Sedimentation

Previously we defined a general equation for flow as

$$J = \sum_{i=1}^{N} L_i X_i$$

(1)

where the contributing forces can be external and internal (diffusion). In order to derive the equations for diffusion, we assumed that the external forces were zero. For electrophoresis, the external force was an electric field.

What if the external force is gravity?

Can we use gravity to determine the molecular weight of biomolecules?
The force does not depend on:    - charge
    - shape
    - chemical composition

\[ F = mg \]

The boundary moves with the same velocity as that of the sedimenting particle (on average).
This technique cannot be applied to biomolecules because of their molecular weight ($M_2$).

Reason:

rate of sedimentation $\propto M_2$

But recall equation 2.7 in the first set of notes:

$$J_{x,2} = - (L_{22} RT/M_2 c_2) \left( \frac{\partial c_2}{\partial x} \right)$$

or

$$J_{x,2} = - D \left( \frac{\partial c_2}{\partial x} \right)$$

For the molecular masses that we are dealing with, this implies that diffusion dominates. Therefore

No sharp boundary
No sedimentation
Gravity is too weak – we need another force.

- Linear force \( F = ma \) Difficult to sustain for extended periods of time

- Centrifugal force \( F = m\omega^2r \)
  
  e.g. \( m = 1g \)
  \( r = 10\text{cm} \)
  \( \omega = 60,000 \text{ rpm} = 1000 \text{ s}^{-1} \)
  
  \[
  F = 0.001 \text{ kg} \times 0.1\text{ m} \times (1000 \text{ s}^{-1})^2 \\
  = 1 \times 10^2 \text{ N}
  \]

  Gravitational force = \( 0.001 \text{ kg} \times 9.8 \text{ m.s}^{-2} \)
  \[
  = 9.8 \times 10^{-3} \text{ N}
  \]

  This means that the radial force is a factor 10000 times larger than gravity!

But diffusion is comparatively still significant – must take it into account!!!!

see: http://www.citycollegiate.com/gravityXb.htm
Let us consider the case where only the sedimentational force (centrifugal force) and a diffusional force are present. We will see later how buoyancy \( (F_b) \) and viscous drag \( (F_d) \) are taken into account.

Let us consider a slice at position \( x \).

As we saw in the first set of lecture notes, we can write

\[
J_{x,2} = L_{22}F_{x,2} + L_{22}^{D}(\partial^{A}\mu_2/\partial x)
\]

for a binary system (i.e. solvent (1) and solute (2)).

ref: http://www.ultrascan.uthscsa.edu
Putting in the equation for the centrifugal force (per gram – so we divide by m) yields

\[ J_{x,2} = L_{22} \omega^2 x - L_{22}^D(\partial \mu^\Lambda_{2}/\partial x). \]

Let us now define the *sedimentation coefficient*, \( s \), which is given by

\[ s = \frac{v}{\omega^2 x} = \frac{1}{\omega^2 x} \frac{dx}{dt} \quad \text{[units = seconds]} \]

where \( v \) is the constant drift velocity – i.e. the velocity of the particle if there is no diffusion. The sedimentation coefficient is a function of the size and shape of the sedimenting particle.

At the boundary \( x \), we have a concentration \( c_2 \) of solute. These solute particles are moving with a velocity \( v = s \omega^2 x \), so the flux is

\[ J_2 = c_2 s \omega^2 x - L_{22}(\partial \mu^\Lambda_{2}/\partial x) \]

where the subscript \( x \) has been dropped for simplicity.
Finally, putting the flux due to diffusion into the equation (Fick’s first law), we get that the flux at boundary $x$ is

$$J_2 = c_2 s \omega^2 x - D (\partial c_2 / \partial x). \quad (2)$$

Now we want to calculate the change in mass of solute ($m_2$) over time in a volume element, between a boundary at $x$ and one at $x+dx$. Recall that we did the same when deriving Fick’s second law, i.e.

$$\frac{dm_2}{dt} = J_2 A(x) - J_2 A(x+dx)$$

Previously, we had unit area – now the area is the area of a surface at radius $x$, which is $a x \phi$. 
Fick’s Second Law

\[ J_{x2} = \frac{Dc_2(x) - Dc_2(x + dx)}{dx} = -D \frac{\partial c_2}{\partial x} \]

Let us consider the change in weight of the solute in the volume bounded by \( x \) and \( x+dx \).

