A. One-Substrate Reactions
   (1) Kinetic concepts
   
   (2) Kinetic analysis
      (a) Briggs-Haldane steady-state treatment
      (b) Michaelis constant ($K_m$)
      (c) Specificity constant
   
   (3) Graphical analysis
   
   (4) Practical enzymology

B. Enzyme Inhibition
   
   (1) Classification
      (a) Competitive
      (b) Noncompetitive
      (c) Uncompetitive
      (d) Substrate

C. Multisubstrate Reactions
   
   (1) Convention
   
   (2) Mechanisms

D. Substrate binding
   
   (1) Derivation
   
   (2) Methodology
(1) Kinetic Concepts

Reaction Rates

- Chemical kinetics deals with the rates of chemical reactions
- Law of Mass action "the rate of a chemical reaction is proportional to the active masses of the reacting substances"
- Thus the forward rate of EQ1: \[ A \rightleftharpoons B \propto [A] \]

For more complex rxns EQ2: \[ 2A + B \rightleftharpoons 3C \] "The rate of a chemical rxn is proportional to the molar concentrations of the reacting substances, with each conc. rounded to the power equal to the stoichiometric coefficient of that substance in the balanced chemical equation"
- Thus EQ:2 rate \( \propto [A]^2 [B] \)

Kinetics addresses the following questions
(a) What is the rate (velocity) of the rxn?
(b) How can the rate of the rxn be altered?
(c) What is the path by which the rxn proceeds?

Rxn Velocity

- speed with which a chemical reaction proceeds
- rate can be described by the appearance of product or the disappearance of a reactant

\begin{align*}
\text{EQ:1} & \quad A \rightleftharpoons B \\
\text{Reaction rate} & \quad V = \frac{-d[A]}{dt} = \frac{d[B]}{dt} \\
\text{the derivative} & \quad d[ ]/dt \text{ refers to change in concn over time} \\
\text{the derivative is the slope of a plot of concn versus time} & \quad \text{may be straight line on a curve}
\end{align*}
Straight line
- slope is fixed and can be determined from any set of 2 points

Curved line
- slope at any point is given by the slope of the tangent to the curve at that point
  - slope is given by \( \frac{\Delta y}{\Delta x} \) as \( \Delta x \) approaches zero
  - slope of the tangent at the origin (\( t = 0 \)) is the initial velocity
- velocity always has a positive value (initial - final, or final - initial)
- For a more complex rxn, the stoichiometric coefficients of the reactant and products must be considered
  e.g. \( 2A + B \leftrightarrow 3C \)
  \[ v = \frac{-1}{2} \frac{d[A]}{dt} = \frac{-1}{3} \frac{d[B]}{dt} = \frac{1}{3} \frac{d[C]}{dt} \]

Rate Constants
- expression of the velocity as a function of reactant concentrations is known as the rate equation (rate law)

Order of reactions
- concn terms in the rate equation may be more complex and may be raised to specific powers. These powers can't be inferred from the stoichiometry of the rxn but rather have to be determined experimentally
- the exponent (powers) of the concn terms in a rate equation define the order of the rxn
  - usually range 0-3 (exponents) but may be fractional values
  - more than 1 substance in a rxn has a nonzero order called "mixed order rxn"
  - rxn that is independent of reactant concn is a "zero order" rxn
  - units of the rate constant are such that the units on the right side of the rate equation must always be identical to those on the left side

(1) Zero Order Rxns
- \( A \leftrightarrow B \)
  Assume that: \( \frac{-d[A]}{dt} = k \)
rewritten: \[ d[A] = -kdt \]
integrate: \[ [A] = -kt + C \] (\(C = \) integration constant)

evaluate integration constant: \( t = 0 \) then \([A] = [A]_0 \) and \( C = A_o \) so:
\[ [A] = -kt + [A]_0 \]
A plot of \([A]\) as function of \(t\) will yield a straight line, slope = -\(k\)

