Exp. #1: Accurate & Precise Measurment of Volume & Dilutions

- Types of pipettes:
  - Transfer / volumetric pipette 
    - ↳ transfer one fixed volume
    - ↳ left the last drop in the tip
  - Graduated / Mohr pipettes 
    - ↳ has graduation ⇒ can deliver intermediate volumes
    - ↳ Do not drain completely ⇒ only drain to the last calibration

  } use suction bulb

  - Single-channel Eppendorf pipette
    - transfer between:
      - Yellow: 10.0 µL to 100.0 µL
        or 2.00 µL to 20.00 µL
      - blue: 100 µL to 1000 µL
    - down to first stop → in liquid → slowly release
      → in beaker → down to first → down to second

- Dilution
  - Units:

Molarity (M): mole of solute (mol) / volume of solution (L)

mg/mL: mass of solute (mg) / Volume of Solution (mL)

% v/v: volume of solute (mL) / Volume of solution (mL) × 100%

% m/v: mass of solute (mg) / mass of solution (mg) × 100%

% m/v: mass of solute (g) / volume of Solution (mL) × 100%

  - Terms
    - dilution factor = $\frac{V_{stock}}{V_{total}}$
    - X/y dilution = X mL of stock : y-x mL of H_2O
    - a X Sample = use $\frac{\text{final total volume}}{A}$ of AX Sample & add H_2O
  - Key Equation:

$$C_1 V_1 = C_2 V_2$$

- Spectrometry

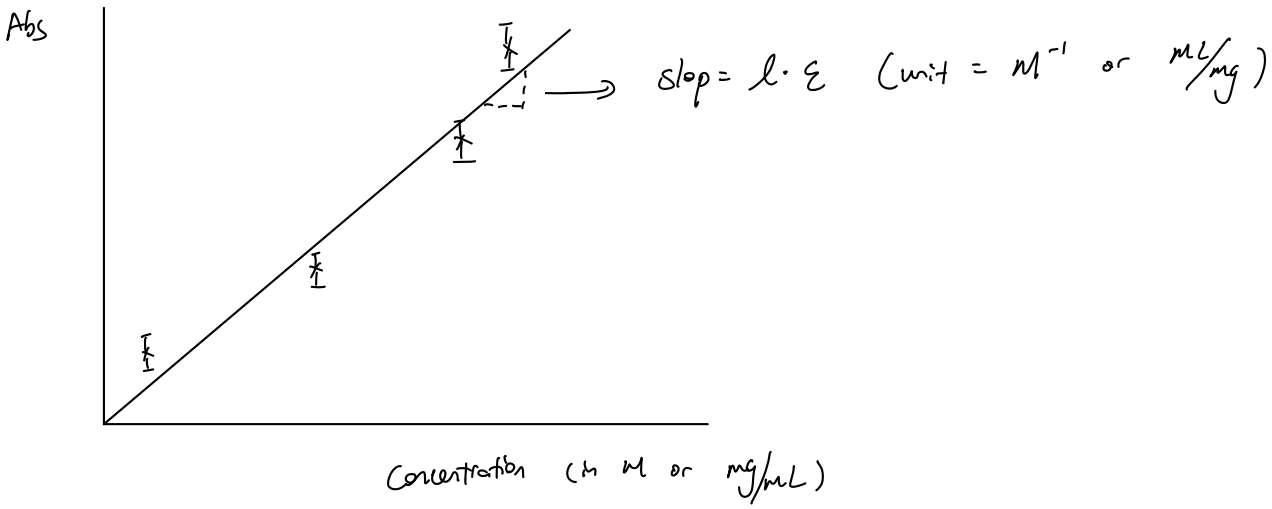
Beer-Lambert Law:

$Abs = l \cdot \varepsilon \cdot C$

- l: path length (usually 1 cm) (cm)
- ε: molar extinction coefficient → constant for a substance ($M^{-1}\ cm^{-1}$)
- C: concentration (M)

* Does not work at high concentrations → usually ensure abs < 1.0

- Data Reporting
  - Average Deviation = $\sum_{n} \frac{1}{n} |x_i - \mu|$, where $\mu = \sum_{n} \frac{1}{n} x_i$
  - From a standard Curve:



- Lab Techniques
  - Load from least concentrated to most concentrated
  - Do not rinse cuvette in between

Exp# 2: Buffering Properties of Phosphate

• Buffer Theory

$$HA \rightleftharpoons H^+ + A^-$$

HA: ↓ weak acid

A^-: ↓ Conjugate base

• Henderson-Hasselbalch Equation:

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

$\Rightarrow pH = pK_a$ if $[A^-] = [HA]$

$\Rightarrow$ Buffering range is usually $pK_a \pm 1$ pH units

• Buffer Capacity

- When diluted ...
  - ↳ pH is approximately the same
  - ↳ buffering capacity (↓) due to less material per volume

- The capacity of buffer should be high to buffer any rxn. that may cause a change in pH $\Rightarrow$ usually 10 - 20 x the concentration of acid/base produced during the rxn.

