I. Introduction

A. There are a group of biophysical techniques that are based on transport processes.
   1. Transport processes involve observing how macromolecules move (are transported) when they experience forces acting upon them.
   2. These techniques include
      a. Sedimentation
      b. Electrophoresis
      c. Diffusion measurements.
   3. Each involves a different force
      a. Sedimentation - centrifugal force
      b. Electrophoresis - electromotive force (electrical potential)
      c. Diffusion - Thermal energy
   4. In addition to their use for isolating macromolecules, these techniques provide information about the following physical properties of a macromolecule in various combinations:
      a. Mass - sedimentation
      b. Size - sedimentation, electrophoresis, diffusion
      c. Shape - sedimentation, electrophoresis, diffusion
      d. Charge - electrophoresis
      e. Degree of hydration - sedimentation, electrophoresis, diffusion
   5. In addition to using these techniques to characterize macromolecules, they are also used to isolate and purify macromolecules.

B. We will focus on sedimentation as a representative example of a technique that is based on transport processes.
   1. We will examine how sedimentation provides information about a macromolecule's mass, size, shape and degree of hydration.

II. Theory of Sedimentation

A. We will focus first on an individual macromolecule and then later expand to consider molar quantities of macromolecules.
   1. We will consider a macromolecule that is dissolved in an aqueous solvent.
      a. This solution may also contain buffer salts and other small molecular weight components; we will consider these as part of the solvent.
      b. The solvent will be treated as a continuum.

B. Centrifugal force, \( F_c \).
   1. In sedimentation, the force that causes a macromolecule to move is a centrifugal force.
2. According to Newton's laws of motion, force is equal to the product of mass \((m)\) times acceleration \((a)\).

\[ F = ma \]

a. When a force acts upon a molecule with mass \(m\), it accelerates:

\[ a = \frac{F}{m} \]

b. When a macromolecule revolves around an axis, as it does in a centrifuge, it experiences a centrifugal acceleration, \(a_c\):

\[ a_c = \omega^2 r \]

i. Centrifugal acceleration is equal to the square of the angular velocity, \(\omega^2\), times the distance from the axis of rotation, \(r\).

1. \(\omega\) has units of \textit{radians/s}.

c. Making this substitution the centrifugal force is

\[ F_c = m\omega^2 r \]

C. Frictional drag, \(F_d\)

1. As a macromolecule in solution moves under the influence of a centrifugal force, it experiences an opposing force.

a. The solvent flowing past it causes this force.

b. This force is proportional to the velocity, \(v\), of the macromolecule:

\[ F_d = fv \]

i. Where \(f\) is the proportionality constant.

ii. \(f\) is called the frictional coefficient.

1. As we will see, it is a property of the macromolecule and is determined by the macromolecule's size and shape.

2. For a sphere, the frictional coefficient is give by 

\textbf{Stoke's equation:}

\[ f_o = 6\pi\eta R_o \]

a. Where \(R\) is the radius of the sphere and \(\eta\) is the viscosity of the solvent.

D. Buoyant force, \(F_b\)

1. As the macromolecule move it displaces the solvent.

a. The displaced solvent must move in a direction opposite to the macromolecule.
b. The displaced solvent will also experience a centrifugal force and because the displaced solven moves in a direction opposite to the macromolecule, this force will oppose the movement of the macromolecule.

Mass of displaced volume, \( m_o \)

\[
m_o = V \rho
\]

\[
F_b = (V \rho) \omega^2 r
\]

i. Where \( V \) is the volume of the macromolecule and \( r \) is the density of the solvent.

ii. The volume of a macromolecule can be determined from its mass, \( m \), and its partial specific volume, \( \bar{V} \),

\[
V = m \bar{V}
\]

1. \( \bar{V} \), can be determined experimentally.

2. For proteins, \( \bar{V} \) can be also be predicted from the amino acid composition.

iii. Making this substitution for \( V \):

\[
F_b = m \bar{V} \rho \omega^2 r
\]

E. Sedimentation velocity

1. The centrifugal and buoyant forces are independent of the macromolecule's velocity, however, the drag force is proportional to the velocity and opposes the movement of the macromolecule.

(See Fig 5.3, p196, diagram of sector cell)

a. As the macromolecule accelerates under the combined influences of the centrifugal and buoyant forces, the drag force increases.

b. When the forces balance one another, the macromolecule will experience no net force and will stop accelerating.

i. It will then move at a constant velocity known as the \textit{steady-state velocity}.

ii. For sedimentation experiment, this velocity is also called the \textit{sedimentation velocity}.

2. At steady-state, the sum of the three forces acting on a macromolecule is 0:

\[
F_c - F_b - F_d = 0
\]

a. The signs of the buoyant and drag forces are negative because they oppose the movement of the macromolecule.
3. Substitution of the expressions derived above for the three forces gives:

\[
m \omega^2 r - m \bar{\nu} \rho \omega^2 r - f v = 0
\]

\[
m \omega^2 r (1 - \bar{\nu} \rho) - f v = 0
\]

4. Solving for the sedimentation velocity, \( v \):

\[
v = \frac{\omega^2 r m (1 - \bar{\nu} \rho)}{f}
\]

F. Sedimentation coefficient

1. The sedimentation velocity is a quantity that can be measured
   a. It depends not only on the properties of the macromolecule and the solvent \( (m, \bar{\nu}, \rho \) and \( f \), but also on how fast the centrifuge is run \( (\omega) \) and how far from the axis of rotation the solution is placed \( (r) \).

