left[h\phi \frac{b^2 - a^2}{2}\right] = \int_a^b \left[\int_a^r \frac{dc}{dr} dr\right] dV(r)$$

$$= \int_a^b \Delta(r)\, h\phi\, r\, dr$$

$$\Rightarrow \quad C^{\circ} - C(a) = \frac{2}{b^2 - a^2} \int_a^b \Delta(r)\, r\, dr$$

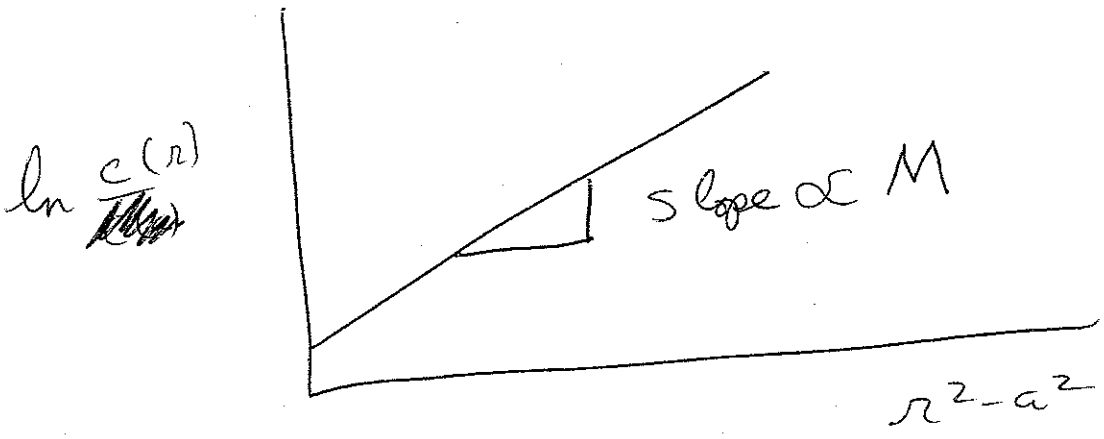
$$\Rightarrow \boxed{C(a) = C^{\circ} - \frac{2}{b^2 - a^2} \int_a^b \Delta(r)\, r\, dr}$$

$$\text{where} \quad \Delta(r) = \int_a^r \left(\frac{dc}{dr}\right) dr$$

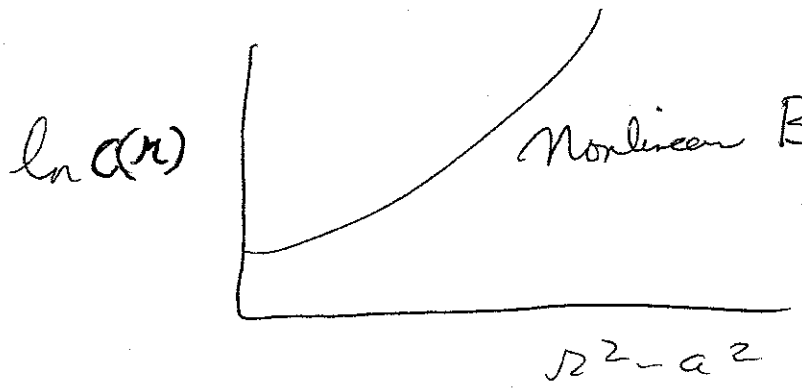
This allows you to get $C(a)$

Then $c(r) = C(a) + \Delta(r)$

You are all set for a plot of $\ln \frac{c(r)}{\rho}$ vs. $r^2 - a^2$



Sed. Equilibrium of Heterogeneous Systems



Sedimentation Equilibrium is a good technique for determining various averages of a distribution. We will only exemplify how to get M_w, but M_N and M_Z can also be obtained.

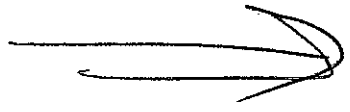
Getting M_w

We had:

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

Before we integrated like this

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



But we can also integrate like this:

$$\int_a^b \left(\frac{dc}{dr}\right) dr = \frac{\omega^2 M (1-\rho\bar{v}_2)}{RT} \int_a^b c(r)\, r\, dr$$

On the left we just have $\int_a^b dc = c(b) - c(a)$

Now we do know how to calculate $c(r)$ — see calculation of $c(a)$; then $c(r) = c(a) + \Delta(r)$.

However, the $\int_a^b c(r)\, r\, dr$ has a neat form so we need not actually plug in $c(r)$ and tough it out graphically.

