

# Lecture 13

Consecutive reactions, steady state approximation  
and enzyme catalysed reactions

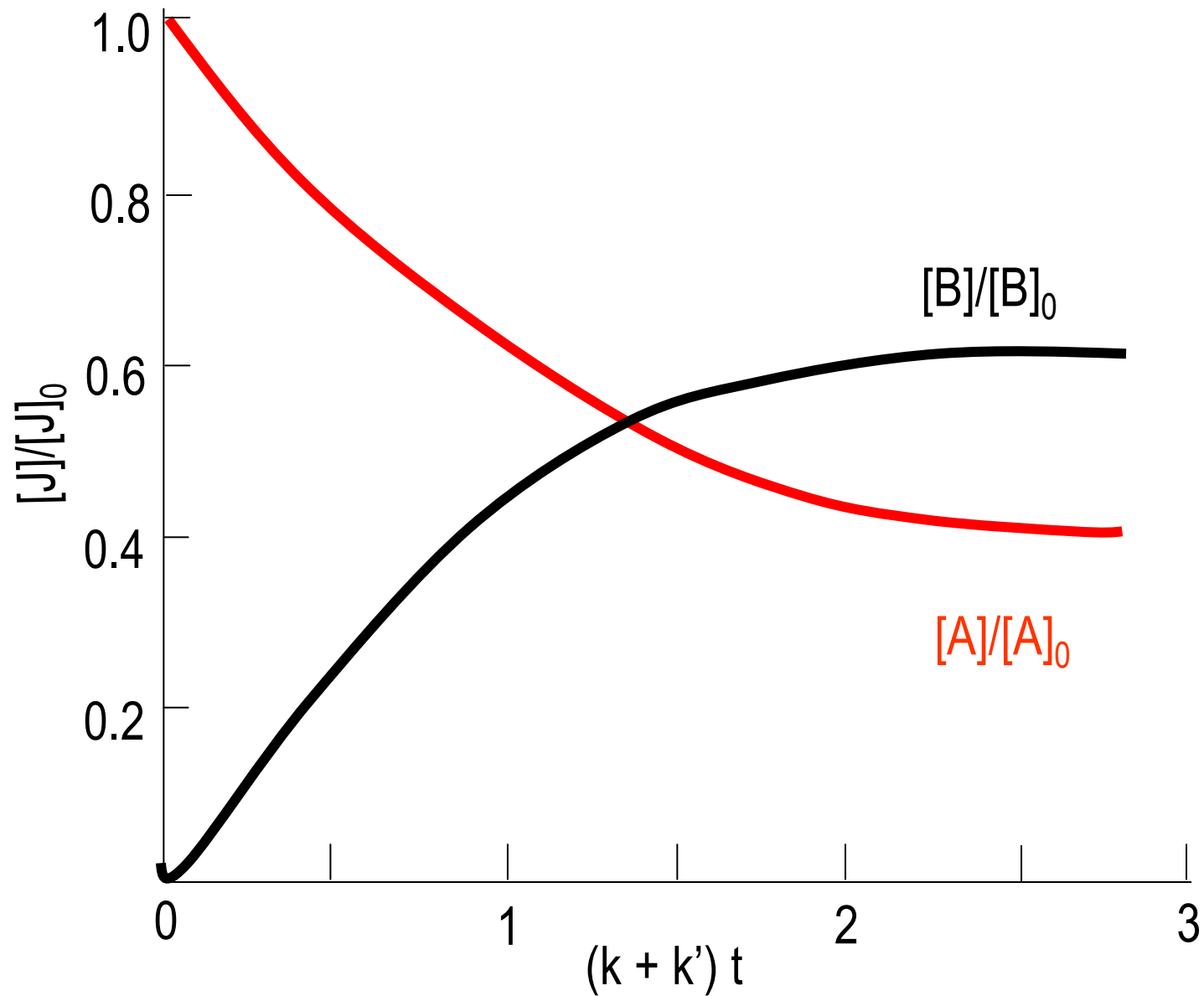


In general, discussions of kinetics disregard reverse reaction. However, this is important when the product concentration is significant.

