ENZYMES

0905423 Biochemical Engineering
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First Semester 2016/2017

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Outline of Enzymes

- **Introduction**
  - Features of enzyme catalysis
- **Enzyme Kinetics**
  - Models for simple enzyme kinetics
  - Effect of pH and Temperature
- **Immobilized Enzyme Systems**
  - Methods of immobilization
  - Diffusional limitations
- **Large-Scale Production of Enzymes**
- **Medical and Industrial Utilization of Enzymes**

What is an Enzyme?

- **An enzyme** is a **protein molecule** that is a biological catalyst that catalyzes chemical reactions.
- Enzymes have high molecule weight (15,000< mw< several million Daltons).
- Enzymes are specific, versatile, and very effective biological catalyst, resulting in much higher reaction rates as compared to chemically catalyzed reactions under ambient conditions.

Enzymes

- **Holoenzyme** is an enzyme contains non-protein group.
  - Such non-protein group is either a **cofactor** such as metal ions, Mg, Zn, Mn, Fe
  - or **coenzyme**, such as a complex organic molecule, NAD, or some vitamins.
- **Apoenzyme** is the protein part of holoenzyme.

Holoenzyme = apoenzyme + cofactor (coenzyme)

Enzyme Nomenclature

Enzyme is named by adding the suffix ***–ase*** to

the end of the **substrate** that is to be converted to the desired product.	the **reaction** catalyzed
Example: Urease	**Example: Alcohol dehydrogenase**
changes urea into ammonium carbonate	catalyzes the removal of hydrogen from alcohol

Enzyme Classification

- International Classification of Enzymes by the International Classification Commission in 1864.
- **Enzymes are substrate specific and are classified according to the reaction they catalyze.**

Enzyme Nomenclature, 1992, Academic Press, San Diego, California, ISBN 0-12-227164-5.

http://www.chem.qmul.ac.uk/iubmb/enzyme/

Enzyme Classification

- Enzymes can be classified into six main classes:

1. **Oxidoreductases:** catalyze the oxidation and reduction
   Example: $CH_3CH_2OH \rightarrow CH_3CHO+H^+$

2. **Transferases:** catalyze the transfer of a functional group (e.g. a methyl or phosphate group) from one molecule (called the donor) to another (called the acceptor).
   $A–\mathbf{X} + B \rightarrow A + B–\mathbf{X}$

Enzyme Classification

3. **Hydrolases:** catalyze the hydrolysis of a chemical bond. $A–B + H_2O \rightarrow A–OH + B–H$
   Example: peptide bond

4. **Lyases:** catalyze the breaking of various chemical bonds by means other than hydrolysis and oxidation, often forming a new double bond or a new ring structure
   Example: $CH_3COCO\text{-}OH \rightarrow CH_3COCHO$
   (dehydratase)

Enzyme Classification

5. **Isomerases:** catalyze the interconversion of isomers.
   Example: -

$$\text{glucose-6-phosphate} \xrightarrow{\text{phosphoglucose isomerase}} \text{fructose-6-phosphate}$$

6. **Ligases:** catalyze the joining of two molecules by forming a new chemical bond, with accompanying hydrolysis of ATP or other similar molecules

ATP + L-tyrosine + tRNATyr ↔ AMP + diphosphate + L-tyrosyl-tRNATyr

Mechanism of Enzyme Catalysis

What is a catalyst?

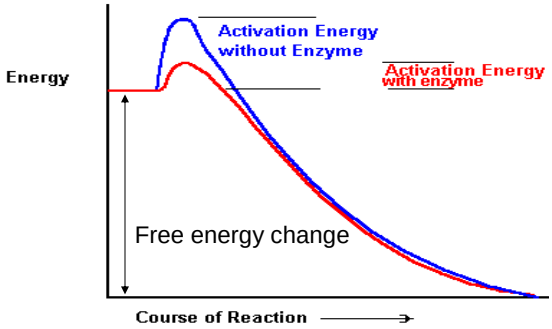
- A catalyst is a substance that accelerates the rate (speed) of a chemical reaction without itself being consumed or transformed.
- It participates in reactions but is neither a chemical reactant nor chemical product.

$$S \leftrightarrow P$$

$$S + C(catalyst) \leftrightarrow P + C(catalyst)$$

Mechanism of Enzyme Catalysis

- Catalysts provide an alternative pathway of lower activation energy for a reaction to proceed whilst remaining chemically unchanged themselves.



