Allosteric Enzymes

3.3.4. Models for More Complex Enzyme Kinetics

3.3.4.1. Allosteric enzymes. Some enzymes have more than one substrate binding site. The binding of one substrate to the enzyme facilitates binding of other substrate molecules. This behavior is known as *allostery* or *cooperative binding*, and regulatory enzymes show this behavior. The rate expression in this case is

$$v = -\frac{d[S]}{dt} = \frac{V_m[S]^n}{K_m'' + [S]^n} \tag{3.18}$$

where n = cooperativity coefficient and $n > 1$ indicates positive cooperativity. Figure 3.8 compares Michaelis–Menten kinetics with allosteric enzyme kinetics, indicating a sigmoidal shape of υ –[S] plot for allosteric enzymes.

The cooperativity coefficient can be determined by rearranging eq. 3.18 as

$$\ln \frac{v}{V_m - v} = n \ln[S] - \ln K_m'' \tag{3.19}$$

and by plotting $\ln v/(V_m - v)$ versus ln[S] as depicted in Fig. 3.9.

Allosteric enzyme kinetics

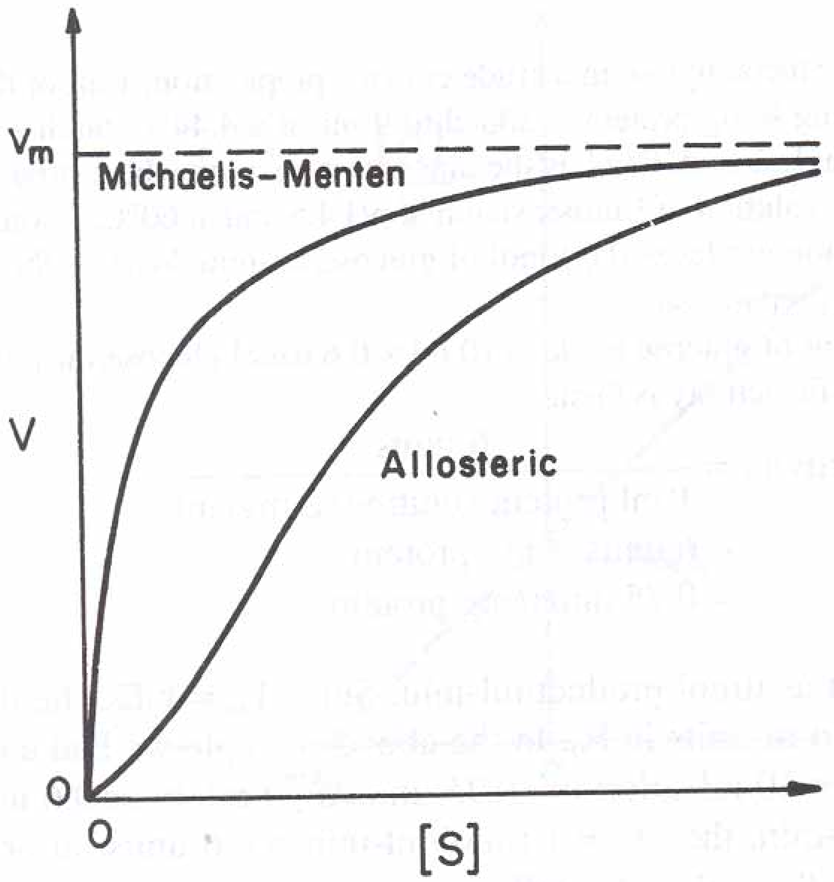


Figure 3.8. Comparison of Michaelis–Menten and allosteric enzyme kinetics.

Determination of cooperativity coefficient

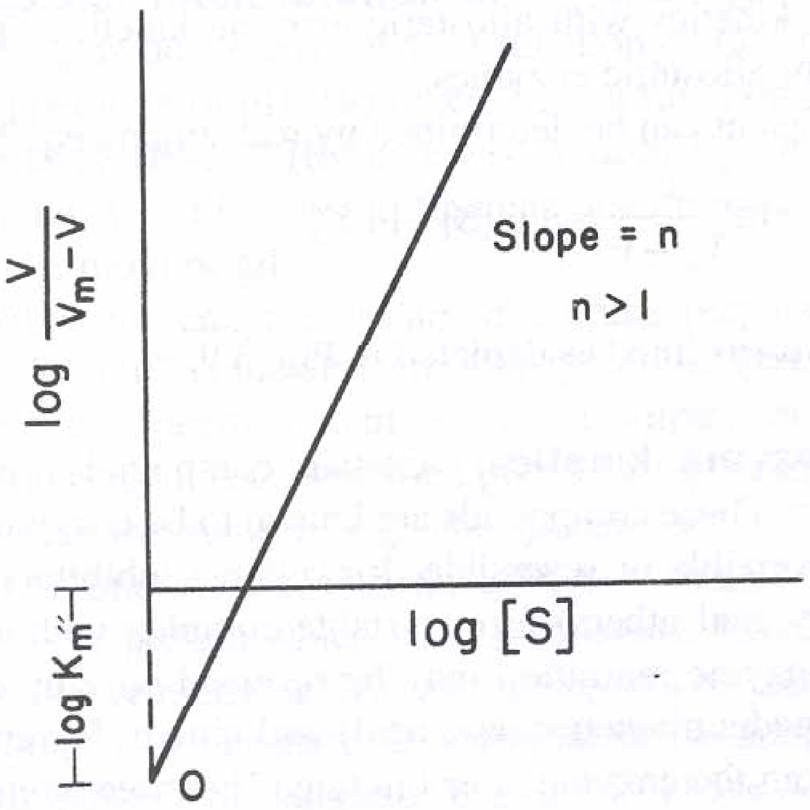


Figure 3.9. Determination of cooperativity coefficient.

Inhibited Enzyme Kinetics

- Inhibitor
  - Inhibitor binds to enzymes and reduces their activity.
- Irreversible inhibitor
  - Heavy metals forms a stable complex with enzymes.
  - Pd, Cd, Hg, and others
  - May be reversed only by using chelating agents such as EDTA and citrate.
- Reversible inhibitor
  - Dissociate more easily from the enzyme

Reversible Enzyme Inhibition

- Competitive inhibition
- Noncompetitive inhibition
- Uncompetitive inhibition
- Substrate inhibition

Competitive Inhibition

$$\begin{array}{l} E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \\ + \\ I \\ \Big\updownarrow K_1 \\ EI \end{array} \qquad (3.20)$$

Assuming rapid equilibrium and with the definition of

$$K'_m = \frac{[E][S]}{[ES]}, \qquad K_I = \frac{[E][I]}{[EI]}$$

$$[E_0] = [E] + [ES] + [EI] \quad \text{and} \quad v = k_2[ES]$$

Assuming rapid equilibrium and with the definition of

$$K'_m = \frac{[E][S]}{[ES]}, \qquad K_I = \frac{[E][I]}{[EI]}$$

$$[E_0] = [E] + [ES] + [EI] \quad \text{and} \quad v = k_2[ES]$$

we can develop the following equation for the rate of enzymatic conversion:

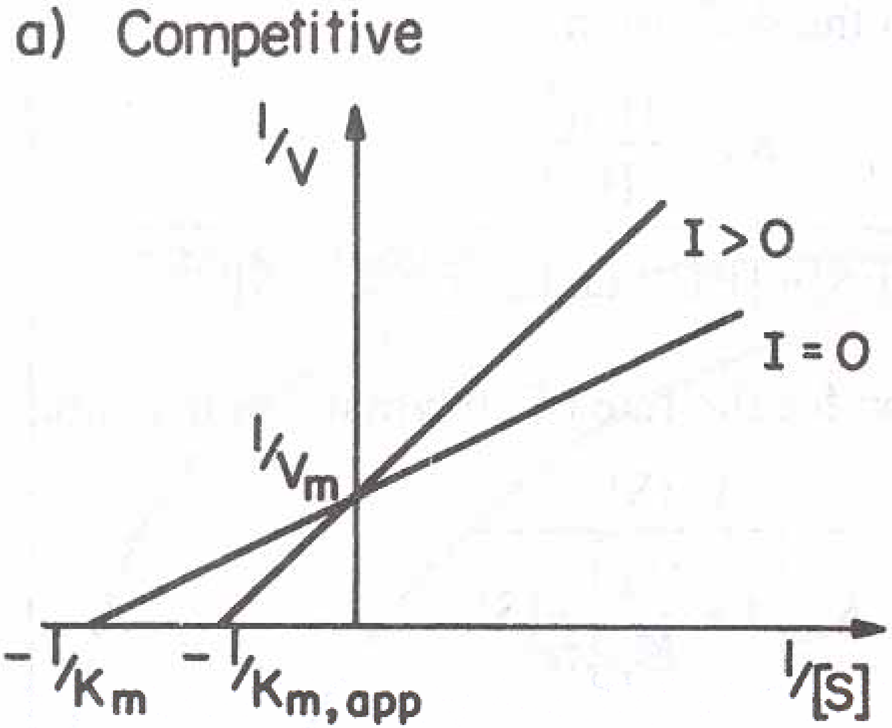
$$v = \frac{V_m[S]}{K'_m\left[1 + \frac{[I]}{K_I}\right] + [S]}$$

or

$$v = \frac{V_m[S]}{K'_{m,\,\text{app}} + [S]}$$

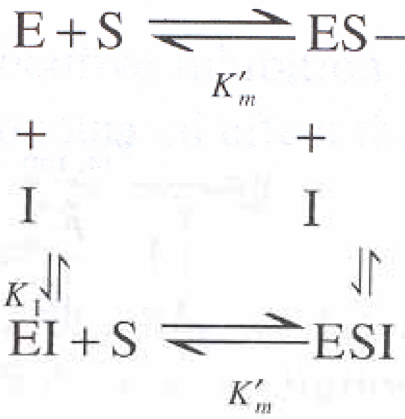
where $K'_{m,\text{app}} = K'_m\left(1 + \frac{[I]}{K_1}\right)$.

L-B Plot



$$v = \frac{V_m[S]}{K'_m\left[1 + \frac{[I]}{K_I}\right] + [S]}$$

Noncompetitive Inhibition



(3.24)

$$K'_m = \frac{[E][S]}{[ES]} = \frac{[EI][S]}{[ESI]}, \qquad K_I = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]} \qquad (3.25)$$

$$[E_0] = [E] + [ES] + [EI] + [ESI] \text{ and } v = k_2[ES]$$

$$K'_m = \frac{[E][S]}{[ES]} = \frac{[EI][S]}{[ESI]}, \qquad K_I = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]} \tag{3.25}$$

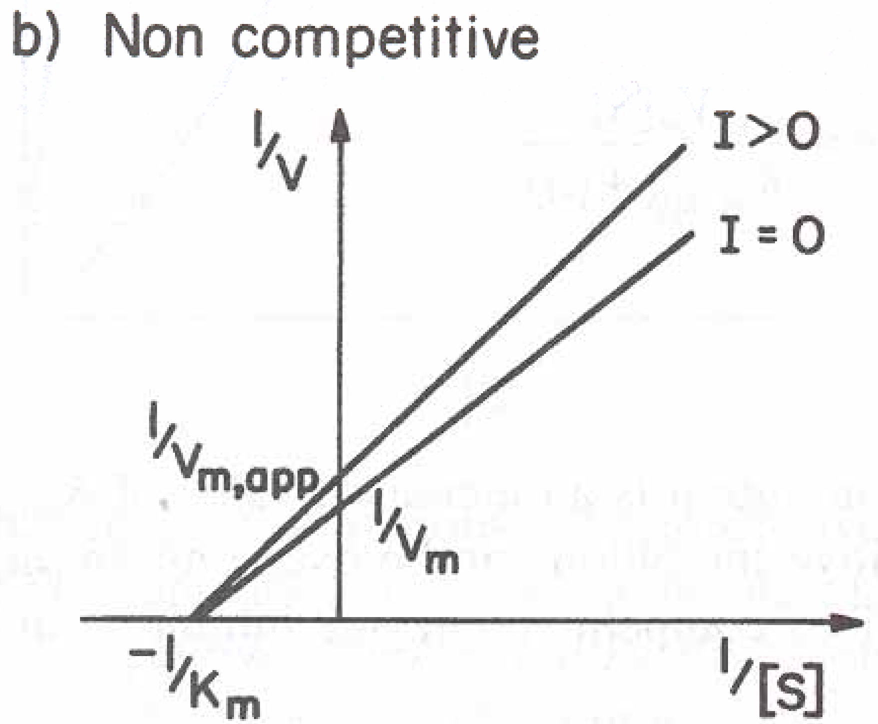
$$[E_0] = [E] + [ES] + [EI] + [ESI] \text{ and } v = k_2[ES]$$

$$v = \frac{V_m}{\left(1 + \frac{[I]}{K_I}\right)\left(1 + \frac{K'_m}{[S]}\right)} \tag{3.26}$$

$$v = \frac{V_{m,\mathrm{app}}}{\left(1 + \frac{K'_m}{[S]}\right)} \tag{3.27}$$

$$\text{where } V_{m,\mathrm{app}} = \frac{V_m}{\left(1 + \frac{[I]}{K_I}\right)}$$

L-B Plot



$$v = \frac{V_{m,\mathrm{app}}}{\left(1 + \frac{K'_m}{[\mathrm{S}]}\right)}$$

$$\text{where} \quad V_{m,\mathrm{app}} = \frac{V_m}{\left(1 + \frac{[\mathrm{I}]}{K_1}\right)}$$

Uncompetitive Inhibition

$$
\begin{array}{c}
E + S \underset{}{\overset{K'_m}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \\
+ \\
I \\
\Big\uparrow\Big\downarrow K_1 \\
ESI
\end{array}
\qquad (3.28)
$$

$$K'_m = \frac{[E][S]}{[ES]}, \quad K_1 = \frac{[ES][I]}{[ESI]} \qquad (3.29)$$

$$[E_0] = [E] + [ES] + [ESI] \quad \text{and} \quad v = k_2[ES]$$

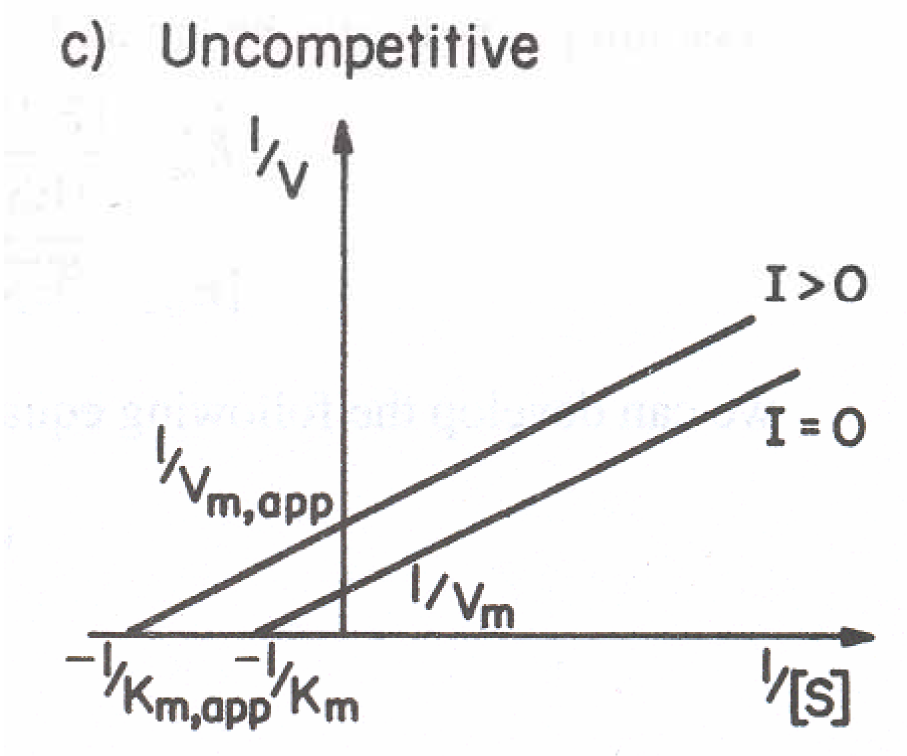
$$K'_m = \frac{[E][S]}{[ES]}, \quad K_1 = \frac{[ES][I]}{[ESI]} \tag{3.29}$$

$$[E_0] = [E] + [ES] + [ESI] \text{ and } v = k_2[ES]$$

$$v = \frac{\dfrac{V_m}{\left(1 + \dfrac{[I]}{K_1}\right)}[S]}{\dfrac{K'_m}{\left(1 + \dfrac{[I]}{K_1}\right)} + [S]} \tag{3.30}$$

$$v = \frac{V_{m,\text{app}}[S]}{K'_{m,\text{app}} + [S]} \tag{3.31}$$

L-B Plot



$$v = \frac{\frac{V_m}{\left(1+\frac{[\mathrm{I}]}{K_1}\right)}[\mathrm{S}]}{\frac{K'_m}{\left(1+\frac{[\mathrm{I}]}{K_1}\right)}+[\mathrm{S}]}$$

$$v = \frac{V_{m,\mathrm{app}}[\mathrm{S}]}{K'_{m,\mathrm{app}}+[\mathrm{S}]}$$

Substrate Inhibition

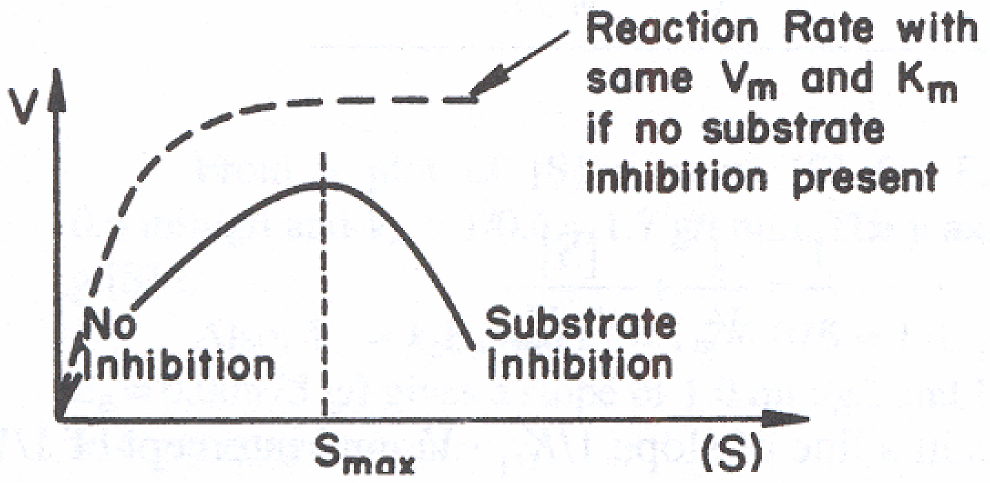


Figure 3.11. Comparison of substrate-inhibited and uninhibited enzymatic reactions.

Substrate Inhibition

$$\begin{array}{ccccc} E + S & \overset{K'_m}{\rightleftharpoons} & ES & \xrightarrow{k_2} & E + P \\ & & + & & \\ & & S & & \\ & & \Big\downarrow\!\!\Big\uparrow K_{S_1} & & \\ & & ES_2 & & \end{array}$$

$$K_{S_1} = \frac{[S][ES]}{[ES_2]}, \quad K'_m = \frac{[S][E]}{[ES]}$$

$$K_{S_1} = \frac{[S][ES]}{[ES_2]}, \quad K'_m = \frac{[S][E]}{[ES]}$$

$$v = \frac{V_m[S]}{K'_m + [S] + \frac{[S]^2}{K_{S_1}}}$$

At low substrate concentrations, $[S]^2/K_{S_1} << 1$, and inhibition effect is not observed. The rate is

$$v = \frac{V_m}{\left[1 + \frac{K'_m}{[S]}\right]} \tag{3.35}$$

or

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K'_m}{V_m}\frac{1}{[S]} \tag{3.36}$$

A plot of $1/v$ versus $1/[S]$ results in a line of slope K'_m/V_m and intercept of $1/V_m$.

$$v = \frac{V_m[S]}{K'_m + [S] + \frac{[S]^2}{K_{S_1}}}$$

At high substrate concentrations, $K'_m/[S] << 1$, and inhibition is dominant. The rate in this case is

$$v = \frac{V_m}{\left(1 + \frac{[S]}{K_{S_1}}\right)} \tag{3.37}$$

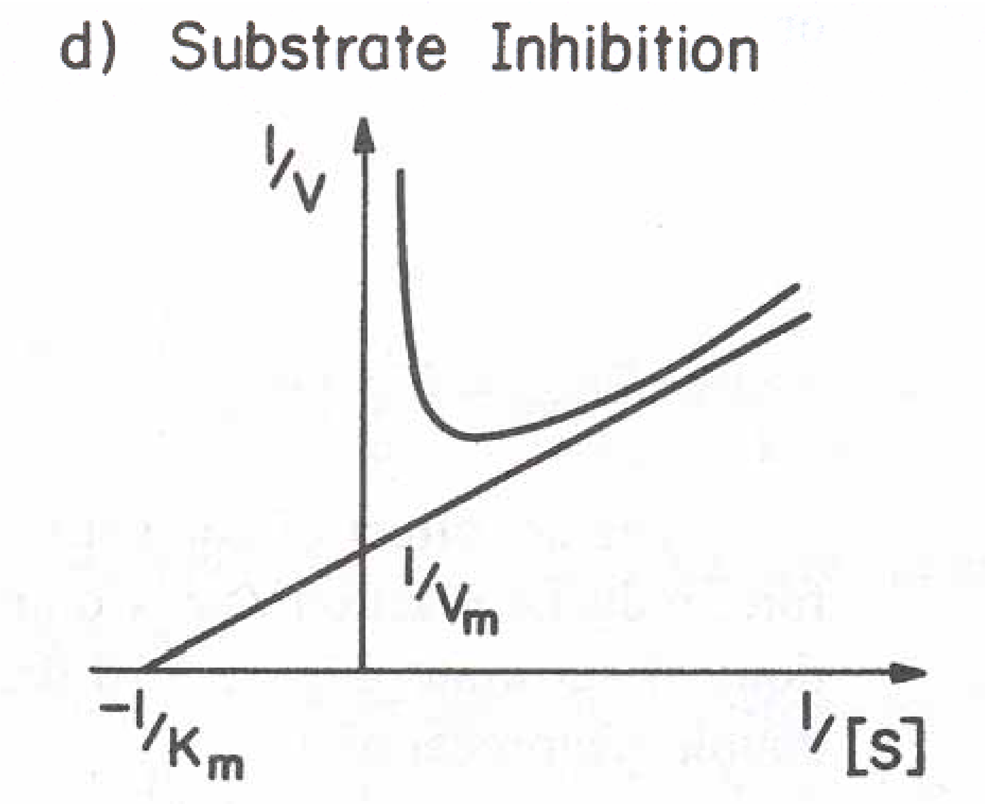
$$\frac{1}{v} = \frac{1}{V_m} + \frac{[S]}{K_{S_1} V_m} \tag{3.38}$$

A plot of $1/v$ versus [S] results in a line of slope $1/K_{s_1} \cdot V_m$ and intercept of $1/V_m$.

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

$$[S]_{max} = \sqrt{K'_m K_{S_1}}$$

L-B Plot



L-B Plot

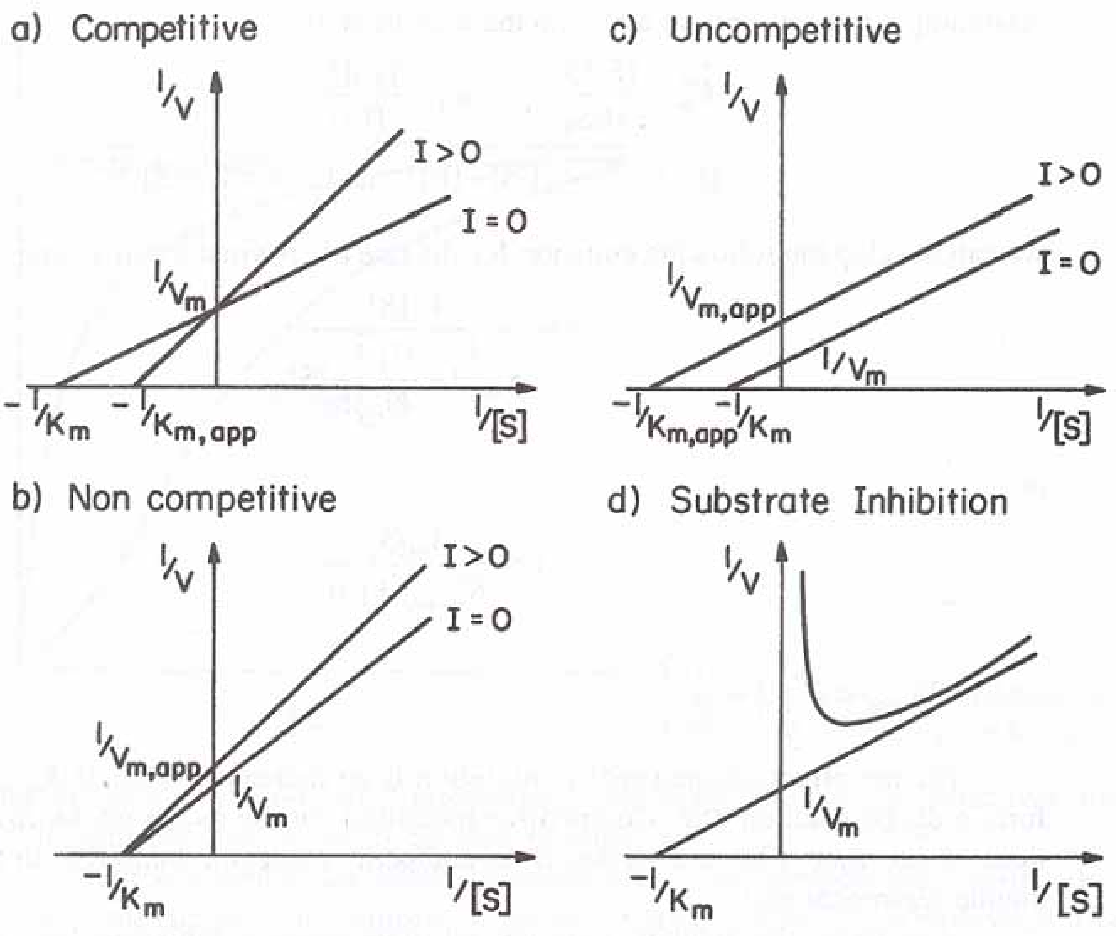


Figure 3.10. Different forms of inhibited enzyme kinetics.