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

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

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



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

In a generalised situation involving multiple steps,

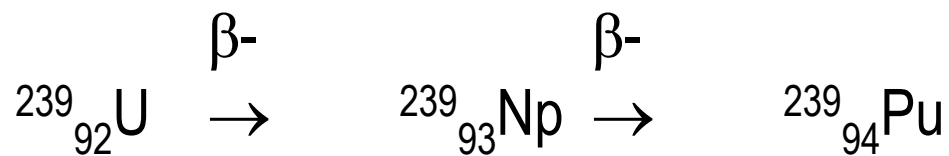
$$K = k_a/k_a' \cdot k_b/k_b' \cdot \dots$$

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,



An examples would be,



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

For the first step,

$$1. \quad dA/dt = -k_1 A$$

$$2. \quad dB/dt = k_1 A - k_1' B \quad \text{since it forms from A and decomposes to C}$$

$$3. \quad dC/dt = k_1' B$$

Relation 1 corresponds to exponential decay. Suppose the concentration of A initially is  $A_0$

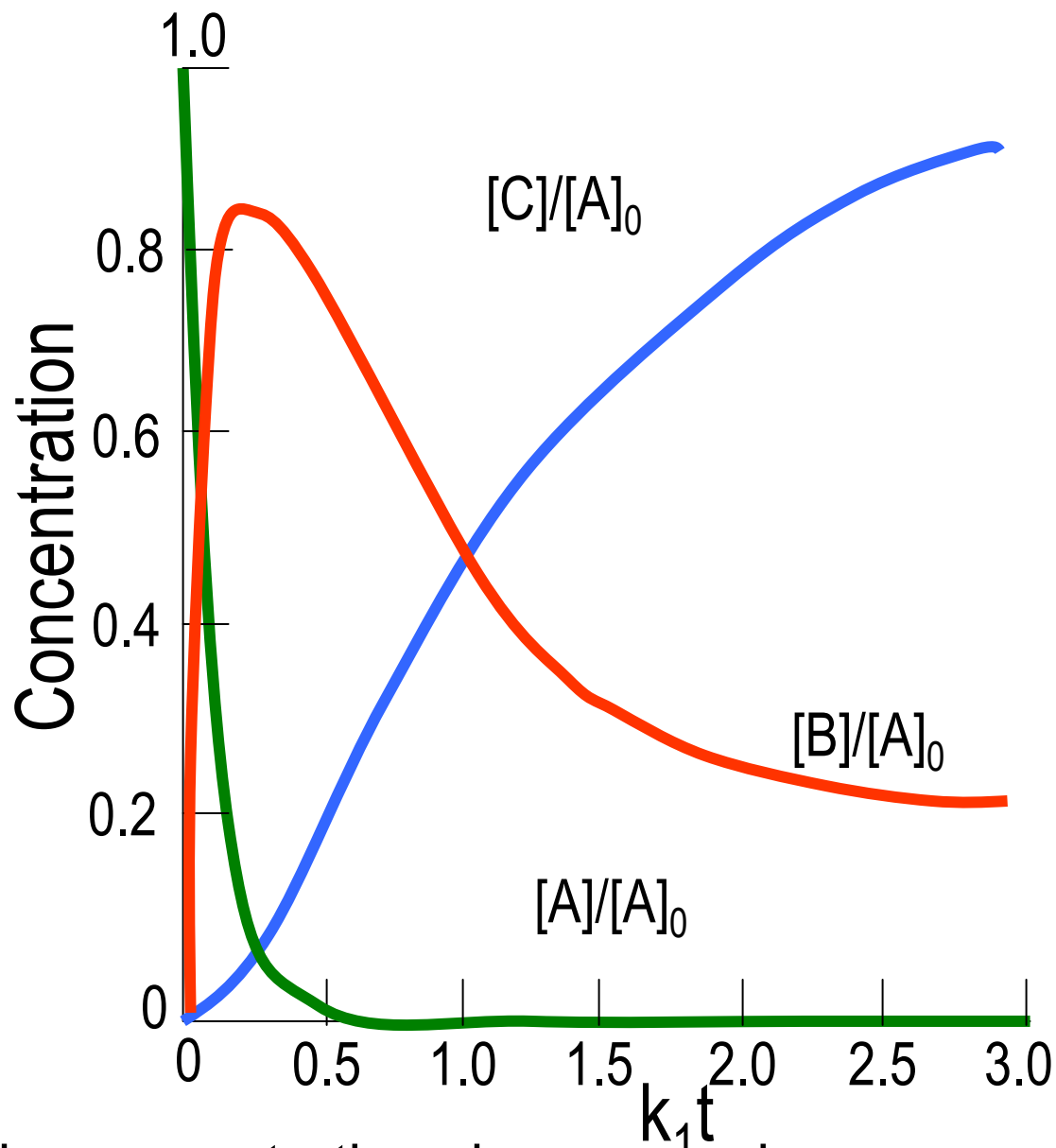
$$A_t = A_0 \exp(-k_1 t)$$

For relation 2, if a condition  $B_0 = 0$  is imposed, we get,

$$B_t = A_0 [k_1 / (k_1' - k_1)] (e^{-k_1 t} - e^{-k_1' t}) \quad (1)$$

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

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



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  $k_1' \gg k_1$ . 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:

$$[C] = \left\{ 1 + \frac{[(k_1 e^{-k_1' t} - k_1' e^{-k_1 t})]}{(k_1' - k_1)} \right\} A_0 \quad (2)$$