(2) First order rxns

- follow an exponential time course
Assume rxn can be described:
\[ -\frac{d[A]}{dt} = k[A] \]
rewritten as:
\[ \frac{-d[A]}{[A]} = kdt \]
integrate: \( \ln[A] = -kt + C \) (\(C = \) integration constant)
evaluate \(C\): \( t = 0 \) then \([A] = [A]_o \) then \(C = \ln[A]_o \)
therefore: \( \ln[A] = -kt + \ln[A]_o \)
or \( A = [A]_o e^{-kt} \)
Thus, a plot of \(\ln[A]\) versus time gives slope = -\(k\) and and intercept of \(\ln[A]_o\)
Alternatively, a plot of \([A]\) versus time will yield an exponential curve

Half Life (\(t_{\frac{1}{2}}\))
- defined as the time required for the concn of a reactant to decrease to one half of its initial value
- so when \( t = t_{\frac{1}{2}} \) then \([A] = \frac{1}{2}[A]_o \)
becomes \( \ln \left( \frac{[A]_o}{2} \right) = -kt_{\frac{1}{2}} + \ln[A]_o \)
\[ \ln 0.5 [A]_o - \ln[A]_o = -kt_{\frac{1}{2}} \]
\[ \ln \left( \frac{0.5[A]_o}{[A]_o} \right) = -kt_{\frac{1}{2}} \]
-0.693 = -\(kt_{\frac{1}{2}}\)
\( t_{\frac{1}{2}} = \frac{0.693}{k} \)

2nd Order Rxn
Consider \( A + A \leftrightarrow B \)
\[ -\frac{1}{2} \frac{d[A]}{dt} = k[A]^2 \]
rearrange: \[-\frac{d[A]}{[A]^2} = 2 \, k dt\]

integrate: \[\frac{1}{[A]} = 2 \, kt + C\]  \[C = \text{integration constant}\]

evaluate: \[[A] = [A_o] \text{ when } t = 0 \text{ so } C = \frac{1}{[A_o]}\]

thus: \[\frac{1}{[A]} = 2 \, kt + \frac{1}{[A_o]}\]

A plot of \[\frac{1}{[A]}\] versus \(t\) gives a slope of \(2 \, k\), intercept = \[\frac{1}{[A_o]}\]

**Determining Rxn order**

1. If a plot of \([A]\) versus \(t\) is linear then zero order rxn
2. If a plot of ln\([A]\) versus \(t\) is linear then 1st order rxn
3. If a plot of \[\frac{1}{[A]}\] versus \(t\) is linear then 2nd order rxn

**Temperature Dependance**

**Rate Constants and Temperature**

1. **Arrhenius Equation**
   - Svante Arrhenius (1889) developed an empirical relationship between rate constants and \(T\)
     - logarithmic relationship between rate constant and \(1/T\) (K)
     - \(K_{eq}\) viewed as ratio of 2 rate constants

\[A + B \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} X + Y \quad K_{eq} = k_1 / k_{-1}\]

Now, since the \(K_{eq}\) varies with \(T\) according to the Van't Hoff equation can postulate that rate constants varying with \(T\) in a like manner

Jacobs Van't Hoff equation: \[\frac{d \ln K}{dT} = \frac{\Delta H^o}{RT^2}\]

- \(K\) - equilibrium constant (concentration)
- \(T\) - absolute temperature
- \(R\) - gas constant
- \(\Delta H^o\) - standard enthalpy change
Arrhenius proposed that rate constant varies with T as:

\[
\frac{d \ln k}{dT} = \frac{E_a}{RT^2}
\]

\(k = \text{rate constant}
\)

\(E_a = \text{energy of activation}
\)

Integration:

\[\ln k = \frac{-E_a}{RT} + \ln A\]

also:

\[\log k = \frac{-E_a}{2.303 RT} + \log A\]

or

\[k = Ae^{-Ea/RT}\]

or its integrated form

\[\log \frac{k_2}{k_1} = \frac{E_a}{2.303 R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right) \quad T_2 > T_1\]

- A is the "Arrhenius factor, preexponential factor, or frequency factor.

