Sedimentation
What is it?

- Technique used to settle particles in solution against the barrier using centrifugal acceleration
Two Types of Centrifuges

Analytical

Non Analytical
History

- 1913 - Dumansky proposed the use of ultracentrifugation to determine dimensions of particles
- 1923 - The first centrifuge is constructed by Svedberg and Nichols.
- 1929 - Lamm deduces a general equation that describes the movement within the ultracentrifuge field
- 1940s - Spinco Model E centrifuge becomes commercially available
- 1950s - Sedimentation becomes a widely used method.
- 1960s - First scanning photoelectric absorption optical system developed
- 1980s - Sedimentation loses popularity due to data treatment being slow and the creation of gel electrophoresis and chromatography
- 1990s - Newer versions of centrifuge gains popularity again
- 2000s - It is now recognized as a necessary technique for most laboratories
How Does It Work?

- Everything has a sedimentation coefficient
  - Ratio of measured velocity of the particle to its centrifugal acceleration
  - Can be calculated from the forces acting on a particle in the cell

![Diagram]

- Buoyant force: \( F_b = -m_0 \omega^2 r \)
- Frictional force: \( F_d = -fu \)

\( \omega \) - angular velocity
\( r \) - distance from the centre of rotation
\( u \) - velocity of molecule
Sedimentation Coefficient

- Usually, determine mass by observing movement of particles due to known forced
  - Use Gravity normally
    - For molecules, force is too small
- Avoid this by increasing PE by putting particles in a cell rotating at a high speed
- Get Sedimentation Coefficient

\[
s = \frac{v}{\omega^2 r} = \frac{M(1 - \bar{\nu} \rho_{sol})}{N_A \bar{\nu} f}
\]

- \( M \) = molecular weight \((m \times N_{AV})\)
- \( s \) = svedberg coefficient
How does it work?

- Rotors must be capable of withstanding large gravitational stress.
- Two types of cells: double sector (accounts for absorbing components in solvent) and boundary forming (allows for layering of solvent over the solution).
- Optical detection systems: Rayleigh optical system (displays boundaries in terms of refractive index as a function of radius), Schlieren optical system (refractive index gradient as a function of radius), and absorption optical system (optical density as a function of radius).
- Data acquisition is computer automated due to the Beckman Instruments Optima XL analytical centrifuge.
Deriving the Lamm Equation

- Describes the transport process in the ultracentrifuge
- Fick’s first equation:
  - $J_x = -D [dC/dx]$
- If all particles in the cell drift in a +x direction at speed, $u$:
  - $J_x = -D [dC/dx] + uC(x)$
  - $u = s \omega^2 x$
  - Therefore, $J_x = -D [dC/dx] + s \omega^2 x C(x)$
  - For ideal infinite cell lacking walls.
Lamm Equation

- For real experimental conditions:
  - Cross-section of a sector cell is proportional to $r$
    - Continuity equation:
      - $\frac{dC}{dt}r = -\frac{1}{r}\left(\frac{drJ}{dr}\right)t$
    - Combine ideal equation with continuity equation to obtain:
      - $\frac{dC}{dt}r = -\frac{1}{r}\left\{\frac{d}{dr}\left[\omega^2 r^2 sC - Dr\left(\frac{dc}{dr}\right)t\right]\right\}t$
  - Describes diffusion with drift in an AUC sector cell under real experimental conditions.
Lamm Equation: Different Boundary Conditions

- Exact Solutions Exist in 2 limiting cases:
  1. “NO DIFFUSION”
     - Homogeneous macromolecular solution
       - \[ C_2(x,t) = \begin{cases} 
       0 & \text{if } x_m < x < x_{avg} \\
       C_0 \exp(-2s\omega^2t) & \text{if } x_{avg} < x < x_b
       \end{cases} \]
  2. “NO SEDIMENTATION”
     - Lamm Equation: \( \frac{dC}{dt} = -D \frac{d^2C}{dt^2} \)
     - Concentration Gradient: \( \frac{dC}{dt} = -C_0(\pi D t)^{1/2} \exp(-x^2/4Dt) \)
     - Diffusion coefficient determined by measuring the standard deviation of Gaussian curve
     - Used for small globular proteins, at low speed, with synthetic boundary cell.
Technology Enabling Analytical Analysis

- Two computer modeling methods enable simultaneous determination of sedimentation, diffusion coefficients, and molecular mass.