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

$$C \sim A_0 (1 - e^{-k_1 t})$$

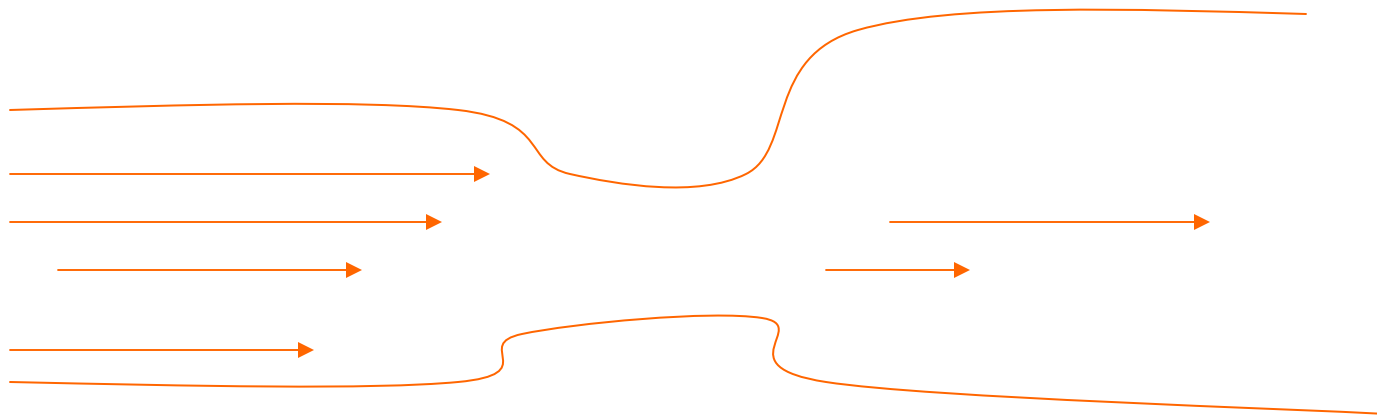
when  $k_1$  in the denominator is neglected in comparison with  $k_1'$ .

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

If  $k_1' \ll k_1$

$$C \sim A_0 (1 - e^{-k_1't})$$

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  $k'_1 \gg k_1$ . Then for the equation of  $B_t$ ,

$$B_t = A_0 [k_1 / (k'_1 - k_1)] (e^{-k_1 t} - e^{-k'_1 t}) \quad (1)$$

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**.

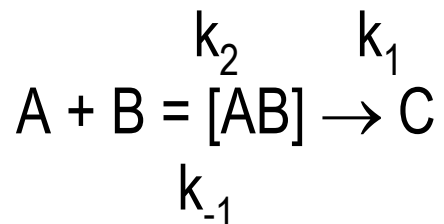
Thus the  $dB/dt$  equation ( $dB/dt = k_1 A - k'_1 B$ ) reduces to,

$$k_1 A - k'_1 B \sim 0$$

$$B \approx (k_1/k'_1) A$$

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

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



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

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

If intermediate is in a **steady state**,

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

or

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

but,  $[AB] = K [A] \cdot [B]$  ←  $K = \text{equilibrium constant}$

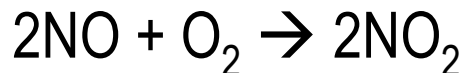
Thus,  $dC/dt = k_1 K [A] \cdot [B]$  or  $dC/dt = k_1 k_2 / k_{-1} [A] \cdot [B]$

Thus, the reaction is overall second order.