In fact we can actually deal with heterogeneity in a neat closed form:

$$\sum_i \int_a^b \left(\frac{dc_i}{dr}\right) dr = \frac{\omega^2 (1-\rho\bar{v}_2)}{RT} \sum_i M_i \int_a^b c_i(r)\, r\, dr \longrightarrow$$

$$\sum_i \int_a^b dc_i = \frac{\omega^2(1-\rho\bar{v}_2)}{RT} \sum_i^1 M_i \int_a^b c_i(r)\, r\, dr$$

But, as long as there is no exchange or pressure induced aggregation we may write from conservation of mass:

total mass of i-mer $\Leftrightarrow$ same

$$h\phi \int_a^b c_i(r)\, r\, dr = c_i^{\circ} V$$

$$= c_i^{\circ} \int_a^b dV(r)$$

$$= c_i^{\circ} \int_a^b h\phi\, r\, dr$$

$$\Rightarrow \int_a^b c_i(r)\, r\, dr = c_i^{\circ} \frac{b^2-a^2}{2}$$

$$\Rightarrow \sum_i \int_a^b dc_i = \frac{\omega^2(1-\rho\bar{v}_2)}{RT} \left(\frac{b^2-a^2}{2}\right) \sum_i^1 M_i c_i^{\circ}$$

$$\Downarrow$$

$$c(b) - c(a) =$$

Now divide both sides by $C^\circ = \sum_i C_i^\circ$

$$\Rightarrow \quad \frac{c(b)-c(a)}{C^\circ} = \frac{\omega^2(1-\rho\bar{v}_2)}{2RT}\left(b^2-a^2\right)\frac{\sum M_i C_i^\circ}{\sum C_i^\circ}$$

But $$\frac{\sum M_i C_i}{\sum C_i} = \frac{\sum g_i M_i}{\sum g_i} = M_w$$

$\therefore$ M_w can be obtained as:

$$\left[\frac{c(b)-c(a)}{C^\circ}\right]\frac{2RT}{\omega^2(1-\rho\bar{v}_2)(b^2-a^2)} = M_w$$

Sedimentation, Contd

C) Density Gradient Methods

It must first be appreciated that particles need not go to bottom of cell. Indeed, particles lighter than the solvent will rise. Particles with density equal to solvent will neither rise nor fall.

[Note: This is a major limitation in light scattering clarification schemes reliant only on centrifuge]

we can see this from:

$$S = \frac{v}{\omega^2 r} = \frac{M(1 - \rho\bar{v}_2)}{RT}$$

when $\bar{v}_2 = \frac{1}{\rho}$ then $S = \frac{v}{\omega^2 r} \Rightarrow 0$

Thus, if the solution density ρ can be adjusted in some innocuous fashion you can stop sedimentation. If the solution density can be made to vary over the length of the centrifuge cell you could actually separate molecules by their partial specific volumes.

Compared to non-gradient methods, the resolution is increased [i.e., in absence of gradient, molecules can be "pelletized" larger ones first, then smaller ones in a less compact "pellet"].

As for regular sedimentation, density gradient has 2 versions:

1) Velocity Density Gradient Sed.

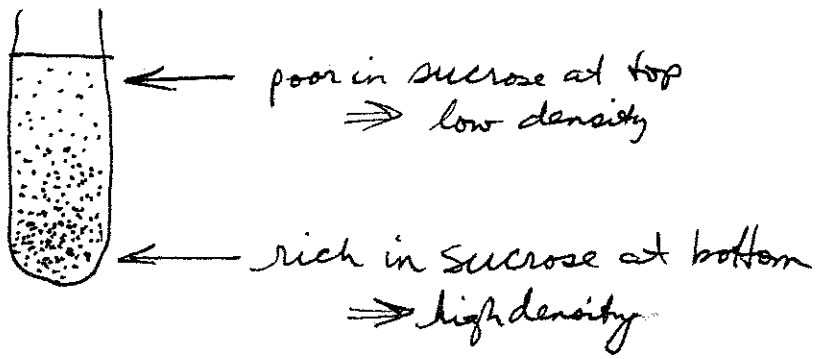
2) Equilibrium Density Gradient Sed.

Take each one at a time.

1) Velocity Density Gradient

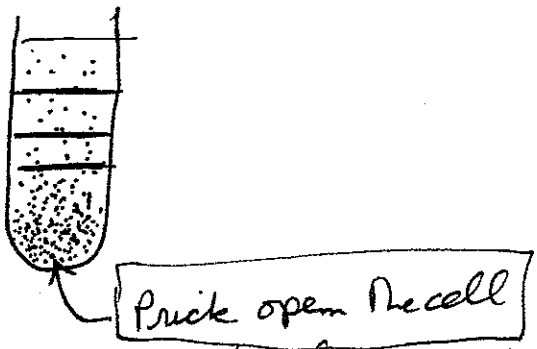
A) Use a gradient former to distribute sucrose slowly into a cell:

Note: gradient is only temporary in this experiment.



