

KINETICS

(Reference: The Chemical Reactor Omnibook. Octave Levenspiel. 1993. OSU Book Stores, Inc., Corvallis, OR. ISN: 0-88246-160-5)

The rate equation: $aA + bB \rightarrow rR + sS$ (1)

Reaction rate for reactant A:

$$-r_A = \left| \begin{array}{l} - \frac{1}{V} \frac{dN_A}{dt} \\ \text{disappearance} \end{array} \right| = \frac{\text{(Amount of A disappearing)}}{\text{(volume) (time)}} \frac{\text{mol}}{\text{m}^3 \text{ s}} \quad (2)$$

$$-r_A = f [\text{temperature terms, conc. terms}] = \underbrace{k}_{\substack{\text{rate} \\ \text{constant}}} C_A^a = k_0 e^{\underbrace{-E/RT}_{\text{temp term}}} C_A^a \quad (3)$$

Let's look at the temperature dependent and concentration dependent terms of the rate.

1. Temperature dependency is often fitted by an Arrhenius law expression:

The relationship between E_a and T was formulated by Arrhenius in 1889.

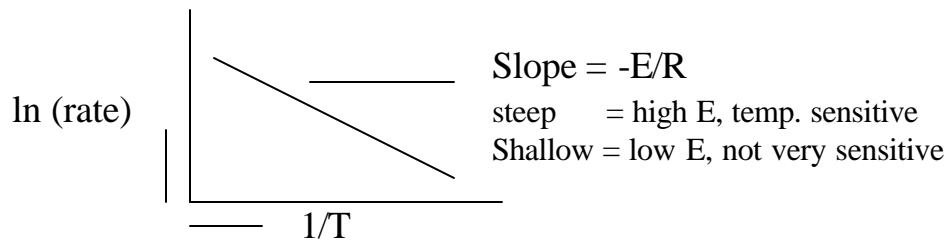
$$k = k_0 e^{-E/RT} \quad (\text{Arrhenius Eqn}) \quad (4)$$

where E = activation energy [J/ mole], k_0 = Arrhenius frequency factor

R = gas constant [8.314 J/mol - K, 8.314 Pa - m³/mol - K, 1.987 cal/mol - K]

Rearrange eqn (4): $\ln \text{rate} = \ln k_0 - E/R (1/T)$ (5)

Semi-log Plot $\ln k$ versus $1/T$ at fixed (constant) C_A ; slope = $-E/R$



E_a = determines that fraction of the total No. of molecules that are sufficiently activated to undergo reaction. The magnitude of E_a is a direct determinant of the RATE of reaction. The larger is E_a , the slower is the reaction.

At two different temperatures, but same concentration:

$$\ln \frac{\text{rate}_1}{\text{rate}_2} = \ln \frac{k_1}{k_2} = \frac{E}{R} \left[\frac{1}{T_2} - \frac{1}{T_1} \right] \quad (6)$$

High E values (200 – 400 kJ/mol) are typical of combustion and free radical reactions.
 Low E values (20 – 80 kJ/mol) are typical of enzyme, cellular and life related reactions.

Example: Given: Rate of reaction $A + B \rightarrow P + Q$ at 35 C is twice the value s the rate at 25 C. Find: Calculate the activation energy (E).

Solution: $\ln k_1/k_2 = E/R (1/308 - 1/298)$; $E = \ln 1/2 (1.987) (1/[1/308 - 1/298])$
 $E = -0.693 (1.987 \text{ cal/mol}) (1/[-0.0001]) = \underline{13,770 \text{ cal/mole}}$

Another question – Is this E value indicative of combustion or enzyme reaction?

2. Concentration and Concentration dependency is often handled by a power rate law expression:

$$-r_A = k C_A^0 \quad (\text{zero order reaction}) \quad (7)$$

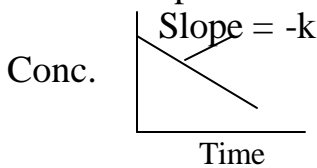
$$-r_A = k C_A^1 \quad (\text{first order reaction}) \quad (8)$$

$$-r_A = k C_A^2 \quad (\text{second order reaction}) \quad (9)$$

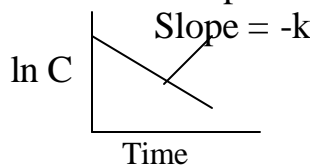
$$-r_A = k C_A C_B \quad (\text{second order reaction that is 1}^{\text{st}} \text{ wrt A \& 1}^{\text{st}} \text{ wrt B}) \quad (10)$$

$$-r_A = k C_A^n \quad (\text{n order reaction})$$

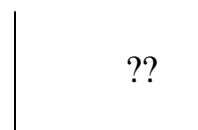
Zero order plot:



First order plot:



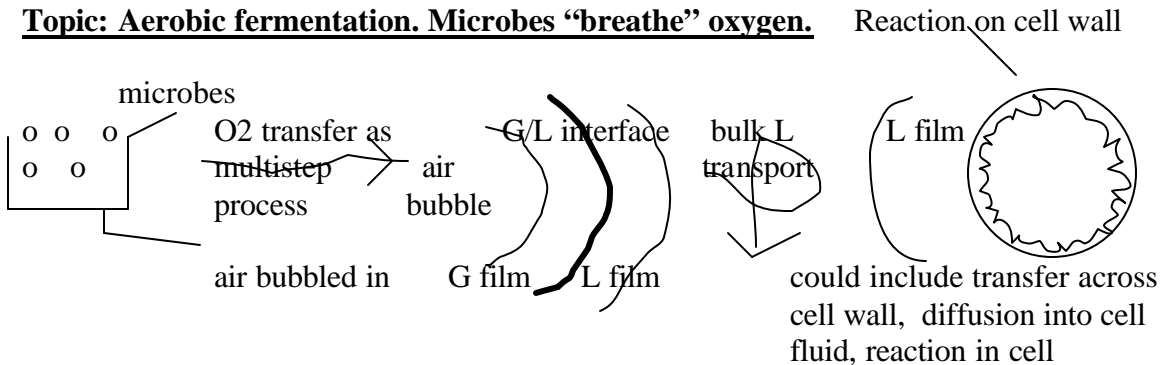
Second order plot:



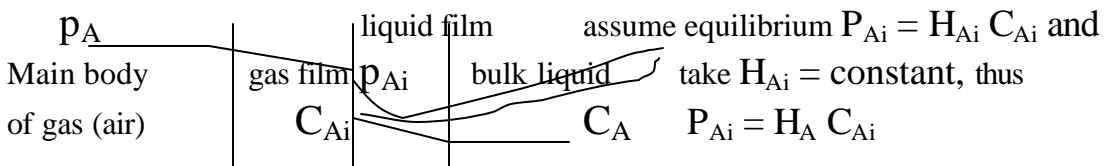
Derive the integrated form of equations for zero, first, second, and n orders.

Multiphase Reactors (Chapter 11)

Topic: Aerobic fermentation. Microbes “breathe” oxygen.

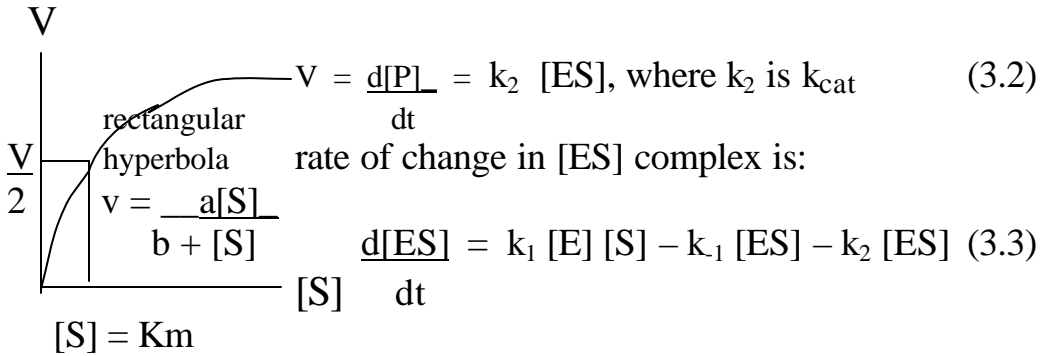


$$-r_A = -\frac{1}{V} \frac{dN_A}{dt} = \frac{\text{mole A reacted}}{\text{volume reactor fluid-time}}$$



3.3.2 (p. 61)

2. Two major approaches used for a rate expression for eq. 3.1
 - i. **Rapid-equilibrium**
 - ii. **Quasi-steady-state approach**
3. Both approaches share initial steps in deriving rate expression, where rate of product formation, in moles/l-sec is:



Since the enzyme [E] is NOT consumed, the conservation equation on the enzyme yields: [E] = [E₀] - [ES] (3.4)

AT THIS POINT, AN ASSUMPTION IS REQUIRED TO ACHIEVE AN ANALYTICAL SOLUTION

3.3.2.1 (p.61) – **Rapid Equilibrium Assumption** – between [E] and [S] to form [ES] complex (Henri, Michaelis & Menten)

1. Use equilibrium coefficient to express [ES] in terms of [S]
2. $K_m = k_{-1} / k_1 = \frac{[E][S]}{[ES]}$ where K_m = dissociation const (3.5)

3. Since [E] = [E₀] - [ES] (conserved enzyme), then

4. $[ES] = \frac{[E_0][S]}{(k_{-1} / k_1) + [S]}$ (3.6)

5. $[ES] = \frac{[E_0][S]}{K_m + [S]}$ (3.7)

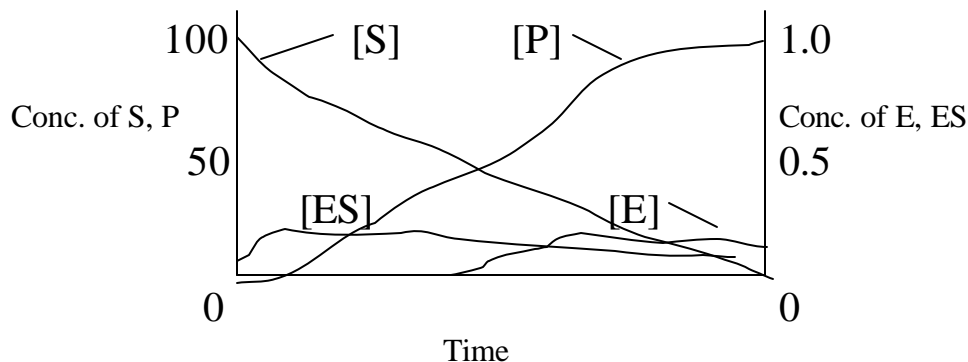
6. Substituting eq. 3.7 into 3.2 yields:

$$V = \frac{d[P]}{dt} = k_2 \frac{[E_0][S]}{K_m + [S]} = \frac{V_m [S]}{K'_m + [S]}, \text{ where } V_{max} = k_2 [E_0] \quad (3.8)$$

7. Let's look at V_{max} :
 - a. Value increases with an increase in enzyme
 - b. Value does NOT increase with an increase in substrate
8. Let's look at K_m' :
 - a. Defined as the Michaelis-Menten constant
 - b. Prime (') reminder of assumption of RAPID EQUIL. in first step
 - c. Corresponds to [S] giving half-maximum reaction velocity

d. Low K_m indicates that E has high affinity for S

3.3.2.2 (p. 62) – **Quasi-Steady-State Assumption** – (G.E. Briggs and J.B.S. Haldane)
Reference: C.F. Water and M.F. Morales, “An Analogue Computer Investigation of Certain Issues in Enzyme Kinetics,” J. Biological Chemistry, 239:1277-1283, 1964.



After a transient moment, [S] and [P] change much more rapidly than [E] or [ES].
Therefore, assume no change with respect to time compared with [S] and [P],
Thus Steady State with respect to $d[ES]/dt$

Rewrite eqn (3.3) using eqm (3.4) as

$$\frac{d[ES]}{dt} = k_1 (E_o - [ES]) [S] - k_{-1} [ES] - k_2 [ES] \quad (3.3a)$$

$$\frac{d[ES]}{dt} = 0 = k_1 E_o [S] - k_{-1} [ES] [S] - k_1 [ES] - k_2 [ES] \quad (3.3b)$$

$$k_1 [E_o] [S] = (k_{-1} [S] + k_{-1} + k_2) [ES] \quad (3.3c)$$

$$\text{Solve for [ES]: } [ES] = \frac{k_1 [E_o] [S]}{K_1 [S] + k_{-1} + k_2} \quad (3.3d)$$

$$\text{Divide by } k_1: [ES] = \frac{[E_o] [S]}{[S] + \frac{k_{-1} + k_2}{k_1}} \quad (3.3e)$$

