

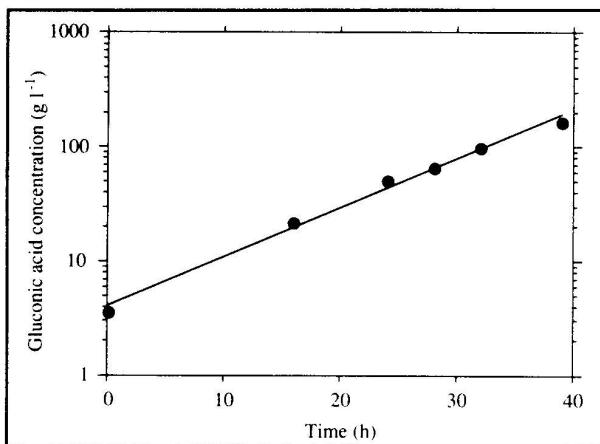
$$r_A = \frac{dC_A}{dt} = k_1 C_A$$

where A denotes gluconic acid. Integrating this equation and taking natural logarithms gives:

$$\ln C_A = \ln C_{A0} + k_1 t.$$

Therefore, a semi-log plot of gluconic acid concentration versus time will give a straight line with slope k_1 . As shown in Figure 11E4.1, the first-order model fits the data well.

Figure 11E4.1 Kinetic analysis of gluconic acid production.



The slope and intercept are evaluated as described in Section 3.4.2; $k_1 = 0.10 \text{ h}^{-1}$, $C_{A0} = 4.1 \text{ g l}^{-1}$.

(b) The kinetic equation is:

$$C_A = 4.1 e^{0.10t}$$

where C_A has units g l^{-1} and t has units h. Therefore, at $t = 20 \text{ h}$, $C_A = 30 \text{ g l}^{-1}$.

11.3.3 Michaelis–Menten Kinetics

The kinetics of most enzyme reactions are reasonably well represented by the *Michaelis–Menten equation*:

$$r_A = \frac{v_{\max} C_A}{K_m + C_A} \quad (11.30)$$

where r_A is the volumetric rate of reaction, C_A is the concentration of reactant A, v_{\max} is the *maximum rate of reaction at infinite reactant concentration*, and K_m is the *Michaelis constant* for reactant A. v_{\max} has the same dimensions as r_A ; K_m has the same dimensions as C_A . Typical units for v_{\max} are $\text{kgmol m}^{-3} \text{ s}^{-1}$; typical units for K_m are kgmol m^{-3} . As defined in Eq. (11.30), v_{\max} is a volumetric rate proportional to the amount of active enzyme present. The Michaelis constant K_m is equal to the reactant concentration at which $r_A = v_{\max}/2$.

K_m values for some enzyme–substrate systems are listed in Table 11.3. K_m and other enzyme properties depend on the source of the enzyme.

If we adopt conventional symbols for biological reactions and call reactant A the *substrate*, Eq. (11.30) can be rewritten in the familiar form:

$$v = \frac{v_{\max} s}{K_m + s} \quad (11.31)$$

where v is the volumetric rate of reaction and s is the substrate concentration. The biochemical basis of the Michaelis–Menten equation will not be covered here; discussion of enzymic reaction models and assumptions involved in derivation of Eq. (11.31) can be found in biochemistry texts [2, 3]. Suffice it to say here that the simplest reaction sequence which accounts for the kinetic properties of many enzymes is:

Table 11.3 Michaelis constants for some enzyme-substrate systems (From B. Atkinson and F. Mavituna, 1991, Biochemical Engineering and Biotechnology Handbook, 2nd edn, Macmillan, Basingstoke)

| Enzyme | Source | Substrate | K_m (mM) |
|------------------------|------------------------------------|------------------|------------|
| Alcohol dehydrogenase | <i>Saccharomyces cerevisiae</i> | Ethanol | 13.0 |
| α -Amylase | <i>Bacillus stearothermophilus</i> | Starch | 1.0 |
| | Porcine pancreas | Starch | 0.4 |
| β -Amylase | Sweet potato | Amylose | 0.07 |
| Aspartase | <i>Bacillus cadaveris</i> | L-Aspartate | 30.0 |
| β -Galactosidase | <i>Escherichia coli</i> | Lactose | 3.85 |
| Glucose oxidase | <i>Aspergillus niger</i> | D-Glucose | 33.0 |
| | <i>Penicillium notatum</i> | D-Glucose | 9.6 |
| Histidase | <i>Pseudomonas fluorescens</i> | L-Histidine | 8.9 |
| Invertase | <i>Saccharomyces cerevisiae</i> | Sucrose | 9.1 |
| | <i>Neurospora crassa</i> | Sucrose | 6.1 |
| Lactate dehydrogenase | <i>Bacillus subtilis</i> | Lactate | 30.0 |
| Penicillinase | <i>Bacillus licheniformis</i> | Benzylpenicillin | 0.049 |
| Urease | Jack bean | Urea | 10.5 |