Mechanism of Enzyme Catalysis

- Catalysts lower the activation energy of the reaction catalyzed by binding the substrate and forming an catalyst-substrate complex which produces the desired product.

- Catalysts lower the activation energy of the catalyzed reaction, but does not affect free energy change or equilibrium constant.

Mechanism of Enzyme Catalysis

- The reaction rate v is strongly affected by the activation energy of the reaction.

$$v = k * f(S)$$

$f(S)$ denotes the function of substrate concentration

k is the rate constant, expressed by Arrhenius equation:

$$k = A * \exp(-E/RT)$$

A is a constant for a specific system

E is the activation energy

R is the universal gas constant

T is the temperature (in degrees Kelvin).

When E is lowered, k is increased, and so is the rate.

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Mechanism of Enzyme Catalysis

- Catalysts do not affect free energy change or equilibrium constant of the catalyzed reaction.
  - Free energy (G) is the energy stored in the bonds of a chemical that can be harnesses to do work.
  - Free energy change (ΔG) of a reaction refers to the change between the free energy in the product(s) and that in the substrate(s).

Mechanism of Enzyme Catalysis

For an example,

$$S \leftrightarrow P$$

$$S + C(catalyst) \leftrightarrow P + C(catalyst)$$

- For uncatalyzed reaction:

  free energy change $\Delta G_{uncatalyzed} = G(P) - G(S)$

- For catalyzed reaction:

  free energy change $\Delta G_{catalyzed} = G(P) - G(S)$

  Therefore, $\mathbf{\Delta G_{uncatalyzed} = \Delta G_{catalyzed}}$

Mechanism of Enzyme Catalysis

For an example,

$$S \leftrightarrow P$$

$$S + C(catalyst) \leftrightarrow P + C(catalyst)$$

- Free energy change determines the reaction equilibrium – the maximum amounts of the product could be theoretically produced.
- Reaction equilibrium is represented by reaction equilibrium constant $K_{eq} = \gamma_p[P] / \gamma_s[S]$

$$-\Delta G_{uncatalyzed} = RT \ln K_{eq}$$

[ ] represents the concentration of the compounds.

γ_p and $\gamma_s \equiv$ activity coefficients of product and substrate, respectively.

Mechanism of Enzyme Catalysis

- Catalysts can not increase the amounts of the product at reaction equilibrium.
- Catalysts can only accelerate the reaction rate to reach the reaction equilibrium.

Characteristics of Enzyme Catalysis

- Effective to increase the rate of a reaction. Most cellular reactions occur about a million times faster than they would in the absence of an enzyme.
- Specific, act with one reactant (called a substrate) to produce products.
- Regulated from a state of low activity to high activity and vice versa.
  Some enzymes are inhibited by formed product
- Versatile: More than 3000 enzymes are identified

Efficiency of Enzyme Catalysis

For an example, in the reaction of decomposition of hydrogen peroxide H_2O_2, the activation energy E_0 of the uncatalyzed reaction at 20°C is 18 kcal/mol, whereas that for chemically catalyzed (Pt) and enzymatically catalyzed (catalase) decomposition are 13 kcal/mol (E_C) and 7 kcal/mol (E_{En}), respectively.

Compare the reaction rates at these three different conditions.

Enzyme catalysis is efficient!

Assuming the reaction is first order:

- If it takes **1 h** to complete the reaction with enzyme,
- it will take **1.5×10^8 hours = 6,250,000 days = 17,100 years** to complete the same reaction without enzyme catalysis, or
- **30,000 hours = 1250 days = 3.4 years** with chemical catalyst!

Work with your partner to prove that these numbers are correct! (R=1.987 cal/mol. K)

Specificity of Enzyme Catalysis

- Much of the catalytic power of enzymes comes from their bringing substrates together in favorable orientations to promote the formation of the transition states in enzyme-substrate (ES) complexes.

$$E + S \rightarrow ES \rightarrow E + P$$

- The substrates are bound to a specific region of the enzyme called the **active site**.
- Most enzymes are highly selective in the substrates that they bind. The catalytic specificity of enzymes depends in part on the specificity of binding.