Introduce velocity, $v_f = k_2 [ES]$, so multiply both sides by k_2

$$V_f = k_2 [ES] = k_2 \frac{[E_o] [S]}{[S] + \frac{k_{-1} + k_2}{k_1}} \quad (3.3f)$$

And $V_{max} = k_2 E_o$, so

$$v = \frac{V_{max} [S]}{K_m + [S]} \quad \text{Michaelis-Menton Equation} \quad (3.12.b)$$

rate const = K_m

Use of K_m (versus K'_m) from derivation of steady-state assumption, is more common in the literature. [$K'_m = k_{-1}/k_1$]. It is generally impossible to tell whether K_m or K'_m is more suitable. End of notes from Walter and Morales, 1964, reference.

Derivation provided in textbook (pp. 62-63)

- Initial $[S] > 100 [E]$ (see Fig. 3.4, p. 63)
 1. Since $[E_0]$ is small, $d[ES]/dt \approx 0$ (QSS assumption)

2. Apply QSS to eq. 3.3 →

$$[ES] = \frac{k_1 [E] [S]}{k_{-1} + k_2} \quad (3.9)$$

3. Substitution of Conservation Eq. 3.4 into Eq 3.9, yields

$$[ES] = \frac{k_1 ([E_0] - [ES]) [S]}{k_{-1} + k_2} \quad (3.10)$$

4. Solve Eq. 3.10 for $[ES]$:

$$[ES] = \frac{[E_0] [S]}{\frac{k_{-1} + k_2}{k_1} + [S]} \quad (3.11)$$

5. Substitute Eq. 3.11 into 3.2 yields:

$$v = \frac{d[P]}{dt} = k_2 \frac{[E_0] [S]}{\frac{k_{-1} + k_2}{k_1} + [S]} \quad (3.12a)$$

or,
$$v = \frac{V_m [S]}{K_m + [S]} \quad (3.12b)$$

Where K_m indicates the QSS assumption, and will be used (p. 63).

Using Eqn 3.12b, consider three cases of $[S]$ with respect to K_m :

1. $[S] \gg K_m$

$$v = \frac{V_m [S]}{K_m + [S]} = \frac{V_m [S]}{[S]} = V_m \quad \text{Zero Order}$$

2. $[S] = K_m$

$$v = \frac{V_m [S]}{K_m + [S]} = \frac{V_m [S]}{2 [S]} = \frac{V_m}{2}$$

3. $[S] \ll K_m$

$$v = \frac{V_m [S]}{K_m + [S]} \approx \frac{V_m [S]}{K_m} = \frac{V_m}{K_m} [S] \quad \text{First Order}$$

To define the relationship between v and $[S]$, we need to determine two constants

1. K_m
2. V_{max}

3.3.3 - Experimentally Determined Rate Parameters for M-M Type Kinetics (p.64)

1. Use INITIAL RATE experiments in batch reactors
 - a. "Charge" reactor with INITIAL known $[E_0]$ and $[S_0]$
 - b. Record and plot $[S]$ or $[P]$ versus time
 - c. Estimate INITIAL slope of the plot, i.e.,
 - i. $v = d[P]/dt|_{t=0} = -d[S]/dt|_{t=0}$
 - d. Obtain many values of v and $[S]$ for different $[S]$, $[E]$
 - e. Can use a plot illustrated in Fig. 3.3 (p. 61)
 - f. Other methods are used for accurate determination of K_m

3.3.3.1 – Double-reciprocal Plot (Lineweaver-Burk plot). See Fig. 3.5 (p.64)

1. Linearize Eq. 3.12b in double-reciprocal form:
2.
$$\frac{1}{v} = \frac{[S] + K_m}{V_m [S]} = \frac{[S]}{V_m [S]} + \frac{K_m}{V_m [S]} = \frac{1}{V_m} + \frac{K_m}{V_m} \frac{1}{[S]} \quad (3.13)$$
3. Plot $1/v$ vs $1/[S]$ (see Fig. 3.5)
 - a. Slope = K_m/V_m
 - b. Y-axis intercept = $1/V_m$
 - c. Data points at low $[S]$ have greater influence than high $[S]$

3.3.3.2 – Eadie-Hofstee Plot (Fig. 3.6, p. 65)

1. Rearrange Eq. 3.12b to yield
 - a.
$$v = \frac{V_m}{\frac{K_m+1}{[S]}} = v(K_m+1) = V_m = \frac{vK_m+v}{[S]}; v = V_m - K_m \frac{v}{[S]} \quad (3.14)$$
2. Plot v versus $(v/[S])$ (see Fig. 3.6)
 - a. Slope = $-K_m$
 - b. Y-axis intercept = V_m
 - c. Can be subject to large errors since both coordinates contain v , but there is less bias at low $[S]$.