The flux across the surface at \( x \) from left to right is \( J_2(x) \) and the flux across \( x+dx \) from right to left is \( -J_2(x+dx) \).

The net rate of accumulation of solute mass, \( m_v \), is:

\[ \frac{dm_v}{dt} = J_2(x) - J_2(x+dx) \quad (2.8) \]

i.e. flux of material going into slab of width \( dx \) – flux of material going out.
As before, we want this in terms of concentration, i.e.

\[
dc_2/dt = (1/V)(dm_2/dt) = (1/A(x)dx)(dm_2/dt)
\]

\[
= \frac{1}{ax\phi} \frac{dm_2}{dx} \frac{dx}{dt}
\]

so that

\[
dc_2/dt = \frac{1}{ax\phi} [J_2A(x) - J_2A(x+dx)]
\]

\[
= \frac{-1}{ax\phi} d(J_2A)
\]

\[
= \frac{-1}{ax\phi} d(J_2(ax\phi))
\]

Finally

\[
dc_2/dt = \frac{-1}{x} d(xJ_2)
\]

\[
(3)
\]
Combining equations 2 and 3 results in

\[
\frac{dc_2}{dt} = \frac{1}{x} \frac{d}{dx} \left[ x[D(\frac{\partial c_2}{\partial x}) - c_2 s \omega^2 x] \right]
\]  

(4)

This is the Lamm equation (!) and describes mass transport of a two-component system in an ultracentrifuge.

If we consider that the solute is present in low concentrations, then we can assume that \(D\) and \(s\) are independent of concentration and we can write equation 4 as

\[
\frac{dc_2}{dt} = D \left[ \frac{d^2c_2}{dx^2} + \frac{1}{x} \frac{dc_2}{dx} \right] - s \omega^2 \left[ x \frac{dc_2}{dx} + 2c_2 \right]
\]

(5)

• if no radial force, then only the first term remains – Fick’s second law
The Lamm equation can be solved analytically only for special cases. For example, if $s$ is a constant and there is no diffusion, i.e. when...
This can occur because of an experimental observation made by van Holde et al.:

Sedimentation is a transport process proportional to the first power of time, while Diffusion is a transport process which is proportional to the square-root power of time.

At infinity transport due to diffusion will be negligible compared to transport due to sedimentation - i.e., all components will separate out if the rotorspeed is fast enough.
In order to get $c_2(x,t)$ in this case, we need to solve

$$\frac{dc_2}{dt} = -s\omega^2 \left[ x \frac{dc_2}{dx} + 2c_2 \right]$$

We can use our trick of the Fourier transform again and the result is

$$c_2(x,t) = 0 \quad \text{if} \quad x_m < x < x$$
$$c_2(x,t) = c_0 \exp\{-2s\omega^2 t\} \quad \text{if} \quad x < x < x_b$$

where $x = x_m \exp\{s\omega^2 t\}$ describes the boundary condition and the other parameters are as defined in the figure.
Paradox?

Concentration is independent of $x$, i.e. $c_2(x,t)$ does not depend on $x$ (?)

but at the same time

Concentration in region decreases exponentially with time?

$$c_2(x,t) = c_0 \exp\{-2s\omega^2t\} \quad \text{if} \quad \bar{x} < x < x_b$$
Radial dilution

\[ c_2(x,t) = c_0 \exp\{-2 s \omega^2 t\} \]
OD

Meniscus

1-component

Plateau Concentration

$\text{t}_1$

$\text{t}_2$

x

CHEM 305
To show that the plateau concentration is constant with respect to x, we need to show that all zones are subject to the same radial dilution. For this we can show that the relative volume change of a zone is independent of position.

\[ V(t_1) = A \left[ x_2(t_1) - x_1(t_1) \right] \]

Recall \( A(x_1) = ax_1\phi \)
and \( A(x_2) = ax_2\phi \)

such that \( A = \frac{1}{2} [A(x_1) + A(x_2)] \)
\[ = \frac{1}{2} [ax_1\phi + ax_2\phi] \]