- Phosphate Buffer

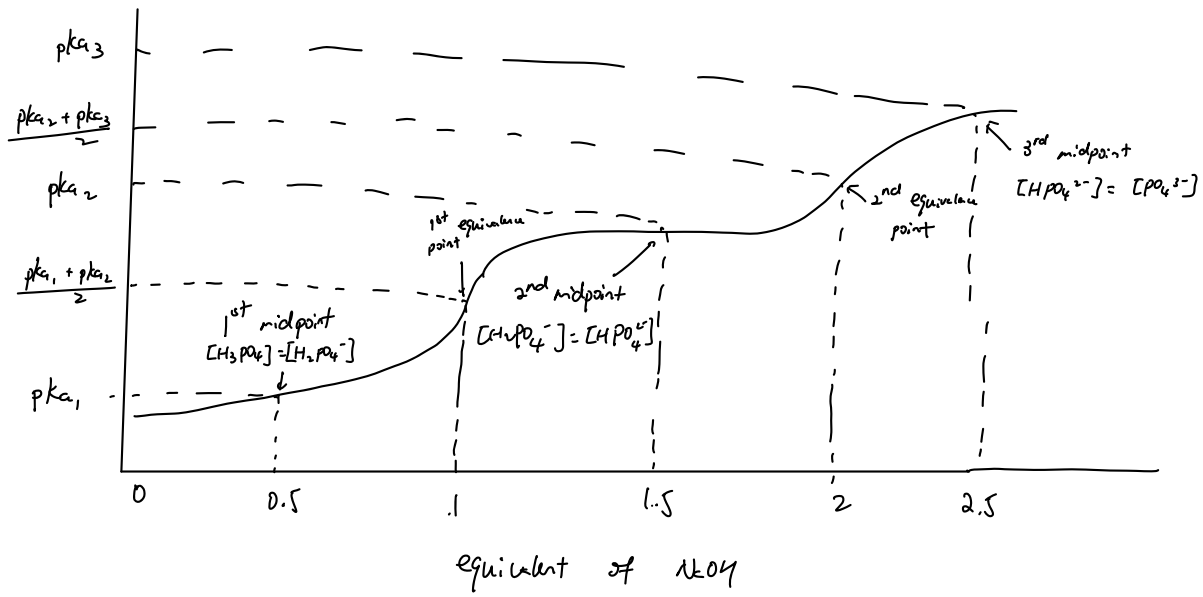
$$H_3PO_4 \rightleftharpoons H_2PO_4^- + H^+ \qquad pka_1 = 2.15$$

$$H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+ \qquad pka_2 = 6.80$$

$$HPO_4^{2-} \rightleftharpoons PO_4^{3-} + H^+ \qquad pka_3 = 12.37$$

- At each $pH = pka$, $[A^-] = [HA]$
  - ↳ 1/2 mole of the HA is deprotonated via addition of base
- At equivalence point (1 mole of strong base = mole of original weak acid)
  - no buffering capacity
  - $pH_{equivalence\ \#1} = \frac{pka_1 + pka_2}{2}$.

- Titration of phosphoric acid (H_3PO_4)



- **Making Buffer**
  - Select a appropriate pka based on the pH required
  - Determine the volume of starting material based on the mole of buffer
  - Determine the ratio of A^-/HA based on the pH required
  - Determine the mole of each based on the total mole ($V \times C$) of buffer
  - Determine the mole of HCl/NaOH needed for a given starting material
  - Calculate the volume of HCl/NaOH needed
  - Calculate the volume of H_2O needed based on the required volume

Key Quick Equation:

$$\frac{[A^-]}{[HA]} = \frac{\text{mole } A^-}{\text{mole HA}} = 10^{pH - pka}$$

$$\therefore n\left(10^{pH-pka} + 1\right)[A^-] = n_{tot}$$

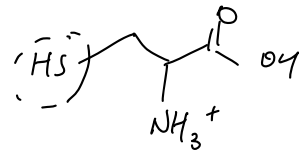
- **Data Analysis**
  - Use a first derivative plot for equivalence points
  - Determine [NaOH] first by using the titration for a known $[H_3PO_4]$