1. vanHolde-Weischet Method:
   - Extrapolation to infinite time must eliminate the contribution of diffusion to the boundary shape.
   - ULTRASCAN software

2. Stafford Method:
   - Sedimentation coefficient distribution is computed from the time derivative of the sedimentation velocity concentration profile
Partial Concentration of faster Component:
37% - 43% = 54% of total boundary

Partial Concentration of slower Component:
43% - 7% = 37% of total boundary

The Remainder (100% - 97% - 7% - 10%) are not accounted

http://www.ultrascan2.uthscsa.edu/tutorial/basics_5.html
Specific Boundary Conditions

- Faxen-type solutions:
  - Centrifugation cell considered infinite sector
    - Diffusion is small
    - Only consider early sedimentation times

- Archibald solutions:
  - $S$ and $D$ considered constant

- Fujita-type solutions:
  - $D$ is constant
  - $S$ depends on concentration
<table>
<thead>
<tr>
<th></th>
<th>Sedimentation Velocity</th>
<th>Sedimentation Equilibrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angular Velocity</td>
<td>Large (according to sedimentation properties)</td>
<td>Small</td>
</tr>
<tr>
<td>Analysis</td>
<td>As a function of time</td>
<td>At equilibrium</td>
</tr>
<tr>
<td>Measurement</td>
<td>Forming a Boundary</td>
<td>Particle distribution in cell</td>
</tr>
<tr>
<td>Calculated Parameters</td>
<td>Shape, mass composition</td>
<td>Mass composition</td>
</tr>
</tbody>
</table>
Sedimentation Velocity

- How we measure the results: Determine the Sedimentation and Diffusion Coefficients from a moving boundary
It takes a While to Run an Experiment!

<table>
<thead>
<tr>
<th>Speed (rpm)</th>
<th>Time at each speed (s)</th>
<th>Svedbergs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6000</td>
<td>15</td>
<td>500</td>
</tr>
<tr>
<td>6000</td>
<td>600</td>
<td>4220</td>
</tr>
<tr>
<td>9000</td>
<td>600</td>
<td>1330</td>
</tr>
<tr>
<td>13000</td>
<td>600</td>
<td>550</td>
</tr>
<tr>
<td>18000</td>
<td>600</td>
<td>250</td>
</tr>
<tr>
<td>25000</td>
<td>600</td>
<td>125</td>
</tr>
<tr>
<td>50000</td>
<td>3600</td>
<td>31</td>
</tr>
</tbody>
</table>
Correcting to Standard Value

\[ S_{20,W} = S^* \frac{(1- \bar{v} \rho)_{20,W} \eta_{T,b}}{(1- \bar{v} \rho)_{T,b} \eta_{20,W}} \]

- Allows for standardization of sedimentation coefficients
Concentration Dependence

- Sedimentation coefficients of biological macromolecules are normally obtained at finite concentration and should be extrapolated to zero concentration.

\[ s = s^0(1 + k'C)^{-1} \]

\[ s = s^0(1 - k''C) \]
Determining Macromolecular Mass

- First Svedberg equation:
  - $M = \frac{sRT}{D(1-\nu_{\text{avg}}\rho_o)}$

- Assumptions:
  - Frictional coefficients affecting diffusion and sedimentation are identical
Sedimentation Equilibrium

- Even if centrifuged for an extended period of time, macromolecules will not join pellet because of gravitational and diffusion force equilibrium.
- Molecular mass determination is independent of shape.
  - Shape only affects rate equilibrium is reached, not distribution.
- No changes in concentration with time at equilibrium
  - Total flux = 0
Binding Constants

- Can measure concentration dependence of an effective average molecular mass.
- Can be used to describe different kinds of phenomenon.
- Dissociation equilibrium constant can be directly determined from the equilibrium sedimentation data
  - \[ C(r) = CA(r)\sigmaA + CB(r)\sigmaB + CAB(r)\sigmaAB \]
Partial Specific Volume

- Needed when determining molecular mass through sedimentation
- Measurement of the density of the particle using its calculated volume and mass
- Very difficult to make precise density measurements needed
Density Gradient Sedimentation

- **Velocity Zonal Method**
  - Layered density gradient
    - Sucrose, glycerol
  - Particles separate into zones based on sedimentation velocity, according to sedimentation coefficients
    - Determined by size, shape, and buoyant density
    - Estimation of molecular masses
  - Potential Problem:
    - Molecular crowding effect due to high sucrose concentration

- **Density Gradient Sedimentation Equilibrium**
  - Density gradient itself formed by centrifugal field
  - Used in experiment by Messelson and Stahl
http://www.mun.ca/biology/scarr/Gr10-23.html
Molecular Shape

- Sedimentation coefficient dependent on particle volume and shape
- Molecules having the same shape, but different molecular mass form a homologous series.

http://web.virginia.edu/Heidi/chapter30/chp30.htm