# Enzymes & Enzyme Kinetics

**The most incomprehensible thing about the world is that it is comprehensible.**

*– Albert Einstein (1879 – 1955)*

# Introduction to Enzymology

**Enzymology** is the study of enzyme activities and functions.

- Enzymes are integral part of most process industries.
- The activities that take place in most living cells whether outside or inside are enzyme enhanced biochemical reactions.
- **Enzymes** are:
    - Biological catalysts that increases the rate of biochemical reactions taking place within living cells without themselves suffering any overall change.
    - Synthesized by living cells.
- Enzyme found in cell are minute and are capable of speeding up chemical reactions associated with life processes.
- Any impairment of enzymes and their activity are reflected by some changes in the cell or even death. There can be no life without enzymes.

- Enzymes function in sequences of reactions called *pathway*.
- Enzymes does not modify:
    - The equilibrium constant and
    - The Gibbs free energy change of a reaction.
- The high specificity of the catalytic function of an enzyme is due to:
    - Its protein nature i.e. the highly complex structure of the enzyme
- Enzymes are thermo labile in character, for example the hydrolysis of proteins by a strong acid at 100 $^\circ$C may require a couple of days but using enzymes, the proteins are fully digested in the gastrointestinal tract at body temperature in just a few hours.

# Nature and Properties of Enzymes

- All Enzymes are proteins.
- However, without the presence of a non-protein component called a *cofactor*, enzyme lack catalytic activity.
- The functional unit of enzyme is called *holoenzyme*, which is made up of *apoenzyme* (inactive protein component) which is a protein part and a *coenzyme* (active part enzyme) which is a non-protein part.

$$Holoenzyme \longrightarrow Apoenzyme + Coenzyme$$

- The cofactor may be an organic molecule (*coenzyme*) or a metal ion.
- The non-protein part is usually called *prosthetic* group whenever it binds covalently with apoenzyme.
- Coenzyme is separable by dialysis while apoenzyme is non-separable.

# Factors Affecting Enzyme Activity

1. **Enzyme Concentration:**
   - The rate of enzyme-catalysed reactions depends directly on the enzyme concentration.
   - In the presence of sufficient substrate, increase in enzyme concentration increases the rate of reaction.

2. **Substrate concentration:**
   - With fixed enzyme concentration, an increase of substrate will result at first in a very rapid rise in velocity or reaction rate.
   - As the substrate concentration continues to increase, the rate of reaction begins to slow down until, with a large substrate concentration, no further change in the velocity is observed.

# Factors Affecting Enzyme Activity (2)

3. **Temperature:**
   - Enzyme are very sensitive to elevated temperatures, because of their protein nature.

   - The rate of enzyme-catalysed reaction increases with temperature up to a certain limit.

   - Above a certain temperature, the activity decreases with temperature because of enzyme denaturation.
     - For example, the optimum temperature for most enzymes is between $40 - 45\ °C$. hence, above $45\ °C$ rapid denaturation will destroy the catalytic function of the enzyme.

4. **pH:**
   - Certain enzyme have ionic groups on their active sites and these ionic groups must be in a suitable form (acid or base form) for the enzymes to function.
   - Variation in pH of the medium results in changes in the ionic form of the active site and changes the activity of the enzyme and hence the reaction rate. Changes in pH values may also alter the three dimensional shape of an enzyme. Hence enzymes are only active over a certain pH range.

# Enzyme Kinetics

## Order of Reaction

Consider a non-enzymatic reaction,

$$S \longrightarrow P$$

Where:

$S$ = substrate

$P$ = product

- The rate or velocity of a reaction, $v$, varies directly with concentration of the reactants. Hence, the rate is given as:

$$\frac{\Delta[P]}{\Delta t} = k[S] \qquad (1)$$

Where $k$ is the rate constant which indicates the speed or the efficiency of the reaction.

- Now consider an enzymatic reaction given as:

$$E + S \longrightarrow ES \longrightarrow E + P$$

# Order of Reaction (2)

- The prime requisite for the enzyme catalysis is that S must combine with $E$ at active site to form $ES$, the enzyme-substrate complex.

- Once combined, after sometime forms the product with the liberation of E.

- More specifically, the reaction is represented as:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_3}{\longrightarrow} E + P$$
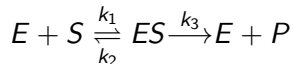
  – Which shows that the formation of ES complex is reversible and the formation of the product is irreversible.

  – The idea of ES complex formation was first conceived by Michaelis-Menten (1913) and has been widely accepted.

## Order of Reaction (3)

- At the beginning of the reaction $E + S \longrightarrow ES \longrightarrow E + P$, the amount of product P formed is very low and so it is negligible.

- The rate of enzymatic reaction depends on the concentration of both the substrate and enzyme.

- When the amount of $S$ is high enough or when the amount of $E$ is much less than the $S$, then the reaction is the *pseudo first order*.
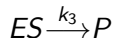
$$[S] \gg [E]$$

$$v = k[E]^1[S]^0$$

$$v = k[E] \tag{2}$$

- In the reaction above, the formation of ES complex and the dissociation of ES complex are happening very fast while the conversion of S to product P is slow.

- Hence, the rate limiting step (slowest step) is the decomposition of *ES* complex to Product.

$$ES \xrightarrow{k_3} P$$

$$v_o = k_3[ES] \tag{3}$$

## Order of Reaction (5)

Various mechanism have been proposed to explain the *ES* complex formation such as:

- Lock and key Model or Emil Fischer's template theory
- Induced Fit theory or Koshland's Model
- Substrate strain Theory

**Lock and key Model**

- The structure of *E* is rigid.
- The substrate fits to the binding site (active site), just as a key fits into a proper lock.
- The active site of *E* is rigid and pre-shaped template where only a specific substrate can bind.

  **Limitation:**

- Does not give any scope for the flexible nature of the enzyme
- Hence fails to explain many facts of enzymatic reactions.

**Induced Fit Theory**

- Presents a more realistic and acceptable model.
- The active site is not rigid and pre-shaped.
- Substrate binding site is present at the nascent active site.
- The interaction of S with E induces a fit or a binding site.

**Substrate Strain Theory**

- The substrate is strained due to the induced conformation change in an enzyme $E$.
- When $S$ binds to the pre-formed active site, the E induces a strain to the substrate.
- The strained S leads to the formation of the product.

# Kinetics of Enzyme-Catalyzed Reactions

## Kinetics of Enzyme-Catalyzed Reactions

- The kinetics of enzyme-catalyzed reactions can be treated using any one of the following approaches:
    - Steady-state approximation (SSA) or Briggs-Haldane Approach
    - Rapid Equilibrium Approach (REA) or Michaelis-Menten Approach

**Steady-State Approximation (SSA)**

- Experiments have shown that a true equilibrium is never achieved in the first step, i.e. $E + S \rightleftharpoons ES$ (fast) as the subsequent step $ES \longrightarrow E + P$ is constantly removing the intermediate $ES$ complex.

Generally,

$$[S] \gg [E]$$

So that

$$[E] \gg [ES]$$

**Hence SSA can be used for the intermediate complex ES**.

# Kinetics of Enzyme-Catalyzed Reactions (2)

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_3}{\longrightarrow} E + P$$
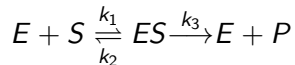
$$r = \frac{-d[S]}{dt} = \frac{d[P]}{dt} = k_3[ES] \tag{4}$$

**For ES Complex**

$$\frac{d[ES]}{dt} = k_1[E][S] - k_2[ES] - k_3[ES] \tag{5}$$

**For SSA**

$$\frac{d[ES]}{dt} = 0$$

$$k_1[E][S] - k_2[ES] - k_3[ES] = 0 \tag{6}$$

# Kinetics of Enzyme-Catalyzed Reactions (3)

Since the free enzyme concentration cannot be measured experimentally, the equilibrium between the free enzyme concentration and the bound enzyme concentration is:

$$[E]_0 = [E] + [ES] \tag{7}$$
$$[E] = [E]_0 - [ES] \tag{8}$$

substituting Eq.(8) into Eq.(6)

$$k_1([E]_0 - [ES])[S] - k_2[ES] - k_3[ES] = 0$$
$$k_1[E]_0[S] - k_1[ES][S] - k_2[ES] - k_3[ES] = 0$$
$$k_1[E]_0[S] = (k_1[S] + k_2 + k_3)[ES]$$

$$[ES] = \frac{k_1[E]_0[S]}{k_1[S] + k_2 + k_3} \tag{9}$$

## Kinetics of Enzyme-Catalyzed Reactions (4)

substituting Eq.(9) into Eq.(4)

$$r = \frac{k_3 k_1 [E]_0 [S]}{k_1 [S] + k_2 + k_3} \tag{10}$$

Dividing the numerator and denominator by $k_1$

$$r = \frac{k_3 [E]_0 [S]}{[S] + \dfrac{(k_2 + k_3)}{k_1}}$$

But

$$\frac{(k_2 + k_3)}{k_1} = K_m$$

$$r = \frac{k_3 [E]_0 [S]}{[S] + K_m} \tag{11}$$

# Kinetics of Enzyme-Catalyzed Reactions (5)

Eq.(12) is called the *Michaelis-Menten Equation*

$K_M$ = Michaelis-Menten constant.

- Further simplification implies that when all the enzymes have reacted with the substrate at high concentrations, the reaction will be going at the maximum rate.
- So free enzyme concentration is not present, i.e. $[E] = 0$

Hence, $[E]_0 = [ES]$

So that Michaelis-Menten equation becomes:

$$r = \frac{k_3[ES][S]}{[S] + K_M} \qquad (12)$$

As the $[S]$ becomes very high, $[S] \to \infty$

$$\frac{d[P]}{dt} = v_{max}$$

Hence,

$$\frac{d[P]}{dt} = v_{max} = k_3[ES]$$

Therefore, Eq. 2.12 becomes

$$r = \frac{v_{max}[S]}{[S] + K_M} \tag{13}$$

But, $r = v_0 =$ initial velocity

$$v_0 = \frac{v_{max}[S]}{[S] + K_M} \tag{14}$$

Three cases arise in using Eq.(14)

# Kinetics of Enzyme-Catalyzed Reactions (7)

- **Case 1**

  When $K_M \gg [S]$, so that Eq.(14) becomes:

  $$v_0 = \frac{v_{max}[S]}{K_M}$$

  $$v_0 = k'[S] \qquad \text{first order reaction}$$

- **Case 2**

  When $[S] \gg K_M$, hence Eq.(14) becomes:

  $$v_0 = v_{max} \qquad \text{zero order reaction}$$

- **Case 3**

  When $[S] = K_M$, so that Eq.(14) becomes:
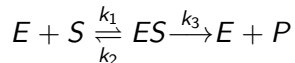
  $$v_0 = \frac{v_{max}}{2}$$
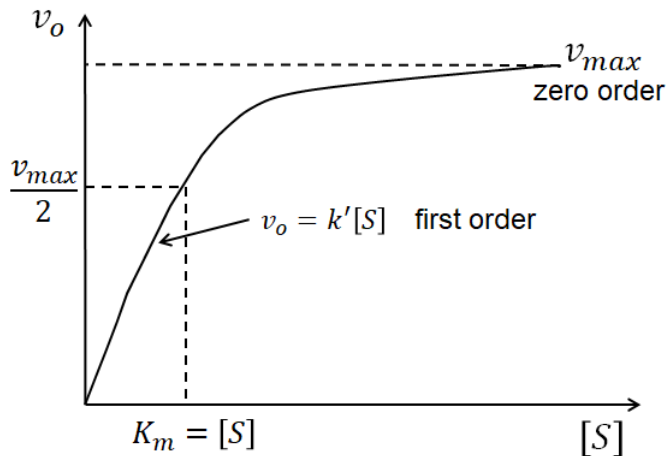
Figure 1: The effect of substrate concentration initial reaction rate

**Rapid Equilibrium Approach (REA)**
Recall that:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_3}{\longrightarrow} E + P$$

Overall reaction

$$S \overset{k_3}{\longrightarrow} P$$

$$r = \frac{-d[P]}{dt} = \frac{d[S]}{dt} \tag{15}$$

- For a rapid equilibrium in the fast step between $E$ and $S$ to form $ES$ complex, we can use the equilibrium coefficient to express $[ES]$ in terms of $[S]$.

$$K_M = \frac{k_2}{k_1} = \frac{[E][S]}{[ES]} \tag{16}$$

Substituting Eq.(8) into Eq.(16)

$$K_M = \frac{k_2}{k_1} = \frac{([E]_0 - [ES])[S]}{[ES]} \tag{17}$$

$$K_M[ES] = [E]_0[S] - [ES][S]$$

Then substituting Eq.(17) into Eq.(2), we have

$$v_0 = \frac{k_3[E]_0[S]}{K_M + [S]}$$

$$v_0 = \frac{v_{max}[S]}{K_M + [S]} \tag{18}$$

- Each molecule of an enzyme has one or several active sites onto which the substrate molecule must bind for a reaction to take place.

- At low substrate concentrations, most of the enzyme active sites remain unoccupied at any point of time. So the rate of reaction is proportional to the substrate concentration, which is first order.

- As the substrate concentration increases, the active site are fully occupied and hence the rate of reaction also increases which is zero order.

# Working Session 2

## Derivation of rate equation

When glucose is converted to fructose by glucose isomerase, the slow product formation step is also reversible as:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES$$

$$ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} P + E$$
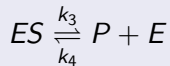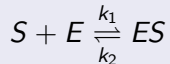
Derive the rate equation using:

(a) the Briggs-Haldane approach (SSA)

(b) the Michaelis-Menten (REA)

## Numerical solution

Show how the concentrations of substrate, product, and enzyme-substrate complex change with respect to time in a batch reactor for the enzyme reactions. The initial substrate and enzyme concentrations are 0.1 and 0.01 mol/L, respectively. The values of the reaction constants are: $k_1 = 40\ L/mol$, $k_2 = 5s^{-1}$, and $k_3 = 0.5s^{-1}$.

## Solution

$$\frac{dC_P}{dt} = k_3 C_{ES}$$
$$\frac{dC_{ES}}{dt} = k_1 C_S C_E - k_2 C_{ES} - k_3 C_{ES}$$
$$\frac{dC_S}{dt} = -k_1 C_S C_E + k_2 C_{ES}$$
$$C_E = C_{E0} - C_{ES}$$
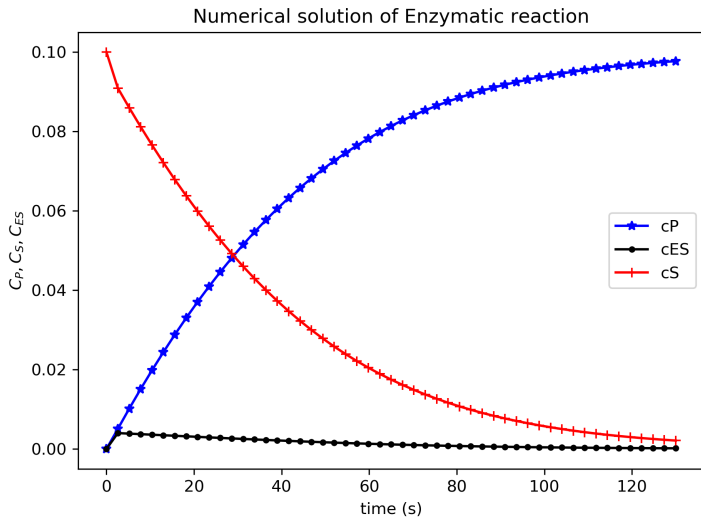$$C_{E0} = 0.01, C_{S0} = 0.1, C_{P0} = 0$$

Figure 2: Numerical solution of working session 2

# Evaluation of Kinetic Parameters

## Evaluation of Kinetic Parameters

- Involves the application of the transformation of Michaelis-Menten Equation (MME).

- The transformation involve the rearrangement of the MME by using the best known three methods:
  - The Lineweaver-Burk Method
  - The Eadie-Hofstee Method
  - The Hanes-Woolf Method

## Lineweaver-Burk method

Recall, $v_0 = \dfrac{v_{max}[S]}{K_m + [S]}$

Taking the reciprocal of both sides:

$$\frac{1}{v_0} = \frac{K_m + [S]}{v_{max}[S]}$$

$$\frac{1}{v_0} = \frac{K_m}{v_{max}[S]} + \frac{1}{v_{max}} \tag{19}$$

Eq. (19) take the form of the standard equation of a line, $y = mx + c$

Making a plot of $\dfrac{1}{v_0} \, vs \, \dfrac{1}{[S]}$ gives Fig. (3).

**Defect**: Data points at low substrate concentrations influence the slope and intercept more than those at high substrate concentrations.
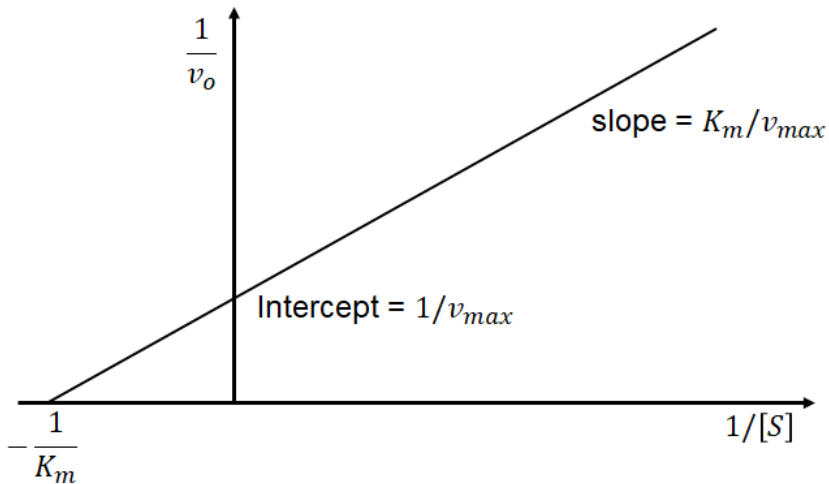
Figure 3: Lineweaver-Burk plot

# Eadie-Hofstee method

Multiplying Eq. (19) by $v_{max} v_0$

$$\frac{1}{v_0} . v_{max} v_0 = \frac{K_m}{v_{max}[S]} . v_{max} v_0 + \frac{1}{v_{max}} . v_{max} v_0$$

$$v_{max} = \frac{K_m v_0}{[S]} + v_0$$

Rearranging the terms in the equation above, we have:

$$v_0 = -K_m \frac{v_0}{[S]} + v_{max} \tag{20}$$

Making a plot of $v_0$ vs $\frac{v_0}{[S]}$, we have Fig. (4).

The Eadie-Hofstee plot can be subjected to large errors since both coordinates contain $v_0$, but there is less bias on the point at low $[S]$.
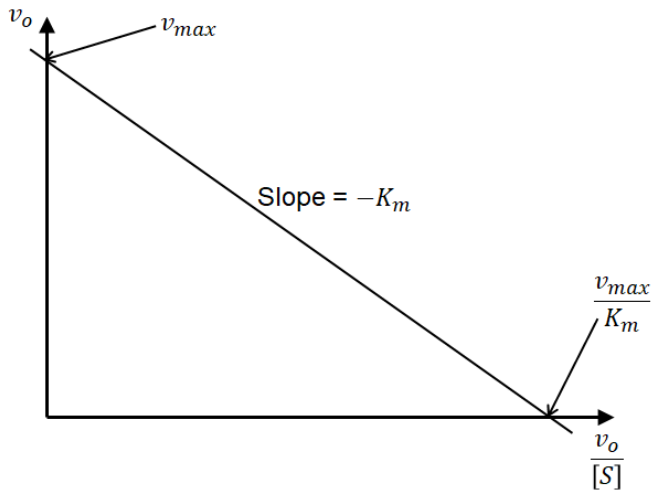
Figure 4: Eadie-Hofstee plot

## Hanes-Woolf method

Recall that:

$$v_0 = \frac{v_{max}[S]}{K_m + [S]}$$

Taking the reciprocal of both sides and multiplying both sides by $[S]$

$$\frac{1}{v_0} \cdot [S] = \frac{K_m}{v_{max}[S]} \cdot [S] + \frac{[S]}{v_{max}[S]} \cdot [S]$$

$$\frac{[S]}{v_0} = \frac{K_m}{v_{max}} + \frac{[S]}{v_{max}} \tag{21}$$

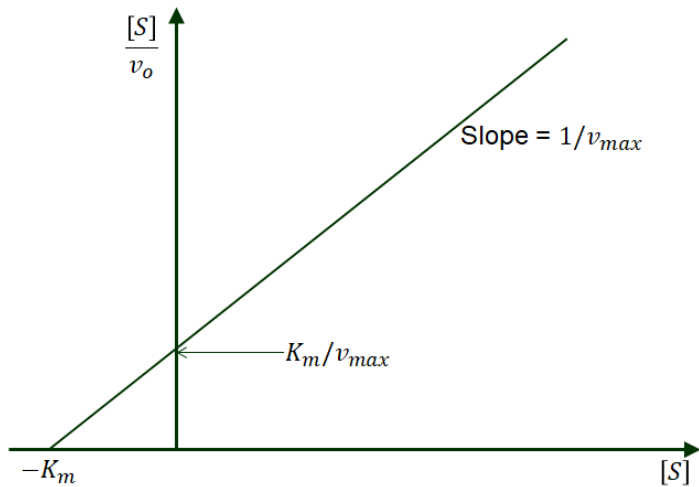Making a plot of $\frac{[S]}{v_0}$ vs $[S]$, we have Fig. (5)

Figure 5: Hanes-Woolf plot

# Working Session 3

## Estimation of kinetic parameters

The data below show the substrate concentrations and the time taken for substrate removal for a waste treatment.

| Substrate concentration $mmolL^{-1}$ | $t=0$ | $t=60s$ | $t=120s$ | $t=180s$ | $t=240s$ | $t=300s$ |
|---|---|---|---|---|---|---|
| 5 | 0 | 161 | 320 | 482 | 598 | 662 |
| 6 | 0 | 194 | 388 | 581 | 745 | 796 |
| 10 | 0 | 263 | 525 | 789 | 998 | 1120 |
| 20 | 0 | 400 | 798 | 1200 | 1520 | 1760 |
| 30 | 0 | 570 | 1150 | 1730 | 2470 | 2460 |

Calculate $K_M$ and $v_{max}$.

## Solution

Using the Lineweaver-Burk method:

- Calculate the rate (initial velocity) for the various [S], as shown below:
- Make a plot of $1/v_0$ vs $1/[S]$

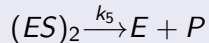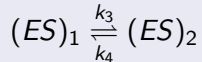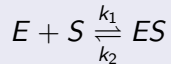| $[S]$ $mmol L^{-1}$ | $v_0 = \frac{[S]_{t=300} - [S]_{t=60}}{t_{300} - t_{60}}$ $mmol L^{-1} s^{-1}$ | $\frac{1}{[S]}$ $L(kmol^{-1})$ | $\frac{1}{v_0}$ $Ls(kmol^{-1})$ |
|---|---|---|---|
| 5 | 2.0875 | 0.20 | 0.48 |
| 6 | 2.5083 | 0.17 | 0.40 |
| 10 | 3.5708 | 0.10 | 0.28 |
| 20 | 5.667 | 0.05 | 0.18 |
| 30 | 7.875 | 0.03 | 0.18 |

# Practice Problems

The Michaelis-Menten approach assumes that the product releasing step is much slower than the first complex forming step of the simple enzyme-reaction mechanism:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES$$

$$ES \overset{k_5}{\longrightarrow} E + P$$
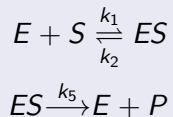
- Derive a rate equation for the case in which the enzyme- substrate formation step is much slower than the product releasing step, that is, $k_1 \ll k_3$, $k_2 \ll k_3$.
- State your assumptions.

## Problem 2

In some enzyme-catalyzed reactions, multiple complexes are involved as follows:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES$$

$$(ES)_1 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} (ES)_2$$

$$(ES)_2 \overset{k_5}{\longrightarrow} E + P$$

Develop a rate expression using:

(a) the Michaelis-Menten approach and

(b) the Briggs-Haldane approach.

## Problem 3

Invertase hydrolyzes cane sugar into glucose and fructose. The following table shows the amount of sugar inverted in the first 10 minutes of reaction for various initial substrate concentrations.

| Substrate Sugar Concentration $g/L$ | Sugar Inverted in 10 min $g/L$ |
|---|---|
| 48.9 | 1.9 |
| 67.0 | 2.1 |
| 98.5 | 2.4 |
| 199.1 | 2.7 |
| 299.6 | 2.5 |
| 400.2 | 2.3 |

Evaluate the Michaelis-Menten kinetic parameters by employing

(a) the Eadie-Hofstee plot

(b) the Lineweaver-Burk plot.

### Problem 4

The $K_M$ value of an enzyme is known to be 0.01 $mol/L$. To determine the maximum reaction rate catalyzed by the enzyme, the initial rate of the reaction was measured and found that 10 percent of the initial substrate was consumed in 5 minutes. The initial substrate concentration is $3.4 \times 10^{-4}$ $mol/L$. Assume that the reaction can be expressed by the Michaelis- Menten kinetics.

(a) What is the maximum reaction rate?

(b) What is the concentration of the substrate after 15 minutes?

## Problem 5

Eadie (1942) measured the initial reaction rate of hydrolysis of acetylcholme (substrate) by dog serum (source of enzyme) and obtained the following data:

| Substrate Concentration $mol/L$ | Initial Reaction Rate $mol/Lmin$ |
|---|---|
| 0.0032 | 0.111 |
| 0.0049 | 0.148 |
| 0.0062 | 0.143 |
| 0.0080 | 0.166 |
| 0.0095 | 0.200 |

Evaluate the Michaelis-Menten kinetic parameters by employing

(a) the Lineweaver-Burk plot.

(b) the Eadie-Hofstee plot.