Common Features of Enzyme Active Sites

- The active site of an enzyme is the region that binds the **substrates** (and the cofactor, if any).
- It also contains the residues that directly participate in the making and breaking of **bonds**.
  These residues are called the **catalytic groups**.
- The interaction of the enzyme and substrate at the active site promotes the formation of the **transition state** (ES).

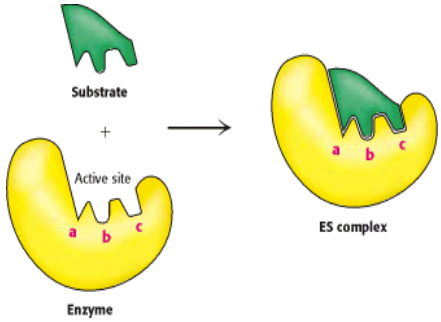
Common Features of Enzyme Active Sites

- The active site is a three-dimensional cleft formed by groups that come from different parts of the amino acid sequence.
- The active site takes up a relatively small part of the total volume of an enzyme.
- The "extra" amino acids serve as a scaffold to create the three-dimensional active site from amino acids that are far apart in the primary structure.
- Substrates are bound to enzymes by multiple weak attractions, like van der Waals forces and hydrogen bonding (much weaker than covalent bonds.)

Specificity of Enzyme Catalysis

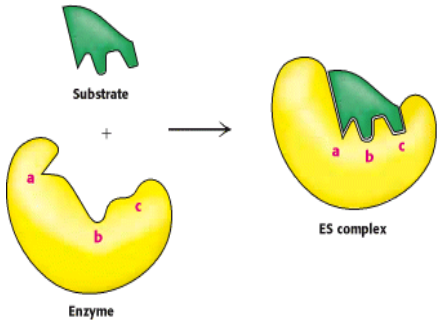
- The specificity of binding depends on the precisely defined arrangement of atoms in an active site.
- **The Lock-and-Key Model** (Emil Fischer, 1890)
  - The enzyme has a fit shape before the substrate is bound.
- **The Induced-Fit Model** (Daniel Koshland, Jr. 1958)
  - Enzymes are flexible and the shapes of the active sites can be markedly modified by the binding of substrate.

Lock-and-Key Model



- In this model, the active site of the unbound enzyme is complementary in shape to the substrate

Induced-Fit Model



- In this model, the enzyme changes shape on substrate binding.
- The active site forms a shape complementary to the substrate only after the substrate has been bound.

Regulated Enzyme Catalysis

Example: Glucose → Ethanol

Used enzymes: Hexokinase, glucose phosphate Isomerase, etc.

- The catalysis is regulated by product concentration.
  - At high product (ethanol) concentration, the enzyme was deactivated when binding with ethanol, the forward reaction is inhibited.

Summary of Introduction

- Enzyme classification
- Enzyme have common catalytic features
  - decrease the reaction activation energy
  - does not affect equilibrium
- Enzyme special catalytic features
  - Efficient
  - Specific
  - Regulated
  - Versatile

ENZYME KINETICS

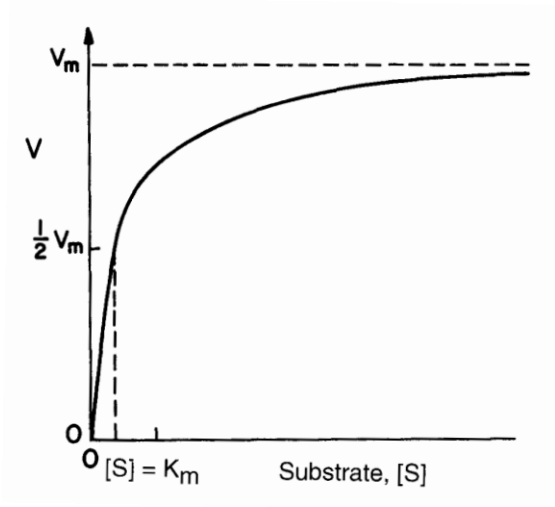
Enzyme Kinetics

- Study the rate of enzyme catalyzed reactions.
- Models for enzyme kinetics
  - Michaelis-Menten kinetics
  - Inhibition kinetics
- Effect of pH and Temperature

Michaelis-Menten Kinetics (Saturation Kinetics)

- This model is based on data from batch reactors with constant liquid volume.
  - Initial substrate, $[S_0]$ and enzyme $[E_0]$ concentrations are known.
  - An enzyme solution has a fixed number of active sites to which substrate can bind.
  - At high substrate concentrations, all these sites may be occupied by substrates or **the enzyme is saturated**.