Using steady state

Using equilibrium constant

Let us look at an example.

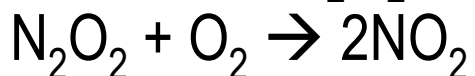


the reaction shows third order kinetics. The assumption that the reaction is termolecular does not appear correct since the reaction rate decreases with temperature which should have increased trimolecular collisions.

Thus we can assume it to involve steps.

Assume the pre equilibrium.

$\text{NO} + \text{NO} = \text{N}_2\text{O}_2$ , the equilibrium constant is K



Applying steady state to  $\text{N}_2\text{O}_2$

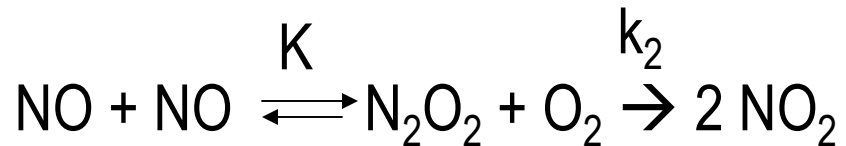
$$[\text{N}_2\text{O}_2] = K [\text{NO}]^2$$

$$\begin{aligned}d[\text{NO}_2]/dt &= k_2 [\text{N}_2\text{O}_2] [\text{O}_2] \\ &= k_2 K [\text{NO}]^2 [\text{O}_2]\end{aligned}$$

this is third order rate law

$$k_3 = k_2 K$$

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



# Michaelis-Menten Mechanism of Enzyme Kinetics

Enzyme kinetics is very efficient.

Basic characteristics:

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

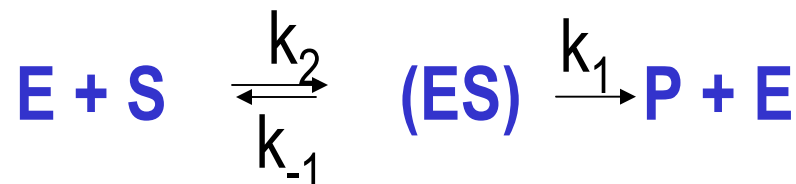
M-M mechanism accounts for these features.



enzyme + substrate  $\rightarrow$  product + enzyme

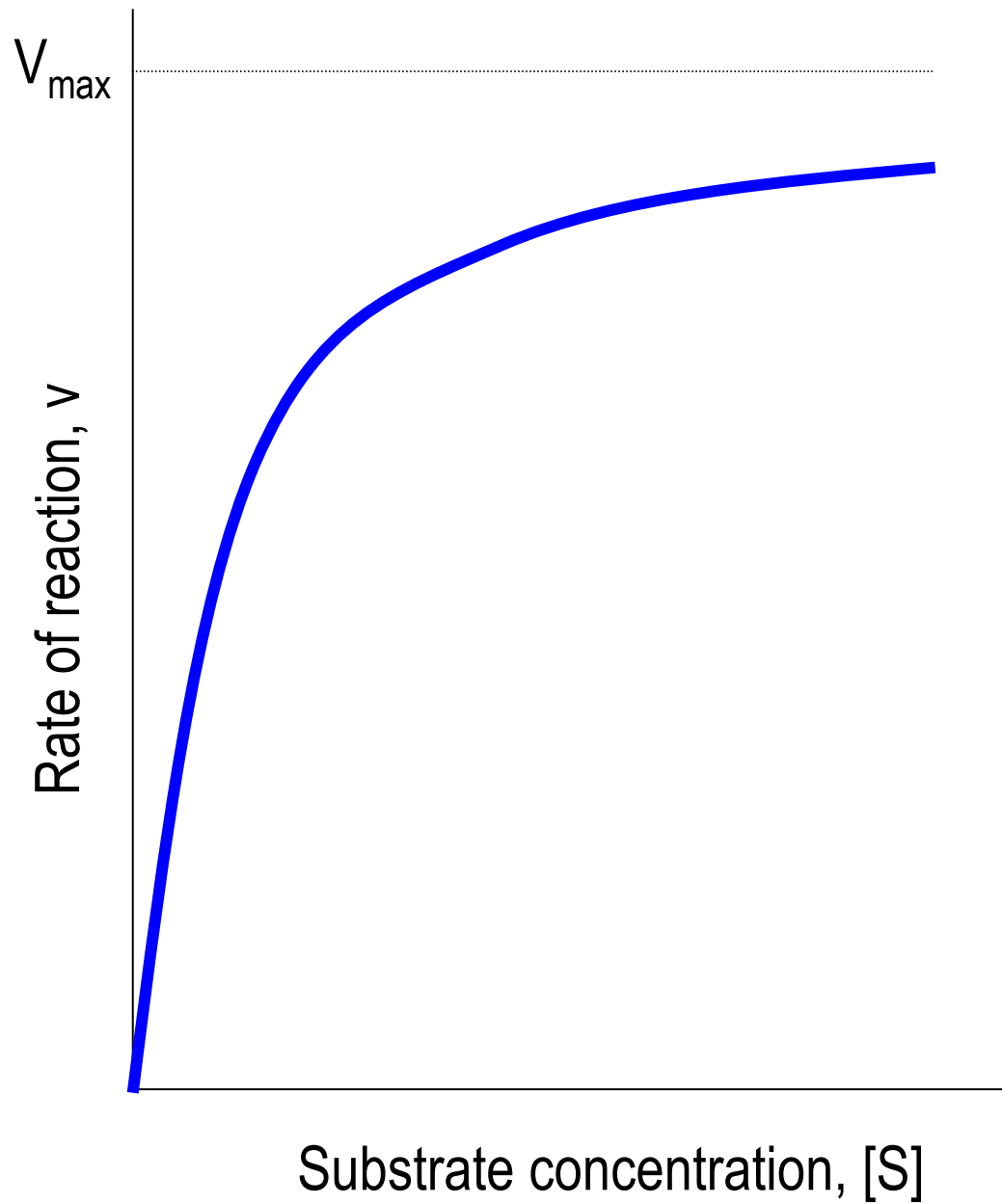
Net  $S \rightarrow P$  but kinetics show that rate depends on E.