2. If the sedimentation velocity, \( v \), is divided by the centrifugal acceleration, \( \omega^2 r \), a parameter is obtained which is independent of the instrument settings:

\[
\frac{v}{\omega^2 r} = \frac{m(1 - \bar{\nu} \rho)}{f}
\]

3. This parameter is represented by the letter \( s \) and is called the sedimentation coefficient:

\[
\frac{v}{\omega^2 r} = \frac{m(1 - \bar{\nu} \rho)}{f}
\]

a. The sedimentation coefficient has units of seconds and for macromolecules has a magnitude of around \( 10^{-13} \) seconds.

b. Sedimentation coefficients are therefore typically given in units of Svedbergs, S.
   i. One Svedberg is equal to \( 10^{-13} \) second.
   ii. The unit is named in honor of Theodor Svedberg, a Swedish chemist who won the Nobel Prize in 1926 for inventing the analytical ultracentrifuge.

4. So far we have been considering only a single macromolecule, if we wish to consider mol of macromolecules, he numerator and denominator of the
equation for the sedimentation coefficient is multiplied by Avogadro's number, $\mathcal{R}$:

\[
\begin{align*}
\mathbf{s} &= \frac{\mathbf{m}(1 - \mathbf{\nu}\mathbf{\rho})}{\mathcal{R}_f} \\
&= \frac{\mathbf{M}(1 - \mathbf{\nu}\mathbf{\rho})}{\mathcal{R}_f}
\end{align*}
\]

c. Where $\mathbf{M}$, represents the molecular weight of the macromolecule.

### III. Moving Boundary Sedimentation

#### A. Determining the sedimentation coefficient

1. In an analytical ultracentrifuge the solution containing the macromolecule is placed in a sector shaped sample cell with windows that allow the solution to be optically analyzed during the centrifugation.  
   (See Fig 5.5, p198, Schematic of centrifuge)

2. As the centrifugation proceeds the region of the sample cell closest to the axis of rotation becomes void of macromolecules.
   a. This produces a solvent/solution boundary.
   b. This boundary moves with a velocity equal to the sedimentation velocity of the macromolecule.

3. There are various optical methods for monitoring the movement of this boundary with time.
   a. One of these is a UV scanner.
      i. Since both proteins and nucleic acids absorb ultraviolet (UV) light, the location of the boundary can be found by measuring the absorption of UV light as a function of the radial distance from the axis of rotation.  
         (See Fig 5.4, p197)

4. The velocity of the boundary is given by derivative equation, $\frac{dr}{dt}$, which can be set equal to the sedimentation velocity at the $r_b$

\[
\begin{align*}
\mathbf{s} &= \frac{\mathbf{v}}{\omega^2 r_b} \\
\mathbf{v} &= r_b \omega^2 \mathbf{s} \\
\frac{dr}{dt} &= r_b \omega^2 \mathbf{s}
\end{align*}
\]
b. Rearranging this equation gives the following differential equation

\[
\frac{dr_b}{r_b} = \omega^2 s \, dt
\]

c. Integrating both sides of this equation gives

\[
\int \frac{dr_b}{r_b} = \int \omega^2 s \, dt
\]

\[
\int \frac{dr_b}{r_b} = \omega^2 s \int dt
\]

\[
\ln(r_b) = \omega^2 s t + \text{constant}
\]

d. This equation has the form of a straight line equation when \(\ln(r_b)\) is plotted against time \(t\).

i. The slope of the line is equal to \(\omega^2 s\) and the y-intercept is equal to the constant.

IV. Corrections and Adjustments to the Sedimentation Coefficient

A. Solvent density and viscosity.

1. The sedimentation coefficient is dependent on properties of the solvent.

2. To see how it depends on solvent properties, we substitute Stoke’s equation for the frictional coefficient:

\[
s = \frac{M(1 - \bar{v}\rho)}{8\pi f}
\]

\[
= \frac{M(1 - \bar{v}\rho)}{8\pi \delta \eta R_e}
\]

a. The only quantities in this equation that depend on the solvent are the density, \(\rho\) and the viscosity, \(\eta\).

3. To produce values for the sedimentation coefficient that are comparable from one determination to the another, the values are typically converted to values expected if the solvent has the viscosity and density of water at 20°C.

(See Table 5.2, p200 for densities and viscosities for water)
\[ s_{20,w} = s_{T,b} \left( \frac{1 - \nu \rho_{20,w}}{1 - \nu \rho_{T,b}} \right) \left( \frac{\eta_{T,b}}{\eta_{T,w}} \right) \left( \frac{\eta_{T,w}}{\eta_{20,w}} \right) \]

b. The viscosity is factored into two terms because \( \frac{\eta_{T,b}}{\eta_{T,w}} \), which is called the relative viscosity, is easily determined by measure the time it takes the buffer to flow through a capillary tube relative to the time that it takes water to flow through the same capillary tube.