Saturation Enzyme Kinetics



M-M Enzyme Kinetics

- Saturation kinetics can be obtained from a simple reaction scheme that involves a reversible step for enzyme-substrate complex formation and a dissociation step of the ES complex.

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_2} E + P$$

where the rate of product formation v (moles/l.s, g/l.min) is

$$v = \frac{d[P]}{dt} = k_2[ES]$$

k_i is the respective reaction rate constant.

Enzyme Kinetics

The rate of variation of ES complex is

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

Since the enzyme is not consumed, the conservation equation on the enzyme yields

$$[E_0] = [ES] + [E]$$

$$[E] = [E_0] - [ES]$$

Enzyme Kinetics

$$v = \frac{d[P]}{dt} = k_2[ES]$$

$$[E] = [E_0] - [ES]$$

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

How to use independent variable $[S]$ to represent v?

Enzyme Kinetics

At this point, an assumption is required to achieve an analytical solution.

- The rapid equilibrium assumption

Michaelis - Menten Approach

- The quasi-steady-state assumption

Briggs and Haldane Approach

Michaelis - Menten Approach

The rapid equilibrium assumption:

- Assumes a rapid equilibrium between the enzyme and substrate to form an $[ES]$ complex.

$$k_1[E][S] = k_{-1}[ES]$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

Michaelis - Menten Approach

- The equilibrium constant K'_m can be expressed by the following equation in a dilute system.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

$$K'_m = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

Michaelis - Menten Approach

Then rearrange the above equation,

$$[ES] == \frac{[E][S]}{K_m^{'}}$$

Substituting $[E]$ in the above equation with enzyme mass conservation equation

$$[E] = [E_0] - [ES]$$

yields,

$$[ES] == \frac{([E_0] - [ES])[S]}{K_m^{'}}$$

Michaelis - Menten Approach

$[ES]$ can be expressed in terms of $[S]$,

$$[ES] == \frac{[E_0][S]}{K_m^{'} + [S]}$$

Then the rate of production formation v can be expressed in terms of $[S]$,

$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{K_m^{'} + [S]} = \frac{V_m[S]}{K_m^{'} + [S]}$$

where $V_m = k_2[E_0]$ represents the maximum forward rate of reaction (e.g. moles/L-min).

Michaelis - Menten Approach

- K'_m is often called the Michaelis-Menten constant, mol/L, mg/L.
  - The prime reminds us that it was derived by assuming rapid equilibrium in the step of enzyme-substrate complex formation.
  - Low value indicates high affinity of enzyme to the substrate.

$$K_m' = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

Michaelis - Menten Approach

- What is the value of v when $[S] = K'_m$?
- **Work with your partner to answer this question!**

- K'_m corresponds to the substrate concentration, giving the half-maximal reaction velocity.
- When $[S] = K'_m$,

$$v = \frac{1}{2} V_m$$

Michaelis - Menten Approach

- V_m is maximum forward rate (e.g. mol/L-s)
- It changes with initial enzyme concentration.

$$V_m = k_2[E_0]$$

- It is determined by the rate constant k_2 of the product formation and the initial enzyme concentration.
- But it is not affected by the substrate concentration.
- The unit of k_2 is determined by the unit of enzyme concentration.