The mechanism,



**Leonor Michaelis 1875-1949**

**Maude Leonora Menten  
1879-1960**



$$dP/dt = k_1 [ES]$$

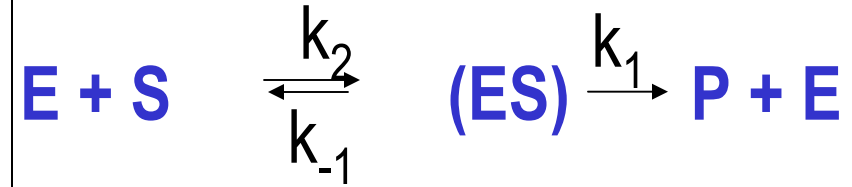
$$d[ES]/dt = k_2[E][S] - k_{-1} [ES] - k_1 [ES]$$

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

$$[S] \approx [S]_0$$

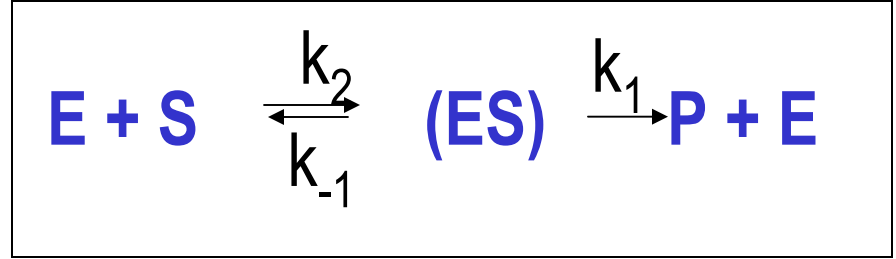
$$[ES] = k_2 \{[E]_0 - [ES]\} [S] / \{k_1 + k_{-1}\}$$

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

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

Thus,

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



Enzymolysis depends linearly on the amount of the enzyme added,

$$=k_1 [E]_0 [S] / \{K_M + [S]\} \quad (1)$$

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,

$$dP/dt = k [E]_0; \text{ where } k = k_1 [S] / \{K_M + [S]\} \quad (2)$$

Thus enzymolysis depends on the amount of enzyme.



Look at the condition,  $[S] \gg K_M$

$dP/dt = k_1 [E_0]$  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  $k_1 [E_0]$  is called the **maximum velocity** of enzymolysis.  $k_1$  is called the **maximum turnover number**.

When  $[S] \ll K_M$ ,

$$dP/dt = \{k_1/K_M\} [E_0][S]$$

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**.