B) Then layer on a protein mixture. Two proteins with same molecular weight will <u>not</u> move at quite the same speed unless [by most unfortunate circumstance] they have same $\bar{v}_2$. In fact, this is true also of regular sedimentation; however, in density gradient one protein might actually come to a nearly complete stop [not quite because the gradient itself will be changing in this experiment].

C) After a time, the macromolecules will have banded:



D) Extract molecules layer-by-layer and record position of bands by dumping, say, every 0.5ml, into different test tubes.

E) Can calculate sedimentation coefficients by calculations that must take into consideration density gradient, etc.

Or.... can use several standard substances to * calibrate the experiment. Note: in this way an estimate for S can be had even without an analytical ultracentrifuge.

Mainly, the technique is good for isolation schemes.

2) Equilibrium Density Gradient Sedimentation

Just mix macromolecules (e.g. proteins, viruses, etc.) with a heavy salt solution, such as CsCl. Under influence of rotor the CsCl <u>and</u> macromolecules will want to achieve an equilibrium concentration profile. However, the CsCl profile will <u>also</u> involve a density gradient. Thus, the protein (or whatever) will stop at some point if conc. of salt is high enough to modify ρ sufficiently at some point in the cell.

* <u>How can we modify the sed. equilibrium theory to take the density gradient into account?</u>

There is nothing inapplicable about our basic sed. eq. equation:

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

<u>BUT</u> $\rho = \rho(r)$ now. { But was ρ assumed constant before?

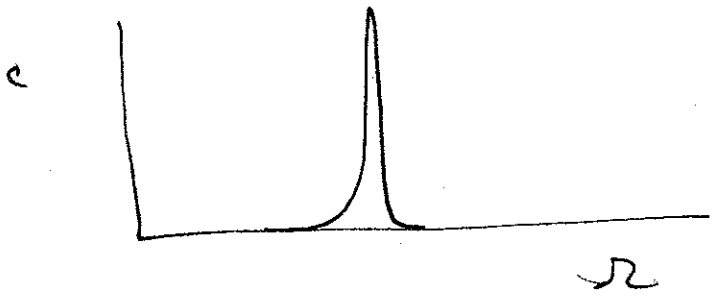
Now $(r-r_0)\,dr \equiv \frac{1}{2}\,d(r-r_0)^2$

$$\therefore \quad \frac{dc}{c} \simeq -\frac{\omega^2 r_0 M}{2RT}\,\frac{d\rho}{dr}\,d(r-r_0)^2$$

$$\Rightarrow \quad \ln c \simeq -\frac{\omega^2 r_0 M}{2RT}\,\frac{d\rho}{dr}\,(r-r_0)^2$$

or ... $$\boxed{c \simeq e^{-\left[\frac{\omega^2 r_0 M}{2RT}\,\frac{d\rho}{dr}\right](r-r_0)^2}}$$

GAUSSIAN ABOUT r_0



* This is a way to measure M!

(But not usually used this way)

The main value of equilibrium density gradient sedimentation is in identifying whether one or more species are present.

See Morawetz p. 211 onward for a description of the role density gradient sedimentation played in determining the DNA replication scheme.
(or.... Meselson, et. al. (1957), PNAS, US, 43, 581.)

Suppose we are interested in some region centered about a point r_0 where $\rho(r_0) = \frac{1}{\bar{v}_2}$. The macromolecule will be centered about this point, but the peak is not infinitely sharp. The ~~density~~ concentration of molecule in the vicinity of r_0 is given by the above equation where, however,

$$\rho(r) = \rho(r_0) + (r - r_0)\left(\frac{\partial \rho}{\partial r}\right)_{r_0}$$

$$\rho(r) = \frac{1}{\bar{v}_2} + (r - r_0)\frac{d\rho}{dr} \quad \left\{\text{valid for region } r \simeq r_0\right\}$$

$$\therefore \quad \frac{1}{c}\frac{dc}{dr} = \frac{\omega^2 r M}{RT}\left\{1 - \bar{v}_2\left[\frac{1}{\bar{v}_2} + (r - r_0)\frac{d\rho}{dr}\right]\right\}$$

$$\frac{1}{c}\frac{dc}{dr} = -\frac{\omega^2 r M}{RT}\left\{(r - r_0)\frac{d\rho}{dr}\right\}$$

$$\Rightarrow \quad \frac{dc}{c} = -\frac{\omega^2 r M}{RT}\frac{d\rho}{dr}(r - r_0)\,dr$$

$r \simeq r_0 \Rightarrow$ (valid for all r of interest)

$$\frac{dc}{c} \simeq -\frac{\omega^2 r_0 M}{RT}\frac{d\rho}{dr}(r - r_0)\,dr$$