or

$$v \approx v_{\max} \quad (11.35)$$

where E is enzyme, S is substrate and P is product. ES is the *enzyme–substrate complex*. Binding of substrate to the enzyme in the first step is considered reversible with forward reaction constant k_1 and reverse reaction constant k_{-1} . Decomposition of the enzyme–substrate complex to give the product is an irreversible reaction with rate constant k_2 ; k_2 is known as the *turnover number*. Analysis of this reaction sequence yields the relationship:

$$v_{\max} = k_2 e_a \quad (11.33)$$

where e_a is the concentration of active enzyme. As expected in catalytic reactions, enzyme E is recovered at the end of the reaction.

An essential feature of Michaelis–Menten kinetics is that the catalyst becomes saturated at high substrate concentrations. Figure 11.5 shows the form of Eq. (11.31); reaction rate v does not increase indefinitely with substrate concentration but approaches a limit, v_{\max} . At high substrate concentrations $s \gg K_m$, K_m in the denominator of Eq. (11.31) is negligibly small compared with s so we can write:

$$v \approx \frac{v_{\max} s}{s} \quad (11.34)$$

Therefore, at high substrate concentrations, the reaction rate approaches a constant value independent of substrate concentration; in this concentration range, the reaction is essentially *zero order* with respect to substrate. On the other hand, at low substrate concentrations $s \ll K_m$, the value of s in the denominator of Eq. (11.31) is negligible compared with K_m , and Eq. (11.31) can be simplified to:

Figure 11.5 Michaelis–Menten plot.

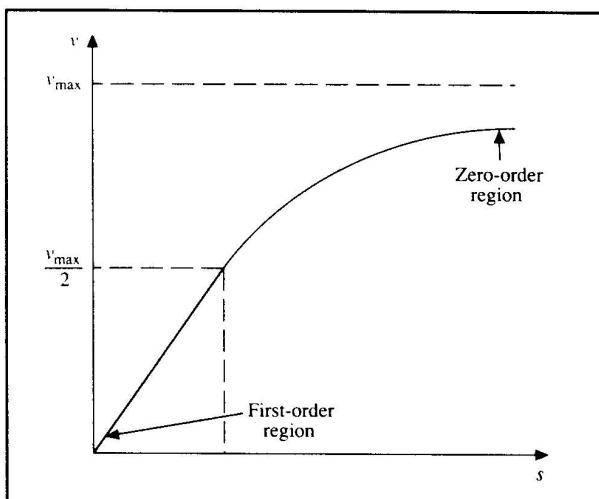
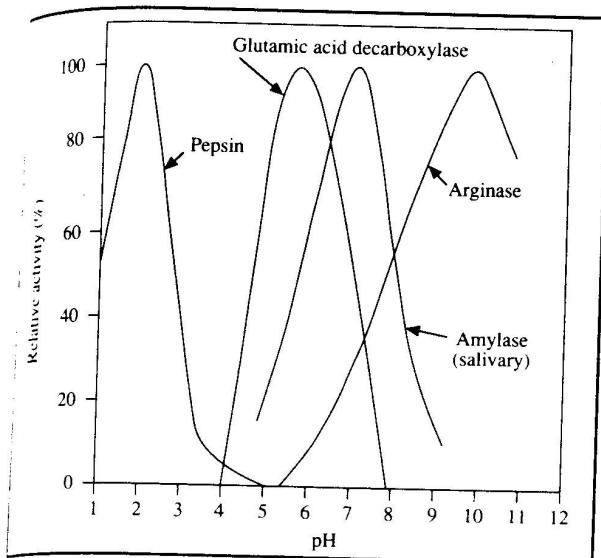


Figure 11.8 Effect of pH on enzyme activity. (From J.S. Prout and S. Simmonds, 1958, *General Biochemistry*, 2nd edn. John Wiley, New York.)



pH has a pronounced effect on enzyme kinetics, as illustrated in Figure 11.8. The reaction rate is maximum at some optimal pH and declines sharply if the pH is moved either side of the optimum value. Kinetic equations have been developed to describe the effect of pH on enzyme activity; however, the influence of pH is usually determined experimentally. Ionic strength and water activity also have considerable influence on rate of enzyme reaction but few correlations are available for prediction of these effects.