**Arrhenius Plots**

- Plot of \(\ln k\) versus \(1/T\) give slope \(-E_a/R + \ln A\)
  - a \(Y\) intercept

**Temperature Coefficient**

- ratio of one velocity at one \(T\) to the velocity at another \(T\) (10°C apart) is known as the \(Q_{10}\) or Temp coefficient of the reaction.
Thus, $Q_{10} = \frac{V \text{ of rxn at (t+10)°C}}{V \text{ of rxn at t°C}}$

Also, $Q_{10} = \frac{10}{T_2 - T_1} \left( \frac{V_2}{V_1} \right) \quad T_2 > T_1 (°K)$

$v_1, v_2$ = velocity at $T_1 + T_2$, respectively

For a 1st order rxn $V = k[A]$

$v_2/v_1 = k_2/k_1$ where $k_1$, $k_2$ = rate constants at $T_1$ & $T_2$

Combine above equations: $Q_{10} = \frac{10}{T_2 - T_1} \left( \frac{k_2}{k_1} \right)$

- term $k_2/k_1$ can be evaluated from the integrated form of the Arrhenius equation
- $Q_{10}$ for a typical chemical and enzymatic rxn is $\approx 2.0$
  i.e., the rate of the rxn is approx. 2 times for $10°C \uparrow$ in $T$

- Temp coefficient (1st order rxn) can be related to the energy of activation by means of the above equations
- for special case where rates are measured at 2 temp. $10°C$ apart ($T_2 - T_1 = 10$) then:

$$E_a = \frac{2.303 RT_1 T_2 \log Q_{10}}{10}$$
Derivation of Rate Equations
- can be simplified by dealing with initial velocities so specific reverse rxn can be ignored

- however, as the extent of rxn ↑'s the experimentally determined results will differ from values predicted

Three methods (1) Direct Computation; (2) Rapid Equilibrium Approx; (3) Steady State Approx.

(1) Direct Computation
- Write differential equations for various species present and solving by computer methods
  - each equation represents the rate of change of a species (difference between rate of formation and rate of consumption)

(2) Rapid Equilibration Approx.
  Assumptions
  (1) There is a rate-determining step
  (2) Conc’n of intermediates preceding this step governed by eqm conditions

  (1) Rate-determining step (RDS)
  - a step in rxn sequence which is significantly slower than all of the other steps
  - overall rate of rxn is determined by rate of rate-determining step

  - simplifies derivation of rate equation

  - RDS is an obvious bottleneck in the reaction sequence and slows rxn at that point
    - provides adequate time for faster steps in the rxn sequence to attain equilibrium

(3) Steady State Approx.
  Assumptions
  (1) Exists a RDS in the mechanism
(2) Concentration of intermediates preceding this step governed by steady state conditions

- difference in equation assumption and steady state assumption lies in assumption #2, above.

(1) Concept of Steady State

Rate of Formation = Rate of Consumption

- A reactive intermediate will undergo reaction as soon as it is formed

- concentration of intermediate never builds up to a significant level during the course of the reaction

- intermediate reacts as rapidly as it is being formed; its concentration is small and constant; hence, the rate of change of its concentration is zero

- this concept widely used in enzymology because:

  (a) some intermediates in enzyme reactions are reactive intermediates for which the steady state treatment seems appropriate

  (b) time made available by slowing down a reaction (due to the RDS) is probably insufficient to allow the intermediates preceding the RDS to attain true equilibrium

  (c) inability to establish equilibrium pertinent for biochemical systems, therefore characterized by a series of consecutive reactions with the product of one reaction serving as a reactant for another reaction