Exp #3: Separating Amino Acids by Column Chromatography

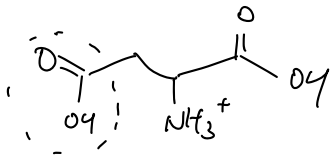
- Amino Acids with titratable side chains (fully protonated form)

charge = 0 side chain (fully protonated)

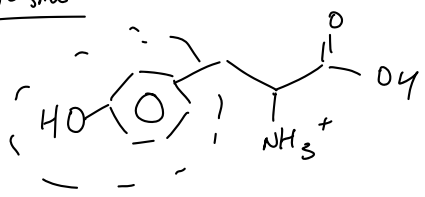
Cystein



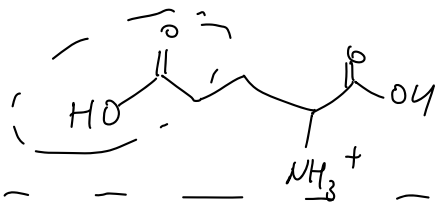
Aspartic Acid



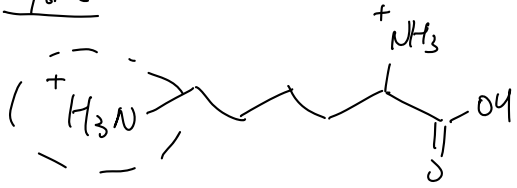
Tyrosine



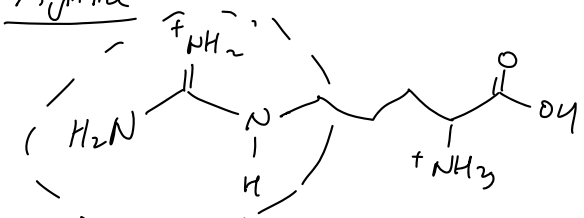
Glutamic Acid



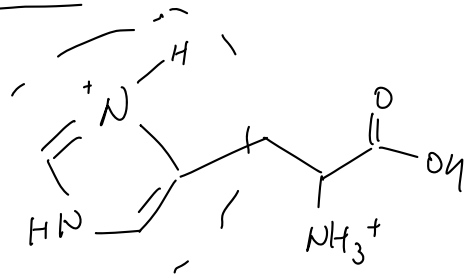
Lysine



Arginine



Histidine



+1 side chain (fully protonated)

- **Ion Exchange Chromatography**
  - Cation exchange binds cation
    - +ve binds, -ve elutes out first, followed by neutral
  - Resolution
    - good resolution means distinct narrow bands
  - Ninhydrin
    - react with amines & become blue purple
    - Absorb at 570 nm
    - most optimal in pH 4 - 8.

- **Fractional charges**

At a particular pH:

$$\begin{cases} \dfrac{C_b}{C_a} = 10^{pH-pka} \\ C_a + C_b = 1 \end{cases} \Rightarrow C_b = \frac{10^{pH-pka}}{1 + 10^{pH-pka}}$$

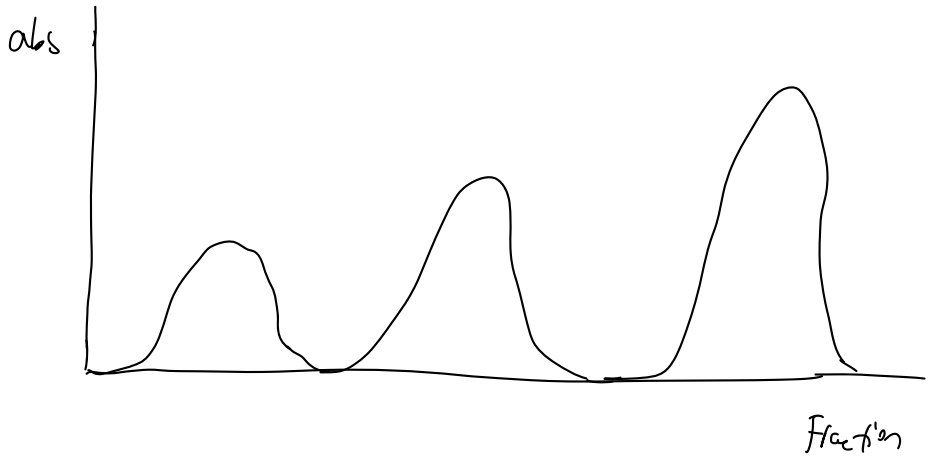
- $pI = \frac{pka_{+1 \to 0} + pka_{0 \to -1}}{2}$