11.4 Determining Enzyme Kinetic Constants From Batch Data

To fully specify the kinetics of Michaelis–Menten reactions, two rate constants, v_{\max} and K_m , must be evaluated. Estimating kinetic parameters for Michaelis–Menten reactions is not as straightforward as for zero- and first-order reactions. Several graphical methods are available; unfortunately some do not give accurate results.

The first step in kinetic analysis of enzyme reactions is to obtain data for rate of reaction v as a function of substrate concentration s . Rates of reaction can be determined from batch concentration data as described in Section 11.2. Typically, only *initial rate data* are used. This means that several batch experiments are carried out with different initial substrate concentrations; from each set of data the reaction rate is evaluated

at time zero. Initial rates and corresponding initial substrate concentrations are used as (v, s) pairs which can then be plotted in various ways for determination of v_{\max} and K_m . Initial rate data are preferred for enzyme reactions because experimental conditions such as enzyme and substrate concentrations are known most accurately at the start of the reaction.

11.4.1 Michaelis–Menten Plot

This simple procedure involves plotting (v, s) values directly as shown in Figure 11.5. v_{\max} and K_m can be estimated roughly from this graph; v_{\max} is the rate as $s \rightarrow \infty$ and K_m is the value of s at $v = v_{\max}/2$. The accuracy of this method is usually poor because of the difficulty of extrapolating to v_{\max} .

11.4.2 Lineweaver–Burk Plot

This method uses a linearisation procedure to give a straight-line plot from which v_{\max} and K_m can be determined. Inverting Eq. (11.31) gives:

$$\frac{1}{v} = \frac{K_m}{v_{\max}s} + \frac{1}{v_{\max}} \quad (11.37)$$

so that a plot of $1/v$ versus $1/s$ should give a straight line with slope K_m/v_{\max} and intercept $1/v_{\max}$. This double-reciprocal plot is known as the *Lineweaver–Burk plot*, and is frequently found in the literature on enzyme kinetics. However, the linearisation process used in this method distorts the experimental error in v (see Section 3.3.4) so that these errors are amplified at low substrate concentrations. As a consequence, the Lineweaver–Burk plot often gives inaccurate results and is therefore not recommended [3].

11.4.3 Eadie–Hofstee Plot

If Eq. (11.37) is multiplied by

$$v \left(\frac{v_{\max}}{K_m} \right)$$

and then rearranged, another linearised form of the Michaelis–Menten equation is obtained:

$$\frac{v}{s} = \frac{v_{\max}}{K_m} - \frac{v}{K_m}. \quad (11.38)$$

According to Eq. (11.38), a plot of ν/v versus v gives a straight line with slope $-1/K_m$ and intercept v_{\max}/K_m ; this is called the *Eadie–Hofstee plot*. As with the Lineweaver–Burk plot, the Eadie–Hofstee linearisation distorts errors in the data so that the method has reduced accuracy.

11.4.4 Langmuir Plot

Multiplying Eq. (11.37) by s produces the linearised form of the Michaelis–Menten equation according to Langmuir:

$$\frac{s}{v} = \frac{K_m}{v_{\max}} + \frac{s}{v_{\max}} \quad (11.39)$$

Therefore, a *Langmuir plot* of s/v versus s should give a straight line with slope $1/v_{\max}$ and intercept K_m/v_{\max} . Linearisation of data for the Langmuir plot minimises distortions in experimental error. Accordingly, its use for evaluation of v_{\max} and K_m is recommended [6].

11.4.5 Direct Linear Plot

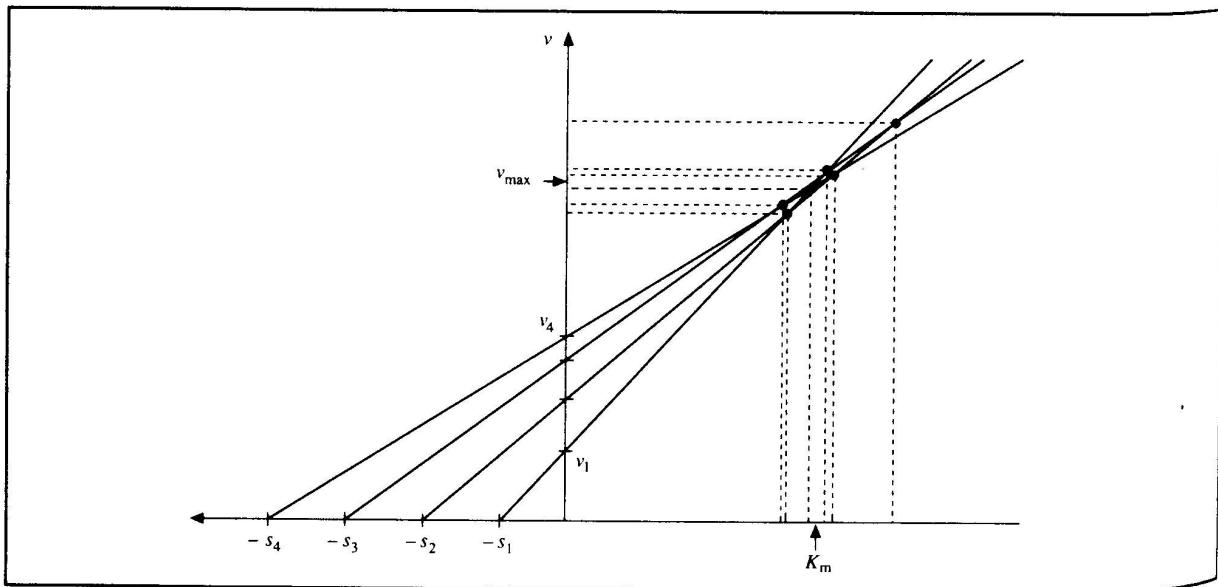
A different method for plotting enzyme kinetic data has been proposed by Eisenthal and Cornish-Bowden [7]. For each