There are two basic treatments of enzyme kinetics: (1) Michaelis-Menten—a rapid equilibrium approximation and (2) Briggs-Haldane—a steady-state assumption.
(1) **Introduction**
- The M-M treatment (rapid equilibrium approximation) is unsuitable for many biochemical systems because:

  (a) Many intermediates in enzyme reactions are reactive intermediates for which the steady-state treatment seems more appropriate

  (b) The time made available by slowing down a reaction (existence of a RDS) is probably insufficient to allow the intermediates, preceding the RDS, to attain a true equilibrium

  (c) Biochemical systems frequently consist of a series of consecutive reactions in which the P of one reaction serves as the reactant for a subsequent reaction; an equilibrium treatment does not appear to be appropriate

- reactive intermediates, ES, is more appropriately evaluated by means of a steady-state rather than an equilibrium treatment

- the RDS $\text{ES} \rightarrow \text{E} + \text{P}$ is not usually slow enough to permit the establishment of a true equilibrium between E, S, and ES.

- lastly, any tendency of E, S, and ES to attain equilibrium is continuously interfered with because any ES formed is siphoned off from the E, S, and ES mixture by being converted to product in the second step of the reaction

- more reasonable to apply the steady-state treatment to assume that the rate of ES formation is equal to the rate of ES consumption over the short time interval defined by the initial velocity

- in 1925 Briggs and Haldane developed the steady-state treatment

(2) **Assumptions**
- The E-S complex is in a steady-state, i.e., the rate at which the complex is being formed from E and S is equal to the rate at which it is consumed by being broken down to E and P; $\frac{d[\text{ES}]}{dt} = 0$
(b) The formation of P is proportional to the concn of ES complex, i.e., the reverse reaction, governed by the reverse rate constant \( k_{-2} \), can be ignored.

implies that the breakdown of ES to E and P is RDS of reaction

implies that reverse reaction \((E + P \rightarrow ES)\) is negligible due to the appropriate conditions i.e., measurement refers to initial velocity

(c) The S concn is much larger than E concn i.e., change in \([S]\) as a result of the formation of ES complex is negligible; the substrate concn is taken to be a constant

-assumptions (b) and (c) are identical to those of M-M treatment

difference in the two treatments lies in the concept of rapid equilibrium between E,S, and ES in the M-M treatment versus the concept of a steady-state for the ES complex in the B-H treatment

(3) Derivation

- assumption (a): \( \frac{d[ES]}{dt} = 0 \) \([K-14] \)

-rate of change of \([ES]\) with time \( (d[ES]/dt) \) represents the difference between the rate of ES formation (appearance) and the rate of ES consumption (disappearance). Must consider all the ways for formation and consumption of ES.

\[
\text{Rate of formation} = k_1[E][S] + k_{-2}[E][P] \quad [K-15]
\]

-based on assumption (b) the second term can be ignored (reverse reaction) so:

\[
\text{Rate of ES formation} = k_1[E][S] \quad [K-16]
\]

\[
\text{Rate of ES consumption} = k_{-1}[ES] + k_2[ES] = (k_{-1} + k_2)[ES] \quad [K-17]
\]

-can rewrite Eq. K-14 by means of Eq. K-16 and K-17:

\[
\frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_2)[ES] = 0 \quad [K-18]
\]

or

\[
k_1[E][S] = (k_{-1} + k_2)[ES] \quad [K-18a]
\]

-conservation equation for total \([E]\) where \([S]\) is constant
\[ [E] = [E_d] + [ES] \quad [K-4] \]

-substituting from Eq. K-4 into K-18(a) and rearranging yields:
\[ ([E] - [ES])[S] = (k_1 + k_2)[ES]/k_1 \quad [K-19] \]

-define a new constant, \( K_M \), such that: \( K_M = (k_1 + k_2)/k_1 \) \quad [K-20]
-called the "Michaelis constant"

-the Michaelis constant is the constant obtained by the B-H treatment; it is not the constant obtained by applying the M-M treatment