  - $pH = pI$: charge is neutral
  - $pH < pI$: charge is positive
  - $pH > pI$: charge is negative

- We consider pH for which $pka - 2 < pH < pka + 2$ to be insignificant

- Data Analysis

  - Wash with NaOH after -ve & neutral has come out at a lower pH (4.2)



Exp #4: Protein Seperation via Size Exclusion Chromatography

- Size Exclusion Chromatography
  - resin has a fractionation range (in Da or g/mol)
  - Larger proteins pass through the column faster than the smaller ones
  - Seperate the molecular weight based on the native conformation
  - Definitions:
    1. Void volume
       - buffer that surrounds the resin beads
       - > upper fractionation limit protein
       - Elute out first
       - Void Volume $\simeq \frac{1}{3}$ Bed Volume $= \frac{1}{3}\pi r^2 h$
    2. Bed Volume
       - total volume of resin + buffer
       - < fractionation range protein eluted at the end
       - Bed Volume $= \pi r^2 h$
    3. Fractionation volume
       - within fractionation range, b/w void & bed

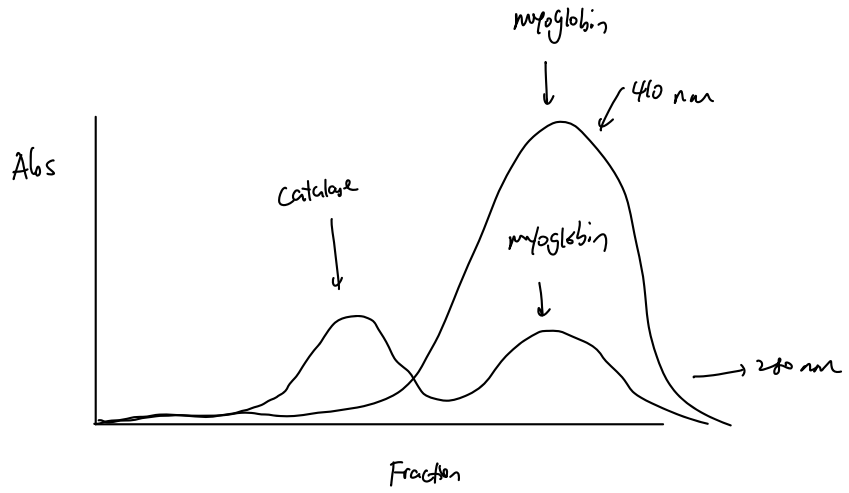
- Detecting proteins
  - Catalase
    - ↳ tetramer
    - ↳ Bubble when H_2O_2 added
  - Myoglobin
    - ↳ Absorbance

  } Can both be confirmed with abs. at 280 nm

  - Molar coefficient of Extinction
    - ↳ $\varepsilon_{280} = x\,\varepsilon_{280\text{-tyrosin}} + y\,\varepsilon_{280\text{-tryptophan}}$

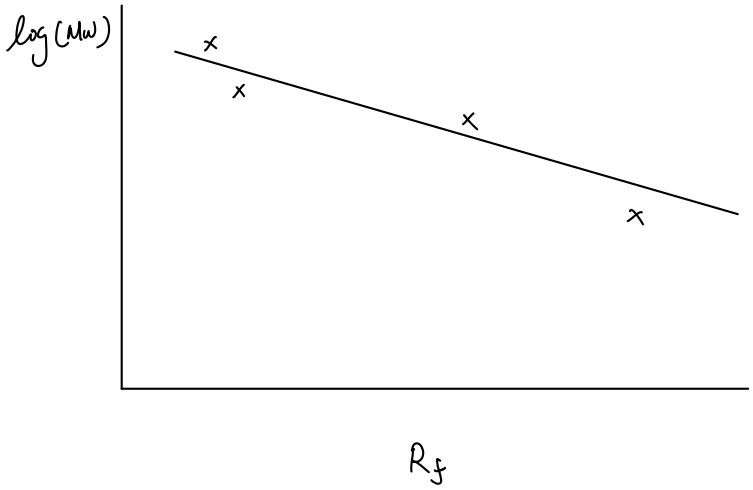
- SDS-PAGE
  - Loading buffer contains:
    - SDS → Denature & give protein -ve charge
      - ↳ migrate towards anode
    - 2-mercaptoethanol → reduces disulfide bonds
    - tracking dye (bromophenol blue)
  - Assume proteins have the same charge to mass ratio
    - ↳ for the same charge, heavier = migrate slower
  - Proteins are denatured into subunits