observation, reaction rate v is plotted on the vertical axis against s on the negative horizontal axis. This is shown in Figure 11.9 for four pairs of (v, s) data. A straight line is then drawn to join corresponding $(-s, v)$ points. In the absence of experimental error, lines for each $(-s, v)$ pair intersect at a unique point, (K_m, v_{\max}) . When real data containing errors are plotted, a family of intersection points is obtained. Each intersection gives one estimate of v_{\max} and K_m ; the median or middle v_{\max} and K_m values are taken as the kinetic parameters for the reaction. This method is relatively insensitive to individual erroneous readings which may be far from the correct values. However a disadvantage of the procedure is that deviations from Michaelis–Menten behaviour are not easily detected. It is recommended therefore for enzymes which are known to obey Michaelis–Menten kinetics.

11.5 Kinetics of Enzyme Deactivation

Enzymes are protein molecules of complex configuration that can be destabilised by relatively weak forces. In the course of enzyme-catalysed reactions, enzyme deactivation occurs at a rate which is dependent on the structure of the enzyme and the reaction conditions. Environmental factors affecting enzyme stability include temperature, pH, ionic strength, mechanical forces and presence of denaturants such as solvents, detergents

Figure 11.9 Direct linear plot for determination of enzyme kinetic parameters. (From R. Eisenthal and A. Cornish-Bowden. 1974, The direct linear plot: a new graphical procedure for estimating enzyme kinetic parameters. *Biochem. J.* 139, 715–720.)



and heavy metals. Because the amount of active enzyme can decline considerably during reaction, in many applications the kinetics of enzyme deactivation are just as important as the kinetics of the reaction itself.

In the simplest model of enzyme deactivation, active enzyme E_a undergoes irreversible transformation to an inactive form E_i :



Rate of deactivation is generally considered to be first order in active enzyme concentration:

$$r_d = k_d e_a \quad (11.41)$$

where r_d is the volumetric rate of deactivation, e_a is the active enzyme concentration and k_d is the *deactivation rate constant*. In a closed system where enzyme deactivation is the only process affecting the concentration of active enzyme:

$$\frac{-de_a}{dt} = r_d = k_d e_a. \quad (11.42)$$

Integration of Eq. (11.42) gives an expression for active enzyme concentration as a function of time:

$$e_a = e_{a0} e^{-k_d t} \quad (11.43)$$

where e_{a0} is the concentration of active enzyme at time zero. According to Eq. (11.43), concentration of active enzyme decreases exponentially with time; the greatest rate of enzyme deactivation occurs when e_a is high.

As indicated in Eq. (11.33), the value of v_{\max} for enzyme reaction depends on the amount of active enzyme present. Therefore, as e_a declines due to deactivation, v_{\max} is also diminished. We can estimate the variation of v_{\max} with time

by substituting into Eq. (11.33) the expression for e_a from Eq. (11.43):

$$v_{\max} = k_2 e_{a0} e^{-k_d t} = v_{\max 0} e^{-k_d t} \quad (11.44)$$

where $v_{\max 0}$ is the initial value of v_{\max} before deactivation occurs.

Stability of enzymes is frequently reported in terms of *half-life*. Half-life is the time required for half the enzyme activity to be lost as a result of deactivation; after one half-life, the active enzyme concentration equals $e_{a0}/2$. Substituting $e_a = e_{a0}/2$ into Eq. (11.43), taking logarithms and rearranging yields the following expression:

$$t_h = \frac{\ln 2}{k_d} \quad (11.45)$$

where t_h is the enzyme half-life.

Rate of enzyme deactivation is strongly dependent on temperature. This dependency is generally well described using the Arrhenius equation:

$$k_d = A e^{-E_d/RT} \quad (11.46)$$

where A is the Arrhenius constant or frequency factor, E_d is the activation energy for enzyme deactivation, R is the ideal gas constant, and T is absolute temperature. According to Eq. (11.46), as T increases, rate of enzyme deactivation increases exponentially. Values of E_d are high, of the order 170–400 kJ gmol⁻¹ for many enzymes [5]. Accordingly, a temperature rise of 10°C between 30°C and 40°C will increase the rate of enzyme deactivation by a factor between 10 and 150. The stimulatory effect of increasing temperature on rate of enzyme reaction has already been described in Section 11.3.4. However, as shown here, raising the temperature also reduces the amount of active enzyme present. It is clear that temperature has a critical effect on enzyme kinetics.

Example 11.5 Enzyme half-life

Amyloglucosidase from *Endomycopsis bispora* is immobilised in polyacrylamide gel. Activities of immobilised and soluble enzyme are compared at 80°C. Initial rate data measured at a fixed substrate concentration are listed below.

| Time (min) | Enzyme activity ($\mu\text{mol ml}^{-1} \text{ min}^{-1}$) | |
|---------------|---|--------------------|
| | Soluble enzyme | Immobilised enzyme |
| 0 | 0.86 | 0.45 |
| 3 | 0.79 | 0.44 |
| 6 | 0.70 | 0.43 |
| 9 | 0.65 | 0.43 |
| 15 | 0.58 | 0.41 |
| 20 | 0.46 | 0.40 |
| 25 | 0.41 | 0.39 |
| 30 | — | 0.38 |
| 40 | — | 0.37 |

What is the half-life for each form of enzyme?

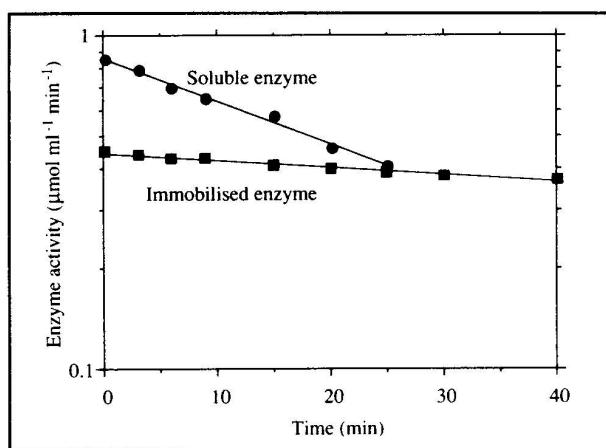
Solution:

From Eq. (11.31), at any fixed substrate concentration, the rate of enzyme reaction v is directly proportional to v_{\max} . Therefore k_d can be determined from Eq. (11.44) using enzyme activity v instead of v_{\max} . Taking natural logarithms gives:

$$\ln v = \ln v_0 - k_d t$$

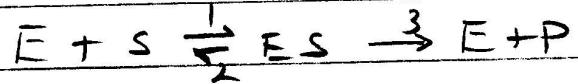
where v_0 is the initial enzyme activity before deactivation. So, if deactivation follows a first-order model, a semi-log plot of reaction rate versus time should give a straight line with slope $-k_d$. The data are plotted in Figure 11E5.1.

Figure 11E5.1 Kinetic analysis of enzyme deactivation.



Enzyme Inhibition Kinetics

Competitive



+

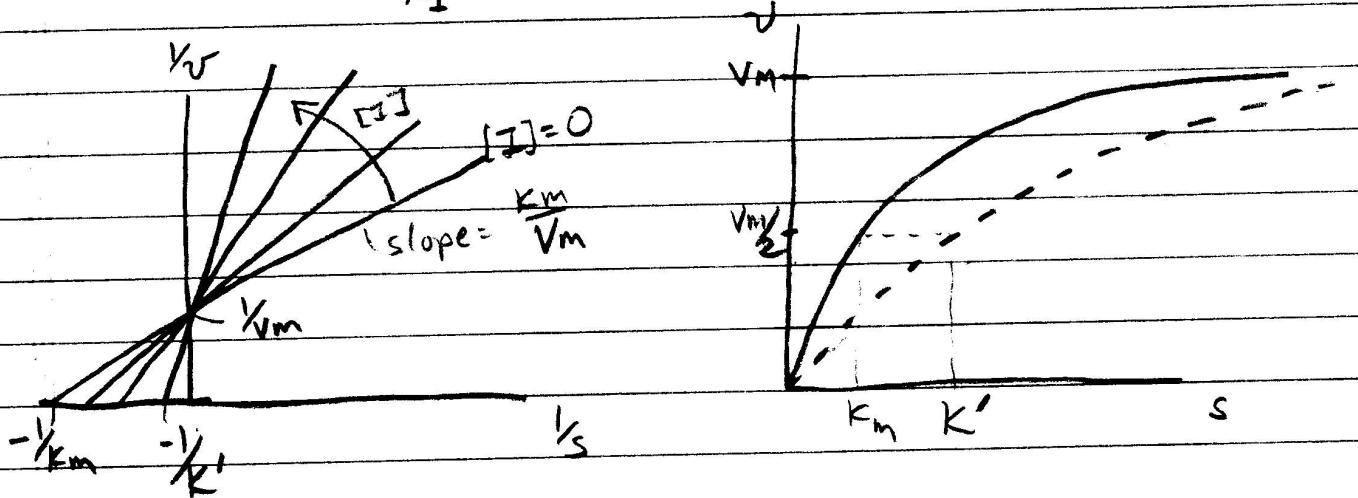
I

I/K_I

EI

$$K_I = \frac{K_S}{K_Y}$$

$$V = \frac{V_m [S]}{K_m + [S] + K_m \frac{[I]}{K_I}} = \frac{V_m [S]}{K' + [S]} \Rightarrow K' = K_m \left(1 + \frac{[I]}{K_I}\right)$$

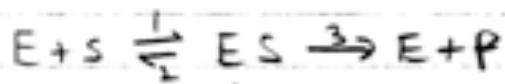


$$\frac{1}{V} = \frac{K_m}{V_m [S]} + \frac{1}{V_m} + \frac{K_m [I]}{V_m K_I} \frac{1}{[S]}$$

$$= \frac{K_m}{V_m} \left(1 + \frac{[I]}{K_I}\right) \frac{1}{[S]} + \frac{1}{V_m}$$

note: intercept unchange
only slope changes

Uncompetitive inhibition



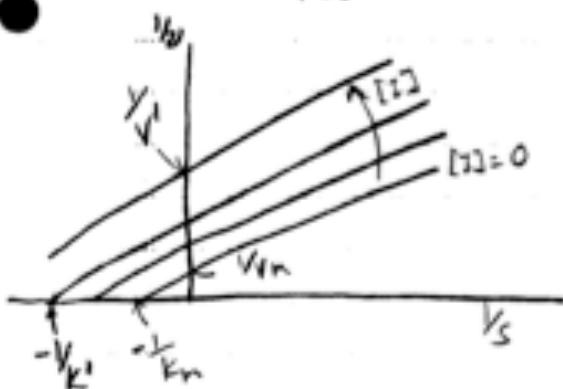
+
I

$\frac{4}{4+K_I}$

ESI

$$K_I = \frac{K_S}{k_4}$$

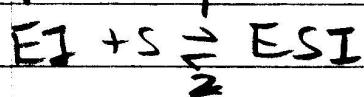
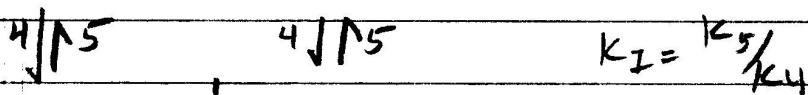
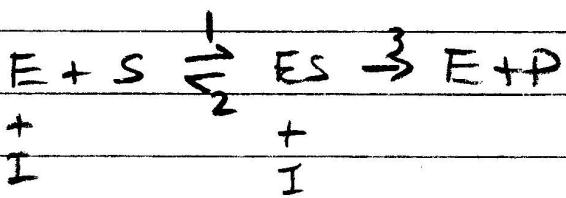
$$V = \frac{\frac{V_m}{(1 + \frac{[I]}{K_I})} [S]}{\frac{K_m}{(1 + \frac{[I]}{K_I})} + [S]} = \frac{V' [S]}{K' + [S]}$$



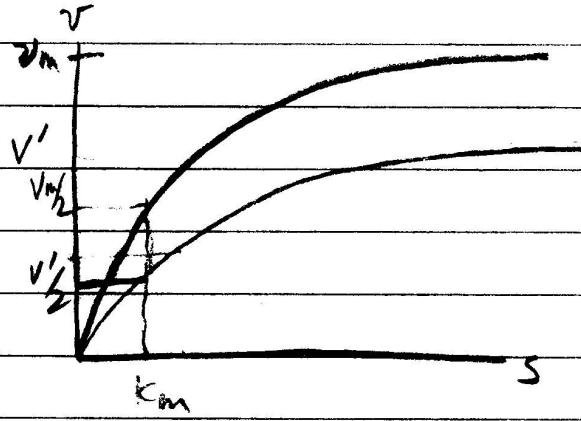
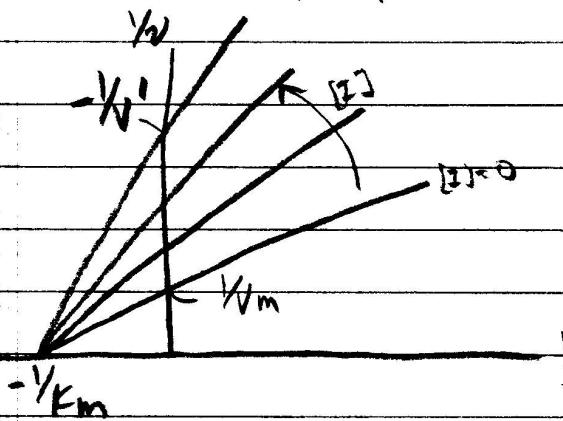
Note: $\frac{V'}{K'} = \frac{V_m}{K_m} \therefore$ slope unchanged

but lines are II, so both intercepts change

Non-competitive

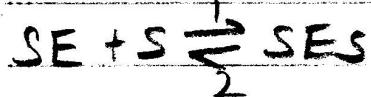
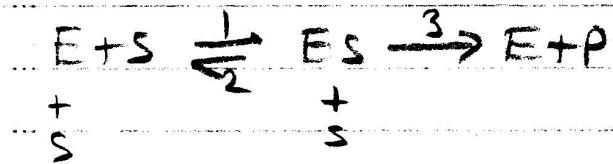


$$V = \frac{V_m \left(\frac{1}{1 + \frac{[I]}{K_I}} \right) [S]}{K_m + [S]} = \frac{V' [S]}{K_m + [S]}$$



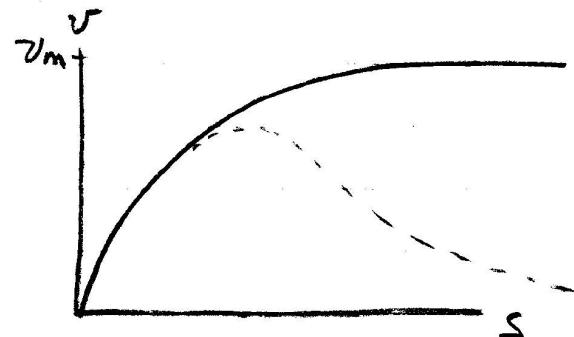
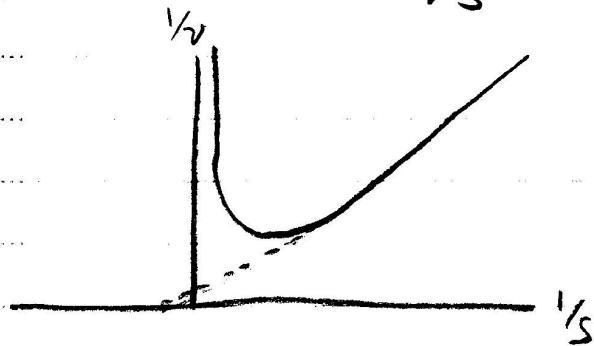
note: K_m unchanged but
slope changes.

Substrate Inhibition



$$K_I = \frac{K_3}{K_4}$$

$$v = \frac{v_m [S]}{K_m + [S] + \frac{[S]^2}{K_s}}$$



Notes:

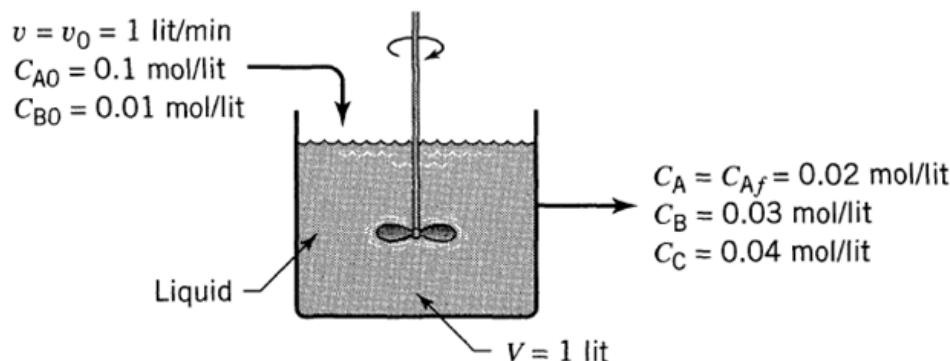
At low $[S]$, $v \approx \frac{v_m [S]}{K_m + [S]}$ \Rightarrow M-M kinetics

At high $[S]$, $v \approx \frac{v_m}{1 + \frac{[S]}{K_s}}$

MFR Practice from Lecture 14

Problem 1

One liter per minute of liquid containing A and B ($C_{A0} = 0.10 \text{ mol/liter}$, $C_{B0} = 0.01 \text{ mol/liter}$) flow into a mixed reactor of volume $V = 1 \text{ liter}$. The materials react in a complex manner for which the stoichiometry is unknown. The outlet stream from the reactor contains A, B, and C ($C_{Af} = 0.02 \text{ mol/liter}$, $C_{Bf} = 0.03 \text{ mol/liter}$, $C_{Cf} = 0.04 \text{ mol/liter}$), as shown in Fig. E5.1. Find the rate of reaction of A, B, and C for the conditions within the reactor.



Solution:

$$-r_A = \frac{C_{A0} - C_A}{\tau} = \frac{C_{A0} - C_A}{V/v} = \frac{0.10 - 0.02}{1/1} = \underline{\underline{0.08 \text{ mol/liter} \cdot \text{min}}}$$

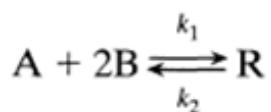
$$-r_B = \frac{C_{B0} - C_B}{\tau} = \frac{0.01 - 0.03}{1} = \underline{\underline{-0.02 \text{ mol/liter} \cdot \text{min}}}$$

$$-r_C = \frac{C_{C0} - C_C}{\tau} = \frac{0 - 0.04}{1} = \underline{\underline{-0.04 \text{ mol/liter} \cdot \text{min}}}$$

Thus A is disappearing while B and C are being formed.

Problem 2

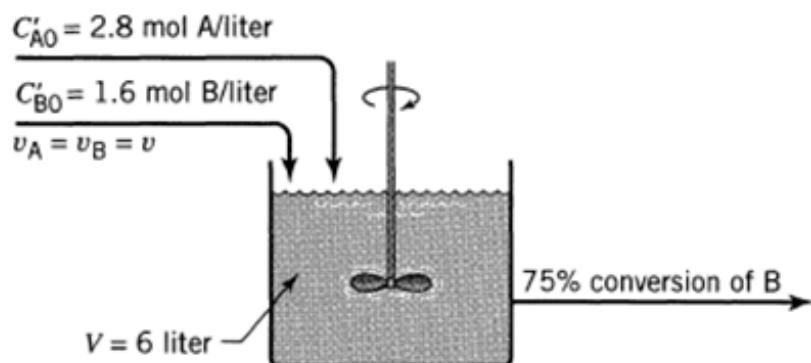
The elementary liquid-phase reaction



with rate equation

$$-r_A = -\frac{1}{2} r_B = (12.5 \text{ liter}^2/\text{mol}^2 \cdot \text{min}) C_A C_B^2 - (1.5 \text{ min}^{-1}) C_R, \quad \left[\frac{\text{mol}}{\text{liter} \cdot \text{min}} \right]$$

is to take place in a 6-liter steady-state mixed flow reactor. Two feed streams, one containing 2.8 mol A/liter and the other containing 1.6 mol B/liter, are to be introduced at equal volumetric flow rates into the reactor, and 75% conversion of limiting component is desired (see Fig. E5.3). What should be the flow rate of each stream? Assume a constant density throughout.



Solution:

$$C_A = 1.4 - 0.6/2 = 1.1 \text{ mol/liter}$$

$$C_B = 0.8 - 0.6 = 0.2 \text{ mol/liter} \quad \text{or} \quad 75\% \text{ conversion}$$

$$C_R = 0.3 \text{ mol/liter}$$

Writing the rate and solving the problem in terms of B we have at the conditions within the reactor

$$\begin{aligned}-r_B &= 2(-r_A) = (2 \times 12.5)C_A C_B^2 - (2 \times 1.5)C_R \\&= \left(25 \frac{\text{liter}^2}{\text{mol}^2 \cdot \text{min}}\right) \left(1.1 \frac{\text{mol}}{\text{liter}}\right) \left(0.2 \frac{\text{mol}}{\text{liter}}\right)^2 - (3 \text{ min}^{-1}) \left(0.3 \frac{\text{mol}}{\text{liter}}\right) \\&= (1.1 - 0.9) \frac{\text{mol}}{\text{liter} \cdot \text{min}} = 0.2 \frac{\text{mol}}{\text{liter} \cdot \text{min}}\end{aligned}$$

For no density change, the performance equation of Eq. 13 gives

$$\tau = \frac{V}{v} = \frac{C_{B0} - C_B}{-r_B}$$

$$\text{tau} = (6L)/vo = (0.8 - 0.2) / 0.2$$

$$vo = vf = 2 \text{ L/min}$$