-substituting the Michaelis constant into Eq K-19 yields:
\[ ([E] - [ES])[S] = K_M[ES] \quad \text{or} \]

-velocity of reaction based on assumption (b) and so:
\[ v = k_2[ES] \quad [K-7] \quad \text{or} \quad [ES] = v/k_2 \]

-substituting from this equation into K-21 gives the rate equation:
\[ [E][S] - v/k_2 * [S] = K_M * v/k_2 \quad [K-22] \]
-rearranging Eq. K-22 gives
\[ k_2[E][S] - v[S] = K_MV \quad [K-22a] \]

-recall: \( V_{max} = k_2[E] \) \quad [K-9]

-replace first term of K-22a with K-9:
\[ V_{max}[S] - v[S] = K_MV \quad \text{and rearrange} \]
\[ V_{max}[S] = K_MV + v[S] \]
\[ V_{max}[S] = v(K_M + [S]) \]
\[ v = V_{max}[S]/(K_M + [S]) \quad [K-23] \]

-Eq K-23 is the "Michaelis-Menten Equation"
-confusion exists because the M-M equation is derived by Briggs-Haldane using the steady-state approx. not by M-M using the equilibrium approx.

(4) Description of $v$ versus $[S]$ curve

i. When $[S]$ is very small c.f. $K_M$ then Eq. K-23 becomes:

$$v = \frac{V_{max}[S]}{(K_M + [S])} = \frac{V_{max}[S]}{K_M} = \frac{V_{max}}{K_M} = k''[S] \ [K-24]$$

where $k'' = \frac{V_{max}}{K_M} = \text{constant}$

-Eq. K-24 is rate equation for first order reaction with $v \propto [S]$; low concn range of $v$ versus $[S]$ curve

ii. When $[S] >> K_M$ then Eq K-23 becomes:

$$v = \frac{V_{max}[S]}{(K_M + [S])} = \frac{V_{max}[S]}{[S]} = V_{max} \ [K-25]$$

-Eq K-25 is rate equation for a zero order reaction with $v$ independent of $[S]$; high end of $v$ versus $[S]$ curve (Fig K-1b)

iii. Central part of curve in Fig K-1b is described by intermediate range of $S$ concn

-special case when $[S] = K_M$ then Eq K-23 becomes:

$$v = \frac{V_{max}[S]}{(K_M + [S])} = \frac{V_{max}[S]}{[S]} = \frac{V_{max}}{2}$$

-K$_M$ defined as being equal to that substrate concn which gives one half of the maximum velocity of reaction; $K_M$ has units of concn

-the two treatments (Michaelis-Menten and Briggs-Haldane) differ vastly in the underlying concept; a rapid equilibrium c.f. a steady-state

-both yield the same mathematical form for the rate equation but not identical equations

-the 2 constant, $K_M$ and $K_s$ are not identical

-$K_s$ (substrate constant) is a true equilibrium constant, the dissociation constant for the ES complex; $K_s = k_{-1}/k_1$

-two constants, $K_M$ and $K_s$, are identical if, and only if, $k_2 << k_{-1}$; if $k_2 << k_{-1}$,
then, and only then, the steady-state treatment becomes identical to the rapid equilibrium treatment. In that case, and only in that case, the rate of product formation governed by $k_2$ is so slow that $E$, $S$, and $ES$ may be considered to attain a state of equilibrium.

- assume an enzyme reaction is assayed and data plotted to give a hyperbolic curve. One half of $V_{max}$ is located on graph and the $[S]$ yielding this velocity is determined. Is it $K_s$ or $K_M$?

- answer is that it is $K_M$, which is the more general constant of the two. Only under special conditions where $k_2 << k_{-1}$ will $K_M$ become equal to $K_s$.