3.3.3.3 – Hanes-Woolf Plot (See Fig. 3.7, p.66)

1. Rearrange Eq. 3.12b to yield

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

or,

$$\frac{[S]}{v} = \frac{K_m}{V_m} + \frac{1}{V_m} [S] \quad (3.15)$$

2. Plot $[S]/v$ versus $[S]$
- Slope = $1/V_m$
 - Y-axis intercept = K_m/V_m
 - Determine V_m more accurately than with Eadie-Hofstee

Langmuir Plot (Outside reference: Bioprocess Engineering Principles, P. M. Doran, Academic Press, 1995, p.272)

1. Multiply Lineweaver-Burke Plot equation by $[S]$ to get

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

2. Plot $[S]/v$ versus $[S]$
- Slope = $1/V_m$
 - Y-axis intercept = K_m/V_m
 - Minimizes distortion in experimental error for V_m and K_m

3.3.3.4 – BATCH KINETICS EQUATION DERIVATION (p. 65)

$$v = \frac{d[S]}{dt} = \frac{V_m [S]}{K_m + [S]} \quad (3.12b)$$

Integrate to yield:

$$V_m t = [S_o] - [S] + K_m \ln \frac{[S_o]}{[S]} \quad (3.16)$$

or

$$V_m - \frac{[S_o] - [S]}{t} = \frac{K_m}{t} \ln \frac{[S_o]}{[S]} \quad (3.17)$$

3.3.3.5 – Interpretation of K_m and V_m (p. 66)

1. K_m
 - a. f(rate parameters)
 - b. will change with temp. and pH

2. V_m
 - a. f(k_2 , E_o)

3. Definitions
 - a. Unit – conc. of enzyme that is part of a crude preparation
 - b. Unit – amount of E that gives predetermined amount of activity under specific conditions (e.g., one unit = 1 μmol Prod/min. at specific pH and temp. with $[S] \gg K_m$)
 - c. Specific activity – No. units of activity per amount of total protein (e.g., crude cell lysate with specific activity of 0.2 units/mg protein which when purified is 10 units/mg protein).

d. Example 3.1 – measurement of specific activity.

- i. **Given:** glucoamylase (GA) in crude enzyme prep.
 1. 1 ml prep contains 8 mg protein
 2. added to 9 ml of a 4.44% starch soln
 3. 1 unit activity of GA = amount of E that produces 1 μmol glucose/min in a 4% soln of Lintner starch (pH 4.5, 60C).
 4. Initial rate experiments = 0.6 $\mu\text{mol}/\text{ml}\cdot\text{min}$
- ii. **Find:** Specific Activity of the crude enzyme prep.

iii. Solution:

$$\begin{aligned}
 \text{Specific Activity} &= \frac{10 \text{ ml} \times 0.6 \text{ } \mu\text{mol GA}/\text{ml}\cdot\text{min} = 6 \text{ units}}{(1 \text{ ml protein soln}) (8 \text{ mg/ml})} \\
 &= \frac{6 \text{ units}}{8 \text{ mg protein}} \\
 &= \frac{0.75 \text{ units}}{\text{mg protein}} \quad \leftarrow
 \end{aligned}$$

Note on units – V_m must have units such as $\mu\text{mol product}/\text{ml}\cdot\text{min}$. Since $V_m = k_2 E_o$, the dimensions of k_2 must reflect the definition of units in E_o .
 Above Ex.: Conc. 8 mg protein/10 ml solution \times 0.75 units/mg protein = 0.6 units/ml.
 If, $V_m = 1 \mu\text{mol}/\text{ml}\cdot\text{min}$, then $k_2 = (1 \mu\text{mol}/\text{ml}\cdot\text{min}) / 0.6 \text{ units}/\text{ml} = 1.67 \mu\text{mol}/\text{unit}\cdot\text{min}$

3.3.4 – Models for More Complex Enzyme Kinetics (p. 67)