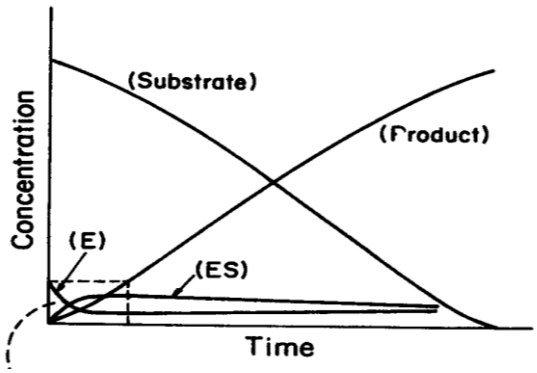
Briggs-Haldane Approach

The quasi-steady-state assumption:

- A system (batch reactor) is used in which the initial substrate concentration [S_0] greatly exceeds the initial enzyme concentration $[E_0]$.
- Since $[E_0]$ is so small,

$$d[ES]/dt \approx 0$$

- It is shown that in a closed system the quasi-steady-state hypothesis is valid after a brief transient if $[S_0] >> [E_0]$.



The quasi-steady-state hypothesis is valid after a brief transient if $[S_0] >> [E_0]$.

Briggs-Haldane Approach

- With such assumption, the equation representing the accumulation of $[ES]$ becomes

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] \approx 0$$

- Solving this algebraic equation yields

$$[ES] == \frac{k_1[E][S]}{k_{-1} + k_2}$$

Briggs-Haldane Approach

Substituting the enzyme mass conservation equation

$$[E] = [E_0] - [ES]$$

in the previous yields

$$[ES] == \frac{k_1([E_0] - [ES])[S]}{k_{-1} + k_2}$$

Using $[S]$ to represent $[ES]$ yields

$$[ES] == \frac{[E_0][S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$$

Briggs-Haldane Approach

Then the product formation rate becomes

$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$$

Grouping the constants results in: $v = \frac{V_m[S]}{K_m + [S]}$

where $V_m = k_2[E_0]$ same as that for rapid equilibrium assumption, and $K_m = \frac{k_{-1} + k_2}{k_1}$

- When $k_2 \ll k_{-1}$, $K_m = K_m' = \frac{k_{-1}}{k_1}$

Comparison of the Two Approaches

	Michaelis-Menten	Briggs-Haldane
Assumption:	$k_1[E][S] = k_{-1}[ES]$	$d[ES]/dt \approx 0$
Equation:	$v = \frac{V_m[S]}{K_m' + [S]}$	$v = \frac{V_m[S]}{K_m + [S]}$
Maximum forward reaction rate:	$V_m = k_2[E_0]$	$V_m = k_2[E_0]$
Constant:	$K_m' = \frac{k_{-1}}{k_1}$	$K_m = \frac{k_{-1} + k_2}{k_1}$

when $k_2 \ll k_{-1}$, $K_m = K_m' = \frac{k_{-1}}{k_1}$

Fumarase

- The enzyme, fumarase, has the following kinetics constants:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

- where $k_1 = 10^9\ M^{-1}s^{-1}$, $k_{-1} = 4.4 \times 10^4\ s^{-1}$, $k_2 = 10^3\ s^{-1}$

a. What is the value of the Michaelis constant for this enzyme? What is the Km in BH approach?

b. At an enzyme concentration of 10^{-6} M, what will be the initial rate of product formation at a substrate concentration of 10^{-3}M? Calculate them using the two approaches.

Experimentally Determining Rate Parameters for Michaelis-Menten Type Kinetics

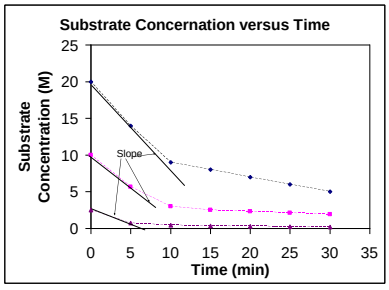
$$v = \frac{V_m[S]}{K_m + [S]}$$

- The determination of V_m and K_m are typically obtained from *initial-rate experiments*.
  - A batch reactor is charged with known initial concentrations of substrate $[S_0]$ and enzyme $[E_0]$ at specific conditions such as T, pH, and Ionic Strength.
  - The product or substrate concentration is plotted against time.
  - The initial slope of this curve is estimated:
    $v = d[P]/dt|_{t=0} = -d[S]/dt|_{t=0}$