**B. Effect of diffusion on the boundary**

1. As sedimentation proceeds the boundary will spread.
   a. This is due to diffusion, which is also a transport process.

2. In diffusion the macromolecules are moving as a consequence of thermal energy.
   a. The movement of an individual macromolecule is random in direction and occurs with equal probability in all directions.

3. Like sedimentation, the movement of the macromolecule is opposed by frictional drag.

4. Einstein derived an equation the related diffusion to the thermal energy, \( RT \), frictional coefficient, \( f \):

   \[ D = \frac{RT}{\mathcal{R}f} \]

   a. Where \( R \) is the ideal gas law constant, and \( T \) is the absolute temperature.

   b. The parameter \( D \) is called the diffusion coefficient, and like the sedimentation coefficient, it is inversely proportional to a macromolecule's frictional coefficient.
      i. It can be determined by measuring the rate of boundary spreading. (We will no discuss the details for determining \( D \))
      ii. Another method of measuring a diffusion coefficient is dynamic light scatter, in which the time dependent fluctuations in scattered light intensity from a solution can be used to determine the diffusion coefficient of the solute molecules in the solution. (The fluctuating scattered light intensity is due to the diffusion of the solute molecules.)

   c. Einstein’s equation can be combined with the sedimentation equation to obtain and equation in which the frictional coefficient is factored out:
\[ s = \frac{M \left(1 - \bar{\nu} \rho\right)}{\Re f} \]

\[ \Re f = \frac{RT}{D} \]

\[ s = \frac{MD \left(1 - \bar{\nu} \rho\right)}{RT} \]

solving for the molecular weight:

\[ M = \frac{s RT}{D \left(1 - \bar{\nu} \rho\right)} \]

iii. The sedimentation and diffusion coefficients for a macromolecule can be combined to determine the molecular weight of a macromolecule.

C. **Effect of concentration**

1. At finite concentrations, interactions between molecules can cause changes in the determined value of the sedimentation coefficient.
   a. To eliminate this effect, the determination of the sedimentation coefficient is done at several concentrations, plotted against the concentration, and extrapolated to zero concentration, where interactions can no longer take place.
      i. The extrapolated, zero concentration value is represented by a “0” subscript.
      ii. \( s_{w,20}^0 \) is a sedimentation coefficient expected at zero concentration at 20°C in water.

D. **Analysis of the frictional coefficient**

1. The frictional coefficient contains information about the size and shape of the macromolecule.
2. The frictional coefficient can be obtained from either the sedimentation or diffusion coefficients:
Using Stokes equation, the radius of a sphere having the same frictional coefficient as the macromolecule can be determined:

\[
f = 6\pi \eta R_S
\]

\[
R_S = \frac{f}{6\pi \eta}
\]

a. This radius, \( R_S \), is called the \textit{Stoke’s radius}.

4. If the mass of a molecule is molded into a perfect sphere would usually be less than the Stoke’s radius
a. This radius, \( R_o \), can be determined from the molecular weight and partial specific volumes of the macromolecule.

\[
V_o = \frac{4\pi}{3} (R_o)^3
\]

\[
R_o = \sqrt[3]{\frac{3V_o}{4\pi}}
\]

\[
= \sqrt[3]{\frac{3}{4\pi} \left( \frac{M\nu}{\eta} \right)}
\]

b. \( R_o \) is usually less than \( R_s \)
   i. This can be due to deviations from a spherical shape
   ii. I can also be due to the binding of solvent molecules to the macromolecule’s surface (hydration), which are being dragged along with the macromolecule and increasing its effective volume.
   iii. The frictional coefficient can be factored into two terms which account for each of these effects:
\[ \frac{f}{f_o} = \left( \frac{f}{f_{sp}} \right) \left( \frac{f_{sp}}{f_o} \right) \]

iv. Where
1. \( f \) is the observed frictional coefficient
2. \( f_o \) is the frictional coefficient for the unhydraded sphere. It can be calculated from \( R_o \):

\[ f_o = 6\pi \eta R_o \]

\[ = 6\pi \eta \left( \frac{3\sigma}{4\pi} \left( \frac{M\nu}{\Re} \right) \right) \]

3. \( f_{sp} \) is the frictional coefficient for a sphere having the same degree of hydration as the macromolecule

v. The ratio \( \left( \frac{f_{sp}}{f_o} \right) \) is called the **hydration factor** and can be used to calculate the volume of solvent bound to the macromolecule per unit volume of the macromolecule:

\[ \left( \frac{f_{sp}}{f_o} \right) = \left( 1 + \delta \right)^{\frac{1}{3}} \]

1. Where \( \delta \) represents the degree of hydration in \( mL \) of solvent per \( mL \) of macromolecule.

vi. The ratio \( \left( \frac{f}{f_{sp}} \right) \) is called the **shape factor** and can be used to estimate the deviation of the shape of the macromolecule from spherical symmetry

1. This is usually done by modeling the macromolecule as either a **prolate** or **oblate** ellipsoid of revolution: