Lecture 14

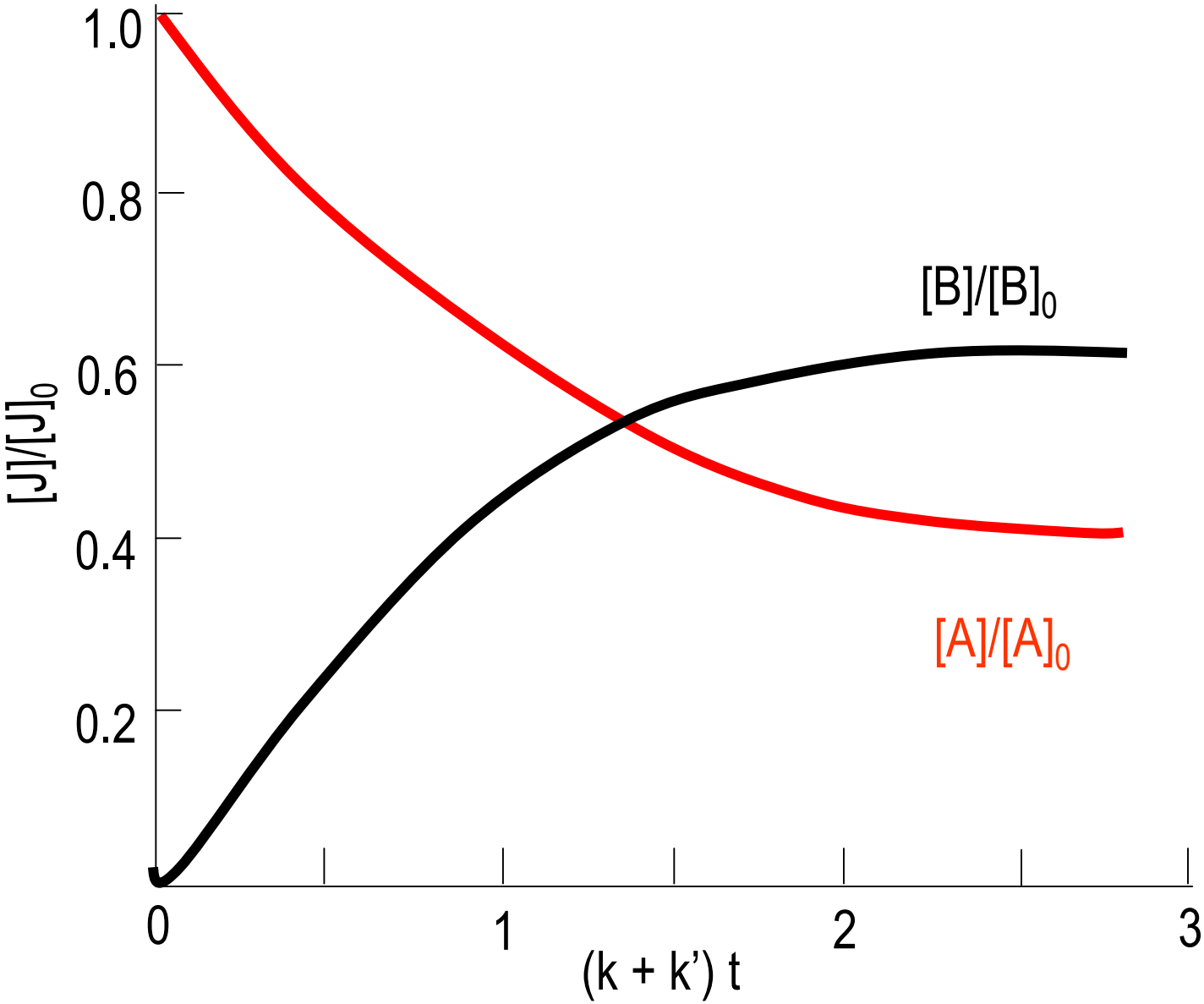
Consecutive reactions, steady state approximation and enzyme catalysed reactions

In general, discussions of kinetics disregard reverse reaction. However, this is important when the product concentration is significant.

Consider the case of A going to B and the reaction is reversible.

At equilibrium, $k[A]_{eq} = k'[B]_{eq}$.

This rearranges to, $K = [B]_{eq}/[A]_{eq} = k/k'$



This is the situation depicted in the figure where k = 2k'.

In a generalised situation involving multiple steps,

$K = k_a/k_a' \cdot k_b/k_b' \cdot \ldots$

Where k_a refers to the forward reaction rate constant of the step a and k_a' refers to that of the reverse reaction.

Consider a consecutive reaction,

$$\mathbf{A} \xrightarrow{k_1} \mathbf{B} \xrightarrow{k_1'} \mathbf{C}$$

An examples would be,

$$^{239}_{92}U \xrightarrow{\beta-} {}^{239}_{93}Np \xrightarrow{\beta-} {}^{239}_{94}Pu$$

The kinetics of this reaction can be studied in the following way,

For the first step,

1. $dA/dt = -k_1A$
2. $dB/dt = k_1A - k_1'B$ since it forms from A and decomposes to C
3. $dC/dt = k'_1B$

Relation 1 corresponds to exponential decay. Suppose the concentration of A initially is A_o

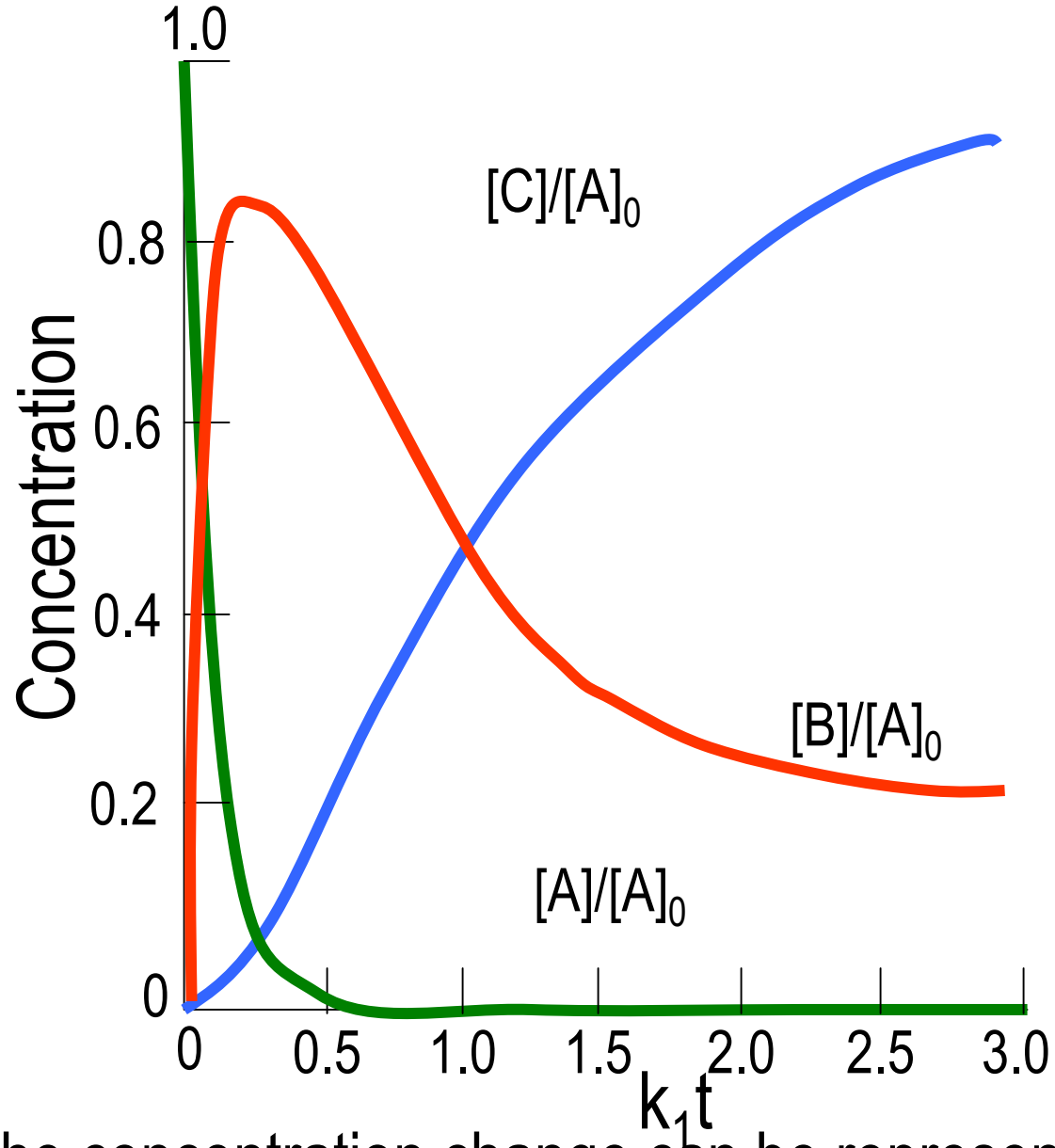
$A_t = A_o \exp(-k_1t)$

For relation 2, if a condition $B_o = 0$ is imposed, we get,

$B_t = A_o [k_1/(k_1'-k_1)] (e^{-k1t} - e^{-k'1t})$ (1)

At all times, $[A] + [B] + [C] = A_0$

$[C] = \{1 + [(k_1e^{-k1't} - k_1'e^{-k1t})/ (k_1'-k_1)]\}A_0$ (2)



All the concentration change can be represented graphically for $[A_t]$, $[B_t]$ and $[C_t]$ using equations, $k_1 = 10k_1'$.

Let us assume that $k_1' >> k_1$. Then every molecule of B formed will decay to C quickly. Then the rate of formation of C depends on the formation of B.

Look at the earlier equation:

$$[C] = \{1 + [(k_1 e^{-k1't} - k_1' e^{-k1t})/ (k_1' - k_1)]\}A_0 \quad (2)$$

If $k_1' >> k_1$, $e^{-k1't}$ is much smaller than e^{-k1t} and may be neglected. $k_1' - k_1 = k_1'$

$$C \sim A_o (1 - e^{-k1t})$$

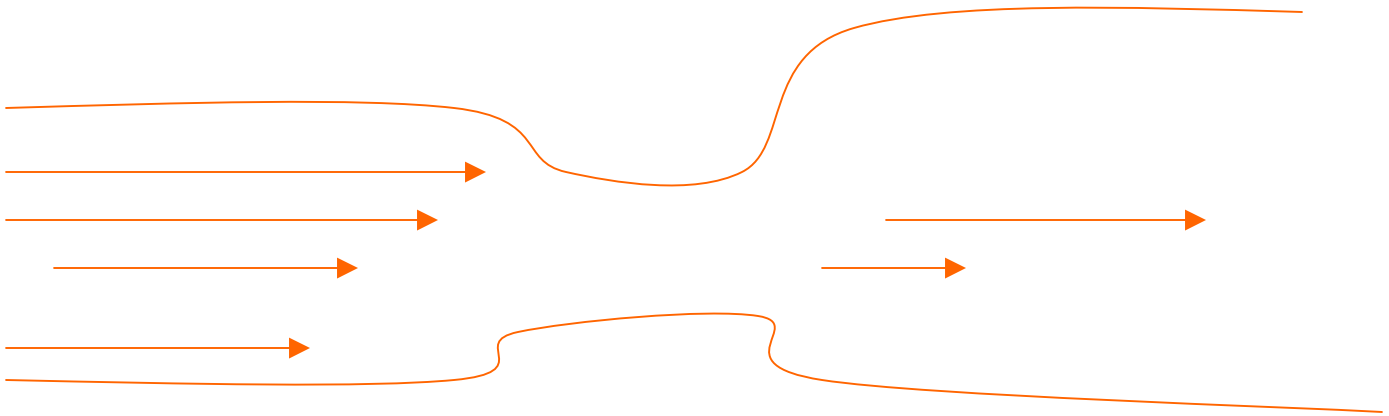
when k_1 in the denominator is neglected in comparison with k'_1.

The concentration of C depends on smaller rate coefficient. The step with smaller rate constant is called the rate determining step.

If $k_1' << k_1$

$C \sim A_0 (1 - e^{-k1't})$

Rate depends upon the rate determining reaction.



Look at the rapidity with which equations become complex. Can we reduce the complexity?

Let us assume that $k'_1 >> k_1$. Then for the equation of B_t,

$B_t = A_o [k_1/(k_1'-k_1)] (e^{-k1t} – e^{-k'1t})$ (1)

it can be seen that the concentration of B_t is lesser than that of A by a factor k_1/k_1'.

Thus if A reacts slowly, it can be seen that the concentration of A remains at the same constant value for a long time such that, $dB/dt \sim 0$

This is not true only in the beginning of the reaction. The assumption the major part of the reaction takes place when the reagent concentration is constant is called the **steady state approximation.**

Thus the dB/dt equation ($dB/dt = k_1A – k_1'B$) reduces to,

$k_1A – k_1'B \sim 0$

$B \approx (k_1/k_1')A$

Thus, $dC/dt = k_1'B \sim k_1A$

Similar type of equation can be used when intermediate attains equilibrium with the reactants called pre equilibrium.

$$A + B \underset{k_{-1}}{\overset{k_2}{=}} (AB) \xrightarrow{k_1} C$$

If the intermediate reacts slowly to form C, k_1 can be neglected in the rate equation.

$d[AB]/dt \sim k_2 [A] . [B] - k_{-1} [AB]$

If intermediate is in a steady state,

$k_2 [A].[B] - k_{-1} [AB] = 0$

or

$[AB] = k_2/k_{-1} [A].[B]$

but, $[AB] = K [A].[B]$

Using steady state

Using equilibrium constant

K = equilibrium constant

Thus, $dC/dt = k_1K [A].[B]$ or $dC/dt = k_1k_2/k_{-1} [A].[B]$

Thus, the reaction is overall second order.

Let us look at an example.

$2NO + O_2 \rightarrow 2NO_2$

the reaction shows third order kinetics. The assumption that the reaction is termolecular does not appear correct since the reaction rate decreases with temperature which should have increased trimolecular collisions. Thus we can assume it to involve steps.

Assume the pre equilibrium.

$NO + NO = N_2O_2$, the equilibrium constant K

$N_2O_2 + O_2 \rightarrow 2NO_2$

Applying steady state to N_2O_2

$[N_2O_2] = K [NO]^2$

$d[NO_2]/dt = k_2 [N_2O_2] [O_2]$

$= k_2 K [NO]^2 [O_2]$

this is third order rate law

$k_3 = k_2K$

The temperature dependence is also explained this way. Although k_2 increases with T, K decreases because the dimerisation is exothermic.

$$NO + NO \overset{K}{\rightleftharpoons} N_2O_2 + O_2 \overset{k_2}{\rightarrow} 2\,NO_2$$

Michaelis-Menten Mechanism of Enzyme Kinetics

Enzyme kinetics is very efficient.

Basic characteristics:

1. For a given substrate concentration $[S]_o$, initial rate is proportional to total enzyme concentration, $[E]_0$.
2. For a given $[E]_o$ and $[S]_o$, rate is proportional to [S].
3. For a given $[E]_o$ and large $[S]_o$, rate is independent of $[S]_o$ and reaches a max. called max. velocity.

M-M mechanism accounts for these features.

E + S → P + E

enzyme + substrate → product + enzyme

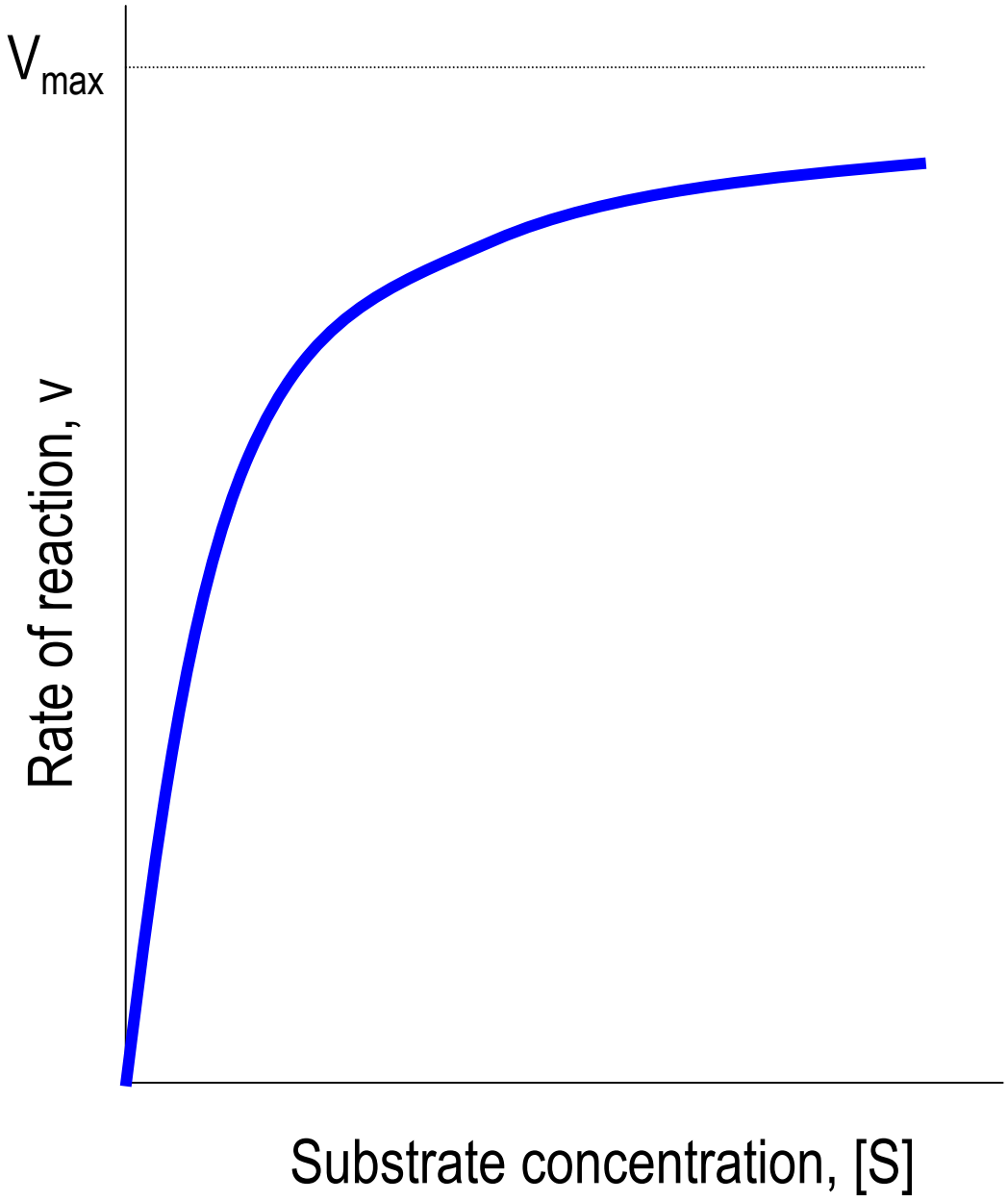
Net S → P but kinetics show that rate depends on E.

The mechanism,

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

Leonor Michaelis 1875-1949

Maude Leonora Menten 1879-1960


V_{max}
Rate of reaction, v
Substrate concentration, [S]

$dP/dt = k_1 [ES]$

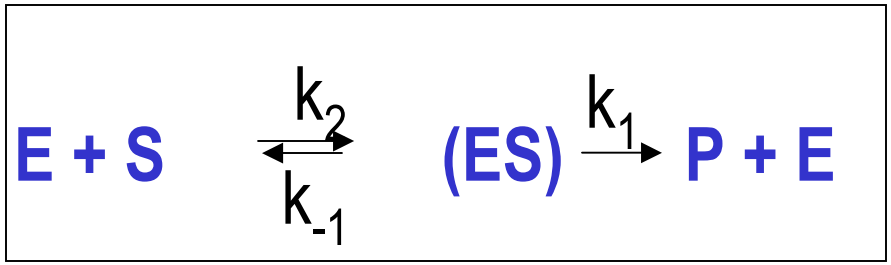
$d[ES]/dt = k_2[E][S] - k_{-1}[ES] - k_1[ES]$

at steady state,

$k_2[E][S] - k_{-1}[ES] - k_1[ES] \sim 0$

So,

$[ES] \sim k_2 [E] [S]/\{k_1 + k_{-1}\}$



[E] and [S] are the free enzyme and substrate concentrations. Enzyme is added only to a small quantity and $[E] + [ES] = [E]_0$, the initial enzyme concentration which is a constant. Only small amount of enzyme is added, and the concentration is much smaller than the substrate, free substrate

$[S] \approx [S]_o$

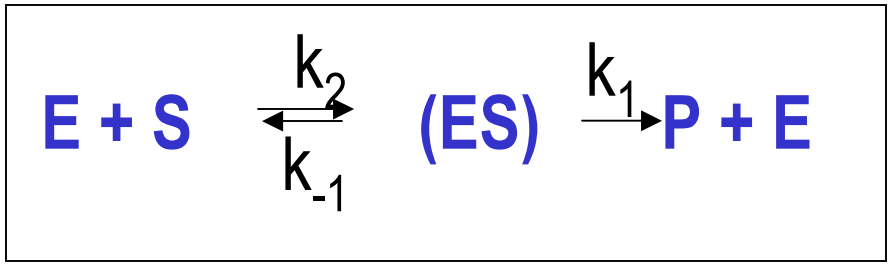
$[ES] = k_2 \{[E]_o - [ES]\} [S]/\{k_1 + k_{-1}\}$

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

$[ES] = k_2 [E]_0 [S]/ \{k_1 + k_{-1} + k_2[S]\}$

Thus,

$dP/dt = k_1k_2 [E]_0 [S]/\{k_1 + k_{-1} + k_2[S]\}$



Enzymolysis depends linearly on the amount of the enzyme added,

$=k_1 [E]_o[S]/\{K_M + [S]\}$ (1)

where $K_M = (k_1 + k_{-1})/k_2$

K_M is the Michaelis constant. This is a constant for a given enzyme and a substrate pair.This mechanism for the mode of action was proposed by Michaelis and Menten in 1913 and therefore called, Michaelis and Menten mechanism of enzyme kinetics.

Equation (1) can be written as,

$dP/dt =k [E]_o$; where $k = k_1[S]/\{K_M + [S]\}$ (2)

Thus enzymolysis depends on the amount of enzyme.

Look at the condition, $[S] >> K_M$

$dP/dt = k_1 [E_o]$ and the reaction is zero order in S. Thus the rate is constant. When S is large, the change in substrate concentration is constant. The rate of formation of product is highest under this condition and $k_1 [E_o]$ is called the **maximum velocity** of enzymolysis.
k_1 is called the **maximum turnover number**.

When $[S] << K_M$,

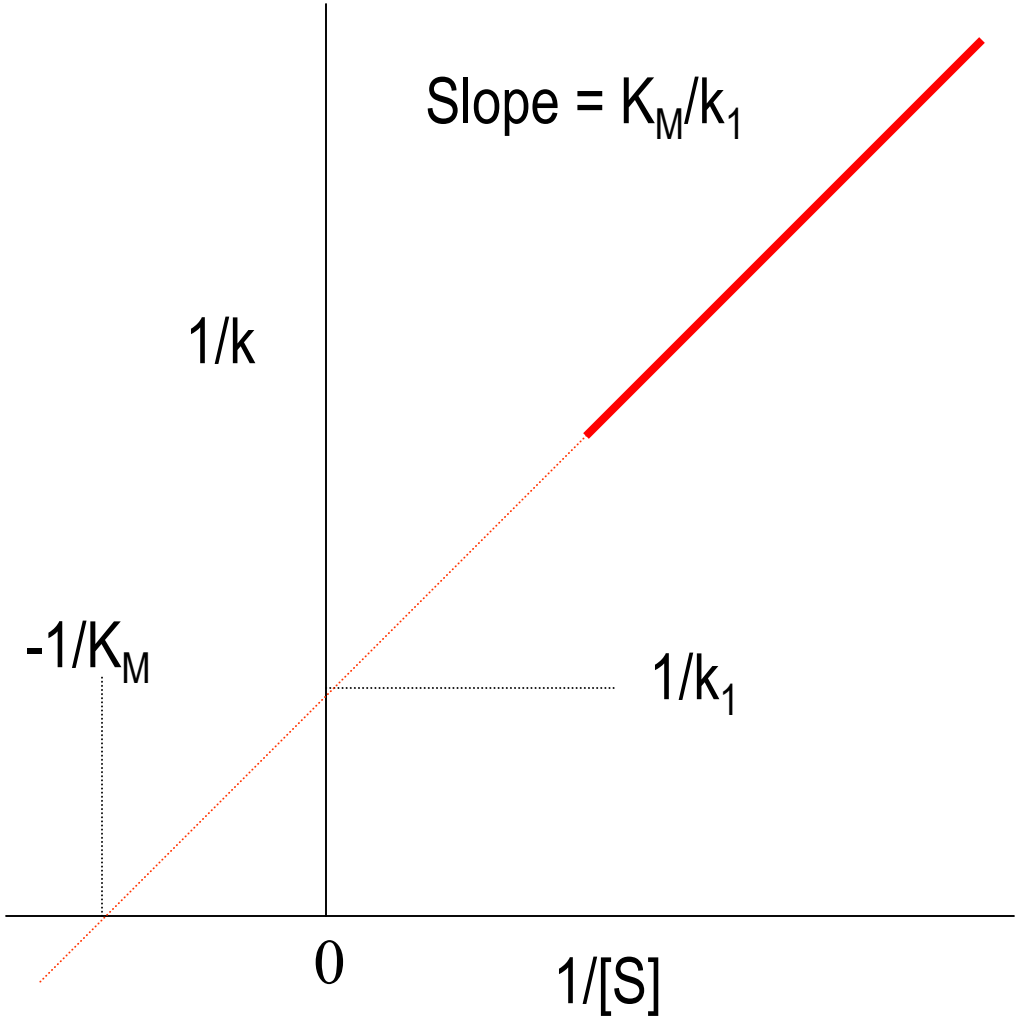
$dP/dt = \{k_1/K_M] [E_o\}[S]$

Rate depends on both enzyme and substrate.

Equation (2) gives,

$$1/k = 1/k_1 + K_M/k_1[S]$$

A plot of 1/k vs. 1/[S] will give intercept ($1/k_1$) when 1/[S] = 0. The slope will give K_M/k_1 and therefore K_M. The method will not give k_2 and k_{-1}, separately. Additional data will be needed to evaluate these. This plot is called the Lineweaver-Burk plot.


Slope = K_M/k_1
1/k
$-1/K_M$
$1/k_1$
0
1/[S]