(1) General Properties
(a) $K_M$ is $[S]$ at which one half of $V_{max}$ of a reaction is observed

(b) $K_M$ has units of concn

(c) $K_M$ is a constant only under rigorously defined conditions of pH, T, ionic strength, etc

- if reaction has 2 or more substrates, the true $K_M$ for a given substrate is that which is observed when all other $S$ are at sat'n concn

- $V_{max}$ is that observed when all substrates are at sat'n concn

(d) Values for $K_M$ vary greatly from E to E
- generally range $10^{-5}$ to 1.0 M

- large $K_M$ means that one half of $V_{max}$ is achieved at a relatively large $S$ concn; a small $K_M$ means that 1/2 of $V_{max}$ occurs at relatively low $S$ concn

(e) $K_M$ is not an equilibrium constant, but rather a complex constant, composed of various rate constants
$K_M = (k_{-1} + k_2)/k_1$ for simple reaction, $E + S \rightleftharpoons ES \rightleftharpoons E + P$

(f) $K_M$ is not a dissociation constant of the ES complex
(g) \( K_M \) is **not** a reciprocal of the affinity (binding, association) constant of the enzyme and the substrate

(h) Physical significance of \( K_M \) cannot be described in general terms; it varies with the specific reaction mechanism under consideration

   - to ascribe physical meaning to the \( K_M \) one must know (1) its description in terms of the various rate constants and (2) the absolute or relative values of these rate constants

(i) For the simplest case: \( K_M = \frac{k_{-1} + k_2}{k_1} \) and is not a true equilibrium constant since the concn of species are not equilibrium concn but **rather** concn existing at steady-state.

(2) **Numerical values**

   - numerical value of \( K_M \) is a useful quantity because:

   (a) \( K_M \) relates velocity of a reaction to \([S]\) and serves to indicate the approximate level of the intracellular \([S]\)

      - true because it is unlikely that intracellular \([S]\) would be significant higher or lower than the \( K_M \)

   (b) \( K_M \) is a characteristic constant for a given \( E \) under defined conditions

      - useful to help identify an enzyme

   (c) \( K_M \) is useful for comparing the activity of different \( E \) and for comparing the "suitability" of alternate substrates for the same \( E \) (a substrate with a lower \( K_M \) is a better substrate)

      - best \( S \) of an \( E \) is one which leads to the highest \( V_{max} \) and the lowest \( K_M \); i.e., a substrate which yields the highest \( V_{max}/K_M \) ratio

   (d) The effective value of \( K_M \) may be altered as a result of ligand binding (allosteric effectors). Unusually high or low \( K_M \) values may be indicative of ligand-induced changes in the \( E \).

   (e) Knowing the value of \( K_M \) permits adjustment of assay conditions, one can vary \( S \) according to the needs of assay, etc. to measure the total \( E \) or \( S \).
(f) \( K_M \) values useful in evaluating the relative roles of forward and reverse reactions in metabolism

**Specificity Constant (SpC)**

- the turnover number of an enzyme (TN or \( k_{cat} \)) is the maximum number of moles of substrate that are converted to product each second per mole of enzyme (or per mole of active sites if the enzyme has more than one active site).

- \( k_{cat} \) is a measure of how rapidly an enzyme can operate once the active site is filled.

\[
 k_{cat} = \frac{V_{max}}{[E]} 
\]

- under physiological conditions, enzymes do not operate at saturating \( S \) conditions.

  - usually the ratio of \( S \) concn to \( K_M \) is in the range of 0.01 to 1.0.

- previously determined that at low [S] then: \( v = \frac{V_{max}[S]}{K_M} \)

- under these conditions, the number of moles of S converted to P per second per mole of enzyme is \( \frac{V_{max}[S]}{K_M}/[E] \), which is the same as \( k_{cat}/K_M \)[S].

- the ratio \( k_{cat}/K_M \) is therefore a measure of how rapidly an enzyme can work at low [S].

- this ratio is referred to as the "specificity constant".

- values for some particularly active enzymes are given in the Table 8.4.