Experimentally Determining Rate Parameters for Michaelis-Menten Type Kinetics

$$v = \frac{V_m[S]}{K_m + [S]}$$

$$v = d[P]/dt|_{t=0} = -d[S]/dt|_{t=0}$$



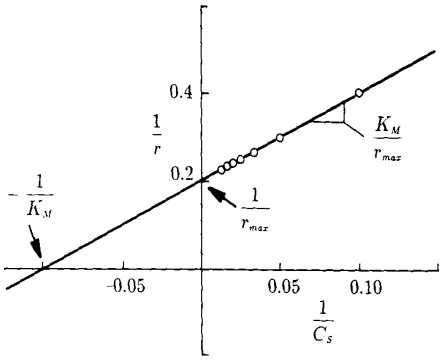
- The value v depends on the values of $[S_0]$ and $[E_0]$

Lineweaver-Burk Plot (Double-Reciprocal Plot)

$$v = \frac{V_m[S]}{K_m + [S]}$$

- Linearizing it in double-reciprocal form:

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m}\frac{1}{S}$$

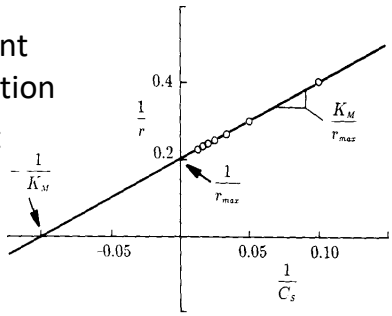


Lineweaver-Burk Plot (Double-Reciprocal Plot)

- slope = K_m/V_m
- y-intercept = $1/V_m$.

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m}\frac{1}{S}$$

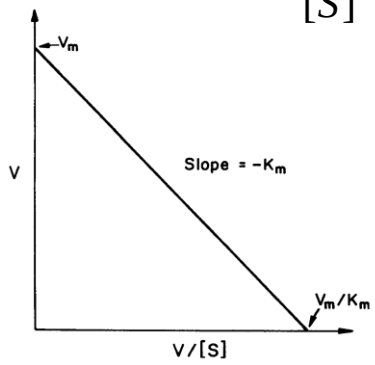
- This plot gives good estimate of V_m but not necessarily on K_m
  - gives undue weight to inaccurate measurement made at low concentration
  - give insufficient weight to more accurate measurements made at high concentration.



Eadie-Hofstee Plot

- the slope = – K_m
- y-axis intercept = V_m
- Can be subject to large error since both coordinates contain dependent variable v, but there is less bias on points at low $[S]$.

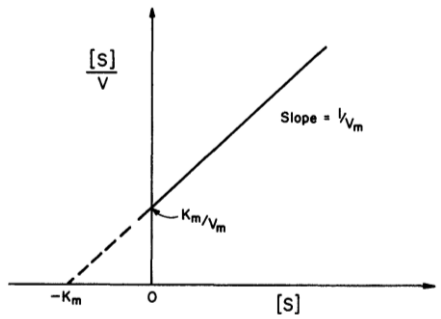
$$v = V_m - K_m \frac{v}{[S]}$$



Hanes-Woolf (Langmuir) Plot

- slope is = $1/V_m$
- y-axis intercept = K_m/Vm
- better fit: even weighting of the data

$$\frac{[S]}{v} = \frac{K_m}{V_m} + \frac{1}{V_m}[S]$$



$$V_m = k_2[E_0]$$

- The unit of V_m is the same as that of a reaction rate (moles/l-min, g/l-s)
- The dimension of k_2 must reflect the units of $[E_0]$
  - if enzyme is highly purified, it may be possible to express $[E_0]$ in mol/l, g/l, then k_2 is in 1/time.
  - if the enzyme is crude, its concentration is in units.
    - A "unit" is the amount of enzyme that gives a predetermined amount of **catalytic activity** under specific conditions.
      - (Textbook, Bioprocessing Engineering, M. Shuler, p.66-67)
  - if V_m is in mmol/ml-min and $[E_0]$ is in units/ml, then k_2 should be in mmol/unit-min

Enzyme Activity

- **Specific Activity** is the number of units of activity per amount of **total protein**.
- Example: A crude cell lysate might have a specific activity of 0.2 units/mg or ml protein upon which purification may increase to 10 units/mg or ml protein.
- One unit would be formation of one µmol product per minute at a specific pH and temperature with a substrate concentration much greater than the value of K_m.