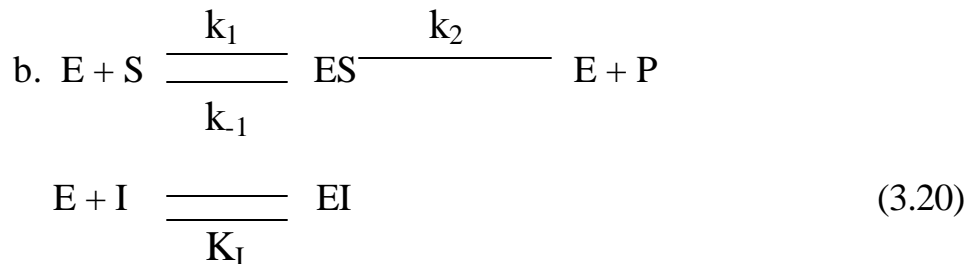
3.3.4.1 – Allosteric Enzymes (see Fig. 3.8, p. 68)

1. Allosteric or cooperative binding - More than one substrate binding site, and the binding of one substrate facilitates binding of other substrate molecules.
2. Rate expression: $v = -\frac{d[S]}{dt} = \frac{V_m [S]^n}{K_m + [S]^n}$ $n = \text{cooperativity coeff}$ (3.18)
3. Determine “n” by rearranging Eq. 3.18: plot $v/(V_m - v)$ versus $\ln[S]$ (Fig. 3.9)

$$\ln \frac{v}{V_m - v} = n \ln[S] - \ln K_m \quad (3.19)$$
4. plot $\ln v/(V_m - v)$ versus $\ln[S]$
 - a. $n = \text{slope of the line}$

3.3.4.2 – Inhibited Enzyme Kinetics (p. 67)

1. Irreversible inhibitors - heavy metals (Pb, Cd, Hg, etc.)
2. Reversible inhibitors –competitive, noncompetitive, uncompetitive
 - a. Competitive inhibitors – substrate analogue that competes with substrate for the active site (increase K_m) (Fig. 3.10)

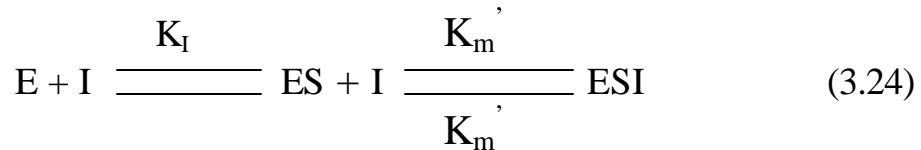
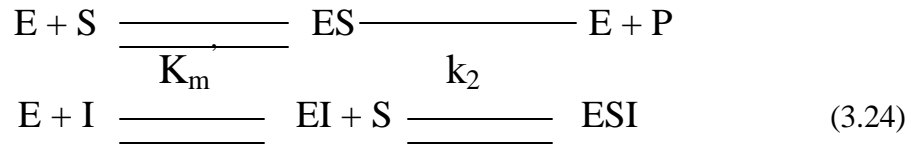


Assuming rapid equilibrium, and with definitions of K_m' and K_I as dissociation constants and conservation of conservation of enzyme:

$$\begin{array}{l}
 \text{i. } K_m' = \frac{[E][S]}{[ES]} \quad K_I = \frac{[E][I]}{[EI]} \quad (3.21) \\
 [E_o] = [E] + [ES] + [EI] \quad \text{and } v = k_2 [ES]
 \end{array}$$

$$\begin{array}{l}
 \text{ii. Can develop rate equation for enzymatic conversion} \\
 v = \frac{V_m [S]}{K_m' \left[1 + \frac{[I]}{K_I} \right] + [S]} \quad (\text{see Fig. 3.10}) \quad (3.22)
 \end{array}$$

- d. Noncompetitive inhibitors – are NOT substrate analogues, but bind to sites other than the active site and reduce enzyme affinity to the substrate (reduction in V_m), (Fig. 3.10)



With definitions:

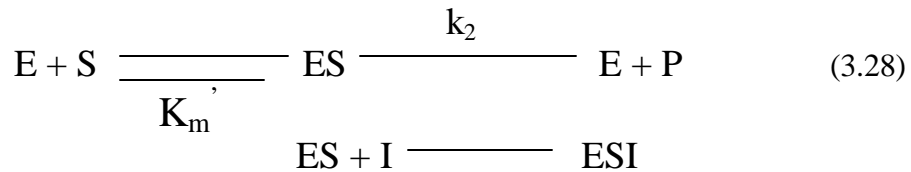
$$K_m' = \frac{[E][S]}{[ES]} = \frac{[E][S]}{[ESI]} \quad K_I = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]} \quad (3.25)$$

$$[E_o] = [E] + [ES] + [EI] + [ESI] \quad \text{and} \quad v = k_2 [ES]$$

Can develop the rate equation for enzymatic conversion:

$$v = \frac{V_m}{[1 + [I]/K_I][1 + K_m'/[S]]} \quad (3.26)$$

- e. Uncompetitive inhibitors – bind to ES only and have NO affinity for the E itself (reduce both V_m and K_m' with greatest reduction in V_m with net reduction in v). (Fig. 3.10)



With definitions:

$$K_m' = \frac{[E][S]}{[ES]} \quad K_I' = \frac{[ES][I]}{[ESI]} \quad (3.29)$$

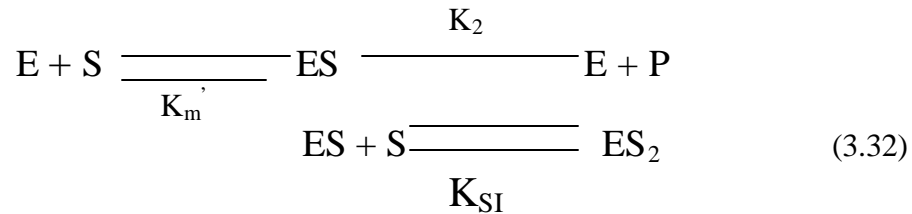
$$[E_o] = [E] + [ES] + [ESI] \quad \text{and} \quad v = k_2 [ES]$$

Can develop the rate equation for enzymatic conversion:

$$v = \frac{\frac{V_m}{[1 + [I]/K_I]} [S]}{\frac{K_m'}{[1 + [I]/K_I]} + [S]} \quad (3.30)$$

(pp. 71 – end of Chapter 3)

a. Substrate Inhibitors–substrate at high concs (Fig. 3.10, 3.11)



With definitions:

$$K_{SI} = \frac{[S][ES]}{[ES_2]} \quad K_m' = \frac{[E][S]}{[ES]} \quad (3.33)$$

Assumption of rapid equilibrium yields:

$$v = \frac{V_m [S]}{K_m' + [S] + [S]^2/K_{SI}} \quad (3.34)$$

At LOW substrate concentration, the term $[S]^2/K_{SI} \ll 1$
 (divide remaining terms by $[S]$)

$$v = \frac{V_m [S]/[S]}{K_m'/[S] + [S]/[S]} = \frac{V_m}{[1 + K_m'/[S]]} \quad (3.35)$$

Or
$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m'}{V_m} \frac{1}{[S]} \quad \text{look at slope \& intercept} \quad (3.36)$$

at HIGH substrate concentration, the term $K_m'/[S] \ll 1$, inhibition is dominant
 (divide remaining terms by $[S]$)

$$v = \frac{V_m [S]/[S]}{[S]/[S] + [S]^2/K_{SI}[S]} = \frac{V_m}{[1 + [S]/K_{SI}]} \quad (3.37)$$

or
$$\frac{1}{v} = \frac{1}{V_m} + \frac{[S]}{K_{SI} V_m} \quad \text{look at slope \& intercept} \quad (3.38)$$

The substrate concentration resulting in the MAXIMUM reaction rate can be determine dby setting $dv/d[S] = 0$. The $[S]_{max}$ is given by:

$$[S]_{max} = \sqrt{K_m' K_{SI}} \quad (3.39)$$

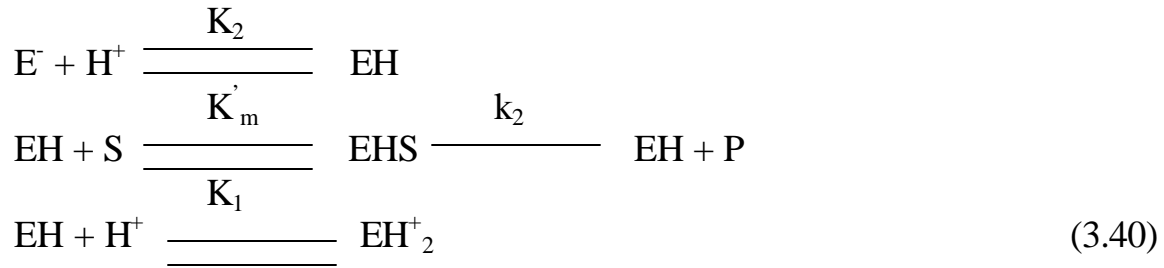
EXAMPLE 3.2 (p. 73) – see textbook

3.3.5 – Effects of pH and Temperature (p. 75)

3.3.5.1 – pH Effects: (Figure 3.14 (p. 77) for trypsin and cholinesterase.)

Ionic groups on active sites, substrate, or E shape/activity

For ionizing enzymes:



With the definition of:

$$K'_m = \frac{[EH][S]}{[EHS]}$$

$$K_1 = \frac{[EH][H^+]}{[EH^+_2]} \quad (3.41)$$

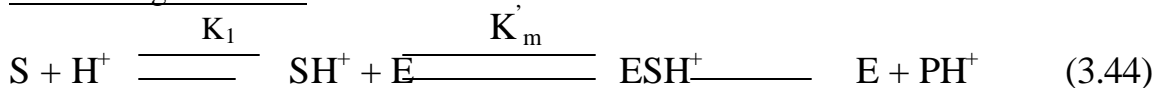
$$K_2 = \frac{[E^-][H^+]}{[EH]}$$

$$E_0 = [E^-] + [EH] + [EHS] + [EH^+_2], \quad v = k_s [EHS]$$

We can derive the following rate expression:

$$v = \frac{V_m[S]}{K'_m [1 + K_2/[H^+] + [H^+]/K_1] + [S]} \quad (3.42)$$

For ionizing substrates:



$$v = \frac{V_m[S]}{K'_m [1 + K_1/[H^+]] + [S]} \quad (3.45)$$

3.3.5.2 – Temperature Effects (See Fig. 3.15, p. 77)

1. Ascending part of Fig. 3.15 = temperature activation, varies according to the Arrhenius equation:

$$v = k_2 [E] \quad (3.46a)$$

$$k_2 = A e^{-E_a/RT} \quad (3.46b)$$

plot $\ln v$ versus $1/T$ yields a line with slope $-E_a/R$

2. Descending part of Fig. 3.15 = temperature inactivation or thermal denaturation,

$$-\frac{d[E]}{dt} = k_d [E] \quad (3.47)$$

or

$$[E] = [E_0] e^{-k_d t} \quad (3.48)$$

k_d (denaturation constant) varies according to Arrhenius equation:

$$k_d = A_d e^{-E_d/RT} \quad (3.49)$$

$$\text{Consequently, } v = A e^{-E_d/RT} E_0 e^{-k_d t} \quad (3.50)$$

3.3.6 – (p. 78) Insoluble Substrates → diffusion of enzyme limits reaction

3.4 – (p. 79) Immobilized Enzyme Systems

3.4.1.1 Entrapment (p. 79, Fig. 3.16)

3.4.1.2 Surface Immobilization (Fig. 3.16, Adsorption, Covalent binding (Table 3.3))

3.4.2 Diffusion limitations in immobilized systems

$$Da = \frac{\text{Maximum rate of reaction}}{\text{Maximum rate of diffusion}} = \frac{V_m'}{k_L [S_b]} \quad (3.52)$$

3.4.2.1 (p.84) Diffusion effects in surface bound enzymes on nonporous supports

$$\text{Fig. 3.17 and 3.18 } J_s = k_L ([S_b] - [S]) = \frac{V_m' [S_s]}{K_m + [S_s]} \quad (3.53)$$

Study Example 3.4

3.4.2.1 (p.86) Diffusion effects in enzymes immobilized in a porous matrix

3.5 – (p. 91) – Large-scale Production of Enzymes

1. Cultivation/production – proteases, pectinases, lactases, lipases, glucose isomerase

2. Separation of cells (intracellular) from media (extracellular)-filtration/centrifugation

3.6 (p. 92) – Medical and Industrial Production of Enzymes

See Table 3.6 (p. 94) for Industrially important enzymes

End Chapter 3.