- Data Analysis
  - Chromatogram



use $Abs = l \varepsilon c$ to obtain the concentration

  - SDS-PAGE
    - R_f = distance migrated / distance migrated by dye front
    - Calibration curve is plotted using $\log(MW_{standards})$ vs. R_f



Use the concentration & MW to determine the mass recovered

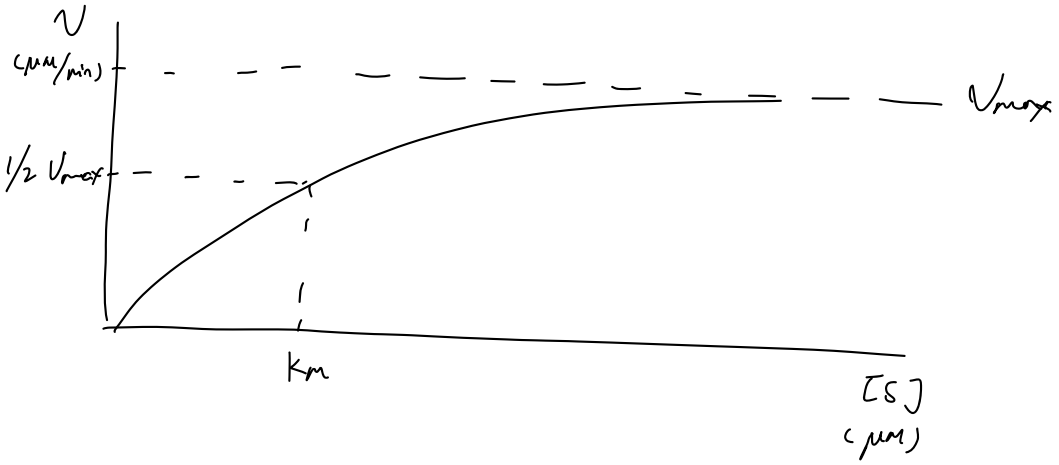
Exp #5 : Enzyme Kinetics of Alkaline phosphate

- **Michaelis - Menten Model**

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

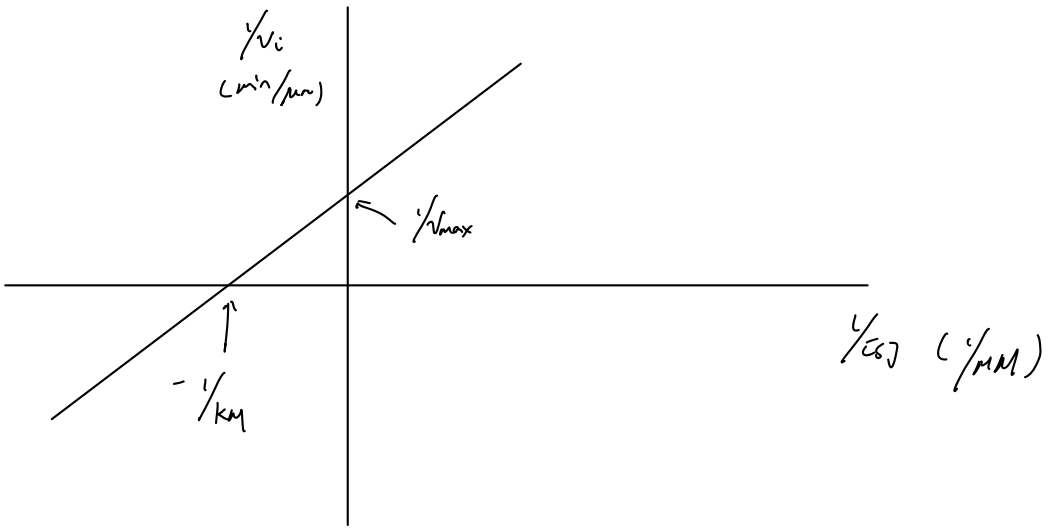
$$\frac{d[P]}{dt} = \frac{V_{max}[S]}{K_M + [S]} = V_i$$

- V_{max} : maximal velocity if enzyme is saturated with substrate
  - k_{cat} (turn-over number) $= \frac{V_{max}}{[E]_{Tot}}$
- K_M : Michaelis constant. the substrate concentration required to reach 1/2 of the maximum velocity
  - high K_M = low affinity
  - Catalytic efficiency $= \frac{k_{cat}}{K_M}$