Thus

\[ V(t_1) = \frac{1}{2}a\phi \left\{ [x_2(t_1) - x_1(t_1)][x_1(t_1) + x_2(t_1)] \right\} \]
\[ = \frac{1}{2}a\phi \left\{ x_2^2(t_1) - x_1^2(t_1) \right\} \]
To calculate the volume at time $t_2$, we need to determine $x_1(t_2)$ and $x_2(t_2)$. We know that

$$v_1 = \frac{dx_1}{dt} = \omega^2 s x_1$$

which is a first order differential equation with a solution of

$$x_1(t_2) = x_1(t_1) \exp\{\omega^2 s(t_2-t_1)\}.$$

Similarly we can write

$$x_2(t_2) = x_2(t_1) \exp\{\omega^2 s(t_2-t_1)\}.$$

This allows us to determine

$$V(t_2) = \frac{1}{2} a \phi \{x_2^2(t_2)-x_1^2(t_2)\}$$

$$= \frac{1}{2} a \phi \{x_2^2(t_1)\exp\{2\omega^2 s(t_2-t_1)\} - x_1^2(t_1) \exp\{2\omega^2 s(t_2-t_1)\}\}$$

$$= \frac{1}{2} a \phi [x_2^2(t_1) - x_1^2(t_1)] \exp\{2\omega^2 s(t_2-t_1)\}$$

$$= V(t_1) \exp\{2\omega^2 s(t_2-t_1)\}$$

Finally

$$V(t_2)/V(t_1) = \exp\{2\omega^2 s(t_2-t_1)\}$$

i.e. is independent of position.
Solving the Lamm equation – real cases

This has only been done for certain cases. Regardless, all of the results show that:

Diffusion only near the boundary – why?
Because even in real cases a plateau will exist (by this we make the assumption that $s$ is a constant) and since the solute concentration will be independent of $x$ at the plateau, we can simplify equation 5

$$\frac{dc_2}{dt} = D \left[ \frac{d^2c_2}{dx^2} + \frac{1}{x} \frac{dc_2}{dx} \right] - s\omega^2 \left[ x \frac{dc_2}{dx} + 2c_2 \right]$$  \hspace{1cm} (5)$$

to

$$\frac{dc_2^P}{dt} = -2s\omega^2 c_2^P$$  \hspace{1cm} (6)$$

where the superscript $P$ refers to the plateau. Equation 6 can be solved to give

$$c_2^P(t) = c_0 \exp\{-2s\omega^2 t\}$$
For more information on solving the Lamm equation, see
http://www.analyticalultracentrifugation.com/LammEqSolutions.htm
We will now derive the relationship between $s$ and molecular properties, i.e. molecular weight, friction coefficient, etc.

Forces acting on the particle:

\[
F_c = \omega^2 mx \quad \text{centrifugal}
\]

\[
F_b = \omega^2 m_s x \quad \text{buoyancy}
\]

\[
F_d = f v \quad \text{viscous drag}
\]

Firstly, we need to consider the effect of hydration on the particle, i.e. $m$ will have to be replaced by $m_h$ (hydrated mass), where

\[
m_h = \frac{M (1 + \delta_1)}{N_A}
\]

$M$ = anhydrous molecular weight (g.mol$^{-1}$); $\delta_1$ = hydration (in g/g)
Therefore,

\[ F_c = \frac{\omega^2 x M (1 + \delta_1)}{N_A} \]  

centrifugal.

For the viscous drag or frictional force, we can replace \( v \) by \( s \omega^2 x \) to yield

\[ F_d = f s \omega^2 x \]  

viscous drag.

Finally, we can rewrite the mass of the displaced solvent as the product of the hydrated volume of the particle times the density of the solvent. Thus,

\[ F_b = \omega^2 x V_h \rho \]  

buoyancy

which can be rewritten as

\[ F_b = \frac{\omega^2 x \rho M (\bar{V}_2 + \delta_1 \bar{V}_1)}{N_A} \]

with \( \bar{V}_1 \) and \( \bar{V}_2 \) being the partial specific volumes of the solvent and solute respectively.
In the cell,

\[ F_c - F_b - F_d = 0 \]

in the steady-state, so putting in the definitions, we get that

\[
\frac{\omega^2 x M (1 + \delta_1)}{N_A} - f s \omega^2 x - \frac{\omega^2 x \rho M (\bar{V}_2 + \delta_1 \bar{V}_1)}{N_A} = 0
\]

\[
\frac{M [1 + \delta_1 - \rho \bar{V}_2 - \rho \delta_1 \bar{V}_1]}{N_A} = f s
\]

In a dilute solution, \( \rho^{-1} \sim \bar{V}_1 \) so the second and fourth terms cancel to give

\[
s = \frac{M [1 - \rho \bar{V}_2]}{N_A f}
\]

This derivation is not rigorous because of the approximation just made. A more rigorous derivation can be made starting from the equation of flux for a two-component system.
Given the equation for the sedimentation coefficient and the equation for the diffusion coefficient, we can now put these two together to get

\[ s = \frac{M [1 - \rho \bar{V}_2]}{N_A f} \quad \text{and} \quad D = \frac{kT = \frac{RT}{f N_A f}}{f} \]

Svedberg equation

\[ M = \frac{sRT}{D [1 - \rho \bar{V}_2]} \]

In combining the two equations above, we have assumed that the friction coefficient under sedimentation is the same as the one for diffusion. One could imagine that this may not always be the case – i.e. under the high forces in the sedimentation experiment, the particle may be distorted

\[ f_s = 6\pi \eta a' \]

will not be equal to a in \( f_D \)!

This however is rare. The Svedberg equation holds for most cases.
From the Svedberg equation, we can obtain the molecular weight, as long as we know the sedimentation coefficient, $s$, the diffusion coefficient, $D$, and the partial specific volume, $\bar{V}_2$, i.e. as long as they are measured under the same conditions. In practice the sedimentation coefficient and the diffusion coefficient are corrected:

$$M = \frac{sRT}{D [1 - \rho \bar{V}_2]}$$

$$D_{20,w} = \frac{293}{T} \frac{\eta_{w,T}}{\eta_{w,20}} \frac{\eta_{sol,T}}{\eta_{w,T}}$$

$$s_{20,w} = \frac{1 - 0.9982(\bar{V}_2)_{w,20}}{s} \frac{\eta_{w,T}}{1 - \rho \bar{V}_2} \frac{\eta_{sol,T}}{\eta_{w,20}} \frac{\eta_{w,T}}{\eta_{w,T}}$$

where $\eta_{w,T}$ is the viscosity of pure water at temperature $T$, $\eta_{w,20}$ is the viscosity of pure water at 20°C, $\eta_{sol,T}$ is the viscosity of the actual solution used at temperature $T$. 0.9982 g.cm$^{-3}$ is the density of water at 20°C.
Since both sedimentation and diffusion depend on the frictional coefficient and since the frictional coefficient depends on the particle shape, both $s$ and $D$ can be used to tell us something about the shape of the biomolecule that we are observing in solution. How?

\[ f = 6\pi \eta a \quad \text{for a sphere} \]

but we can also write

\[ f = 6\pi \eta r_h F \quad \text{for an ellipsoid} \]

where $r_h$ is the hydrated radius of the equivalent sphere and $F$ is the Perrin or shape factor.
We can obtain an equation for the radius of hydration from the hydrated volume. Recall, when we looked at the buoyancy force, we wrote

$$ V_h = \frac{M (V_2 + \delta V_1)}{N_A} $$

We know that the volume of a sphere is given by

$$ V_h = \frac{4}{3} \pi r_h^3 $$

therefore,

$$ r_h = \left[ \frac{3}{4\pi} \frac{M (V_2 + \delta V_1)}{N_A} \right]^{1/3} $$

Finally, we can write

$$ f = 6\pi \eta F \left[ \frac{3}{4\pi} \frac{M (V_2 + \delta V_1)}{N_A} \right]^{1/3} $$
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<th>Axial ratio</th>
<th>F (Prolate)</th>
<th>F (Oblate)</th>
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<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>2</td>
<td>1.044</td>
<td>1.042</td>
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<tr>
<td>3</td>
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<td>4</td>
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<td>5</td>
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<tr>
<td>6</td>
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<tr>
<td>8</td>
<td>1.433</td>
<td>1.374</td>
</tr>
<tr>
<td>10</td>
<td>1.543</td>
<td>1.458</td>
</tr>
</tbody>
</table>
Recall from assignment 2, that the minimum friction coefficient that an anhydrous particle can have is

\[ f_{\text{min}} = 6\pi \eta a_0 \]

with

\[ a_0 = \left( \frac{3M\bar{V}_2}{4\pi N_A} \right)^{1/3} \]

So calculating the ratio between \( f \) and \( f_{\text{min}} \) will yield

\[ \frac{f}{f_{\text{min}}} = \left[ \frac{(\bar{V}_2 + \delta_1 \bar{V}_1)}{\bar{V}_2} \right]^{1/3} F \]

where we still have two unknowns, \( F \) and \( \delta_1 \) (the hydration). These two values be obtained when we consider limiting cases.
Case 1: if all excess friction is due to hydration

\[ F = 1 \]

\[ \frac{f}{f_{min}} = \left( \frac{V_2 + \delta_1^{max}V_1}{V_2} \right)^{1/3} \]

or

\[ \delta_1^{max} = \frac{V_2}{V_1} \left[ \left( \frac{f}{f_{min}} \right)^3 - 1 \right] \]

Case 2: if there is no hydration

\[ \delta_1 = 0 \]

\[ \frac{f}{f_{min}} = \left( \frac{V_2 + \delta_1^{max}V_1}{V_2} \right)^{1/3} F = 1 \]
Determining oligomerization states from sedimentation data

Recall that

\[ s = \frac{M [1 - \rho V_2]}{N_A f} \]

If both the tetramer and the monomer have the same partial specific volume (how can that be?), then we can write

\[ \frac{s_t}{s_m} = \frac{M_t f_m}{M_m f_t} = 4 \frac{f_m}{f_t} \]
The parameter $f_m/f_t$ will depend on the geometry of the tetramer, i.e.

\[
\begin{align*}
\frac{f}{f_m} &= 1.92 & 1.70 & 1.60 \\
\frac{s}{s_m} &= 2.08 & 2.35 & 2.50
\end{align*}
\]
Therefore if $s_m = 1.772 \text{ S} \quad (1 \text{ S} = 10^{-13} \text{ seconds})$,

\[
\begin{array}{ccc}
    s_t &=& 3.70 \text{ S} \\
    4.16 \text{ S} & 4.43 \text{ S} \\
\end{array}
\]

For instance, $s_{20,w}$ for hemoglobin is 4.31 S. Since we know it forms tetramers, we can predict the geometry to be tetrahedral from the calculation above.
Zonal sedimentation

Up to this point, we have only considered a binary system (1 solvent + 1 solute). Could we use ultracentrifugation to separate a multicomponent system?

- centrifugation
- fractionation
Sedimentation results can either be plotted as $c_2$ as a function of $x$

or

$\frac{\partial c_2}{\partial x}$

(For instance, when using Schlieren optics, you measure the gradient).
For separating the red from the green, we expect to see:

- homogeneous solution
- solution with gradient (e.g. sucrose, salt solutions)
In the previous sections, we have considered the case where the macromolecule being centrifuged packs at the bottom of the cell or, at the very least, forms a boundary. What if the ultracentrifugation speeds are still insufficient to sediment our particles?
Some net transport will occur.

No net boundary is formed because diffusion is too strong.
At equilibrium, the flux $J_2$ will be zero everywhere in the sample. Thus we can write:

$$J_2 = \frac{M_2 c_2}{N_A f} \left[ \omega^2 x - \frac{\partial \mu_2}{\partial x} \right]$$

$$= \frac{M_2 c_2}{N_A f} \left[ \omega^2 x - \bar{V}_2 \rho \omega^2 x - \frac{(RT)}{M_2 c_2} \frac{\partial c_2}{\partial x} \left\{ 1 + \frac{\partial \ln \gamma_2}{\partial \ln c_2} \right\} \right]$$

$$= \frac{M_2 (1 - \bar{V}_2 \rho)}{N_A f} c_2 \omega^2 x - kT \frac{\partial c_2}{\partial x} \left\{ 1 + \frac{\partial \ln \gamma_2}{\partial \ln c_2} \right\} = 0$$

Therefore,

$$M_2 \left\{ 1 + \frac{\partial \ln \gamma_2}{\partial \ln c_2} \right\}^{-1} = \frac{RT}{(1 - \bar{V}_2 \rho) \omega^2 c_2 x} \frac{\partial c_2}{\partial x}$$

i.e. the frictional coefficient drops – does not play a role at equilibrium.
At low concentrations, we can solve the equation above. The resulting function is

$$c_2(x) = c_2(x_0) \exp \left\{ [M_2 (1 - \bar{V}_2 \rho) \omega^2 / 2RT] (x^2 - x_0^2) \right\}$$
Equilibrium sedimentation of a monomer-dimer system

\[ 2 \times \text{monomer} + \text{dimer} \]

We can characterize the monomer-dimer equilibrium by the equilibrium constant

\[ k = \frac{c_d}{c_m^2} \]

Will this equilibrium be maintained throughout the ultracentrifugation cell?
Answer: yes!

\[ c_d(x) = c_d(x_0) \exp \{ [2M_m(1-\bar{V}_m\rho) \omega^2/2RT] (x^2-x_0^2) \} \]

\[ c_m(x) = c_m(x_0) \exp \{ [M_m(1-\bar{V}_m\rho) \omega^2/2RT] (x^2-x_0^2) \} \]

Therefore

\[ \frac{c_d(x)}{c_m(x)} = \frac{c_d(x_0)}{c_m(x_0)} \frac{c_m(x)}{c_m(x_0)} \]

\[ \frac{c_d(x)}{c_m^2(x)} = \frac{c_d(x_0)}{c_m^2(x_0)} \]

If load a centrifuge cell with a monomer-dimer mixture at equilibrium, the equilibrium distribution will be preserved at any speed and at all positions in the cell.
Because this equilibrium between monomer and dimer exists, we will not be able to observe either of the two separately. In other words, we will observe a total weight concentration $c_T$ and an average molecular weight $\overline{M}_w$. These are defined as:

$$c_T = c_m + c_d = c_m + k c_m^2$$

$$\overline{M}_w = \frac{\sum c_i M_i}{\sum c_i} = \frac{M_m c_m + M_d c_d}{c_T}$$

$$= \frac{M_m c_m (1 + 2 c_m k)}{c_T}$$

Since we will not be able to observe $c_m$ or $c_d$ experimentally, we need to redefine the equilibrium constant $k$ in terms of the observables. To do this, it is easiest if we take the derivative of the first equation, i.e.

$$\frac{dc_T}{dc_m} = 1 + 2 k c_m$$
We can rewrite this as

\[
\frac{dc_m}{dc_T} = \frac{1}{1 + 2 k c_m} = \frac{c_m M_m}{c_T M_w} = w_m \frac{M_m}{M_w}
\]

where \( w_m \) is the weight fraction of the monomer. We can now take the derivative of \( w_m \) with respect to \( c_T \) to yield:

\[
\frac{dw_m}{dc_T} = \frac{d}{dc_T} \left( \frac{c_m}{c_T} \right)
\]

\[
= c_m \frac{d(c_T^{-1})}{dc_T} + 1 \frac{dc_m}{c_T dc_T}
\]

\[
= -c_m + 1 \frac{dc_m}{c_T^2 dc_T}
\]

This allows us to write

\[
\frac{d(\ln w_m)}{d(\ln c_T)} = \left( \frac{M_m}{M_w} \right)^{-1}
\]
which can be integrated between two positions, \( x \) and \( x_0 \), to give

\[
\ln \left[ \frac{w_m(x)}{w_m(x_0)} \right] = \int_{c_T(x_0)}^{c_T(x)} \left[ \left( \frac{M_m}{M_w} \right)^{-1} \right] \frac{1}{c_T} dc_T
\]

At low concentrations, \( c_T(x_0) \sim 0 \) and \( w_m(x_0) = 1 \). So the equation above becomes:

\[
\ln [w_m(x)] = \int_{0}^{c_T(x)} \left[ \left( \frac{M_m}{M_w} \right)^{-1} \right] \frac{1}{c_T} dc_T
\]

Integral of a plot of this as a function of \( c_T \) will yield a value for \( w_m(x) \)

Finally, this allows us to calculate the equilibrium constant as

\[
k = \frac{(1 - w_m)}{w_m^2 c_T}
\]
To obtain the equilibrium constant as

\[ k = \frac{(1 - w_m)}{w_m^2 c_T} \]

we start from

\[ k = \frac{c_d}{c_m^2} \]

We know that \( c_T = c_d + c_m \), thus

\[ k = \frac{c_T - c_m}{c_m^2} \]

We have also defined \( w_m = \frac{c_m}{c_T} \). Dividing the top and bottom of the equation above by \( c_T \),

\[ k = 1 - \frac{(c_m/c_T)}{c_m^2/c_T} = 1 - \frac{w_m}{w_m^2 c_T} \]