- the specificity constant, \( k_{cat}/K_M \), is useful for comparing the relative abilities of different compounds to serve as a substrate for the same enzyme.

  - if the concn of two S are the same, and are small relative to the values of \( K_M \), the ratio of the rates with the two substrates is equal to the ratio of the specificity constants.

  - another use of SpC is to compare the rate of an enzyme-catalyzed reaction.
with the rate at which the random diffusion of the enzyme and substrate brings the two molecules into collision

-if every collision between a protein and a small molecule resulted in a reaction, the maximum possible value of the second-order rate constant would typically be $10^8$ to $10^9$ M$^{-1}$s$^{-1}$

-some of the values in the above table for the $k_{cat}/K_M$ (second order rate constant) are in this range

-these enzymes have achieved an astonishing state of perfection!

-only way to increase rate is to have the $S$ generated right on the $E$ or in its immediate vicinity or to decrease the size of the $E$ moiety

-first possibility arises when two or more $E$ are combined in a multi-enzyme complex

-the product of one reaction can then be released close to the active site of the next $E$

-second possibility is more difficult since the sizes of $E$ are limited by the need for a certain amount of tertiary structure to create the proper geometry for the active site

-also the large sizes of $E$ are dictated by the need for secondary binding sites for other molecules that act to regulate enzymatic activity
aim is to calculate $K_m$ and $V_{\text{max}}$ using graphs of the terms $v_0$ and $[S]_0$.

(i) Lineweaver-Burk

recall [K23]:
$$v = \frac{V_{\text{max}} [S]}{K_m + [S]}$$

invert:
$$\frac{1}{v} = \frac{K_m}{V_{\text{max}} [S]} + \frac{[S]}{V_{\text{max}} [S]}$$

separate terms:
$$\frac{1}{v} = \frac{K_m}{V_{\text{max}} [S]} + \frac{1}{V_{\text{max}}}$$

so:
$$\frac{1}{v} = \left( \frac{K_m}{V_{\text{max}}} \right) \frac{1}{[S]} + \frac{1}{V_{\text{max}}}$$

plot $\frac{1}{v}$ vs. $\frac{1}{[S]}$

(ii) Hanes

- recall [G2]
$$\frac{1}{v} = \frac{K_m}{V_{\text{max}} [S]} + \frac{1}{V_{\text{max}}}$$

multiply by $[S]$
$$\frac{[S]}{v} = \frac{K_m}{V_{\text{max}}} + [S] \left( \frac{1}{V_{\text{max}}} \right)$$

plot $[S]/v$ vs. $[S]$. 

\quad --slope = $K_m / V_{\text{max}}$
→ best plot to use as indicated by regularity of errors.

(iii) Eadie-Hofstee

recall [G2]:
\[
\frac{1}{v} = \frac{K_m}{V_{max} [S]} + \frac{1}{V_{max}}
\]  

multiply by \(vV_{max}\):
\[
\frac{vV_{max}}{v} = \frac{K_m \cdot vV_{max}}{V_{max} [S]} + \frac{vV_{max}}{V_{max}}
\]

\[
V_{max} = \frac{vK_m}{[S]} + v
\]  

rearrange [14]:
\[
v = V_{max} - K_m \left( \frac{v}{[S]} \right)
\]  

plot \(v\) vs. \(v/[S]\)

slope = \(-K_m\)
(iv) Eadie-Scatchard

recall [G4]: \[ V_{\text{max}} = \frac{vK_m}{[S]} + v \]

divide by \( K_m \):
\[
\frac{V_{\text{max}}}{K_m} = \frac{v}{[S]} + \frac{v}{K_m}
\]

rearrange:
\[
\frac{v}{[S]} = \left( -\frac{1}{K_m} \right) v + \frac{V_{\text{max}}}{K_m}
\]

plot \( v / [S] \) vs. \( v \)

→ also possible to plot kinetic data directly

(i) direct linear plot - \([S]_o \) vs. \( v_0 \)