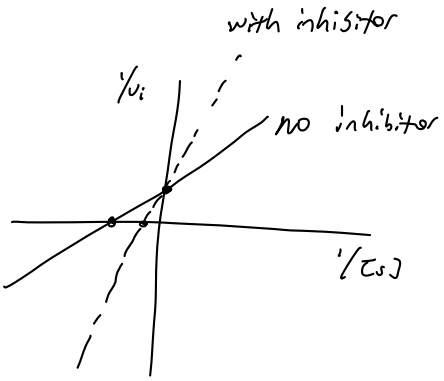


- Lineweaver - Burk plot

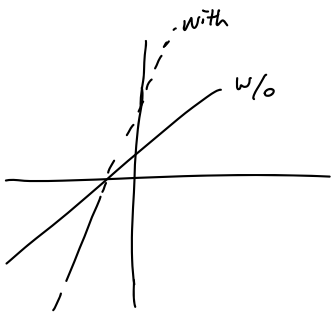
$$\frac{1}{v_i} = \frac{K_M}{V_{max}} \left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$



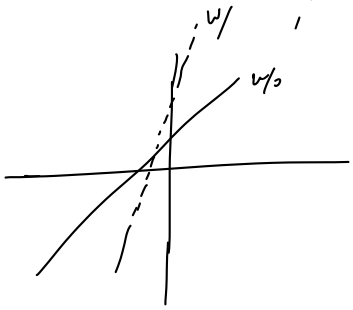
- Inhibition
  - Competitive:
    - Interact at substrate binding site
    - Same V_{max}, increased K_M



  - pure non-competitive:
    - ↓ only max catalytic rate (V_{max})

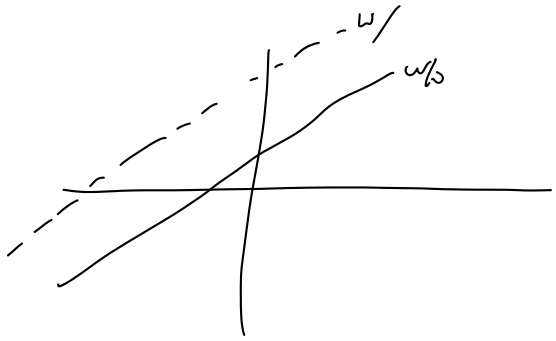


  - mixed-typed inhibition
    - ↓ V_{max} + ↑ K_M



- Uncompetitive

  - ↳ inhibitor binds after ES
  - ↳ parallel lines
  - ↳ V_{max} ↓, K_M ↓



- Alkaline phosphate

  - hydrolize PNPP to give p-nitrophenol w/ yellow colour
  - high Abs = high [P]
  - the competitive inhibitor is Na_2P_i

- Data Analysis

  - Use the initial rates → convert from Au/min to mM/min using $A = \varepsilon l c$
  - Solve the Michaelis-Menten using the Excel Solver

Exp #6 : Enzymatic Determination of Glucose

- Glucose Monitoring
  - ↳ Enzymatic methods provide a high degree of specificity

$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{oxidase}]{\text{Glucose}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{ABTS}_{(\text{reduced})} \xrightarrow{\text{peroxidase}} \text{ABTS}^{\cdot+}_{(\text{oxidized})}$$

(blue)

$\therefore$ Abs for $ABTS^{\cdot+} \propto [\text{glucose}]$

- Controlling Blood Glucose
  - Low blood glucose
    - Pancreatic α-cell $\xrightarrow{\text{glucagon}}$ break down of glucagen $\xrightarrow{\text{phosphorylase}}$ G6P $\longrightarrow$ glucose
  - high blood glucose
    - pancreatic β-cell $\xrightarrow{\text{insulin}}$ activated phosphoprotein phosphotase I $\longrightarrow$ inactivate phosphorylase
      - insulin → ↑ glucose intake
      - insulin → activated glucokinase $\longrightarrow$ glucose $\longrightarrow$ G6P $\longrightarrow$ glycogen
- Reverse Pipetting
  - Second stop $\xrightarrow[\text{liquid}]{\text{into}}$ release $\xrightarrow{\text{transfer}}$ first stop
    - ↳ ensure that there is no bubble

- 96 - well plate
  - path length is dependent on the V of sample in the well
  - when loading, minimize # of columns
- Data Analysis
  - Determine the concentration using a standard plot