Exercise 1: Using the Michaelis-Menten Equation

Problem:
An enzyme has a maximum velocity ($Vmax$) of $200\mu mol{\cdot}L{-}1{\cdot}min{-}1$
and a Michaelis constant (Km) of $4mM$

.

- **Question:** Calculate the initial velocity ($V0$) when the substrate concentration $[S]$ is $12mM$

  .**Solution:**
  Use the formula:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$V0=Vmax[S]/Km+[S]$

$$V_0 = \frac{200 \times 12}{4 + 12} = \frac{2400}{16} = 150\mathbf{\mu mol} \cdot \mathbf{L}^{-1} \cdot \mathbf{min}^{-1}$$

$V0=200\times124+12=240016=\mathbf{150\mu mol{\cdot}L{-}1{\cdot}min{-}1}$

Exercise 2: Lineweaver-Burk Plot (Double-Reciprocal)

Problem:
The linear regression equation for a Lineweaver-Burk plot of an enzyme-catalyzed reaction is:

$$y = 0.4x + 0.05$$

$y=0.4x+0.05$

(where
$y = 1/V_0$ y*=1/*V*0*
in
$(L \cdot min)/\mu mol$ *and* $x = 1/[S]$ x*=1/*$[S]$
in mM^{-1}
mM*−1).*

- **Question:** Determine $Vmax$and Km

- **Solution:**

1. **Find $Vmax$**

   : The y-intercept is $1/Vmax=0.05$

   $Vmax=1/0.05=\mathbf{20 µmol·L^{-1}·min^{-1}}$.

2. **Find Km**

   : The slope is $Km/Vmax=0.4$

   .$Km=0.4×Vmax=0.4×20=\mathbf{8mM}$.

Exercise 3: Identifying Inhibition Types

Problem:

You are analyzing an enzyme with and without an inhibitor. You observe the following:

- **Without Inhibitor:**

  $Vmax=100, \quad Km=2.$

- **With Inhibitor:**

  $Vmax=50, \quad Km=2.$

- **Question:** What type of inhibition is occurring?
- **Answer:** This is **Non-competitive inhibition**. In this type, the inhibitor reduces the overall turnover rate ($Vmax$ decreases), but it does not affect the binding affinity of the substrate (Km stays the same

Exercise 4: Catalytic Efficiency ($kcat$)

Problem:

A purified enzyme solution has a total concentration $[E]t$
of $2nM$ ($2×10^{-9}M$). The measured $Vmax$ is $0.8\mu M·s^{-1}$(
$×10^{-7}M·s^{-1}$
).

- **Question:** Calculate the turnover number ($kcat$).

Solution:

$$k_{cat} = \frac{V_{max}}{[E]_t}$$

$kcat$=$Vmax/[E]t$

$$k_{cat} = \frac{8 \times 10^{-7} M \cdot s^{-1}}{2 \times 10^{-9} M} = 400\mathbf{s}^{-1}$$

$kcat$=8×10−7M·s−12×10−9M=**400s−1**

(Meaning one enzyme molecule converts 400 substrate molecules into product every second).

Enzyme Kinetics Question 5 Detailed Solution

Concept:

Catalytic efficiency is a measure of how efficiently an enzyme converts a substrate into a product. It is defined as the ratio of the turnover number (k_{cat}) to the Michaelis constant (K_m). The equation for catalytic efficiency is given by:

$$\text{Catalytic Efficiency} = \frac{k_{cat}}{K_m}$$

and

$$k_{cat} = \frac{v_{max}}{[E]_o}$$

The Michaelis-Menten equation is used to describe the rate of enzymatic reactions and is expressed as:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

On simplifying the equation:

$$\frac{1}{V_o} = \frac{1}{V_{max}} + \frac{k_m}{V_{max}[S]}$$

Where:

- (V_0) is the initial reaction velocity.
- (V_{max}) is the maximum reaction velocity.
- (S) is the substrate concentration.
- (K_m) is the Michaelis constant.

Explanation:

- Given the intercept is ($4 \times 10^3 \mathrm{mol}^{-1}\mathrm{dm}^3\mathrm{s}^{-1}$)
  - $\frac{1}{v_{max}} = 4 \times 10^3$
  - $v_{max} = 2.5 \times 10^{-4}$
- The turnover number (k_{cat}) is:
  - $k_{cat} = \frac{2.5\times10^{-4}}{10^{-4}} = 2.5$
- Thus, the catalytic efficiency is:
  - $\text{Catalytic Efficiency} = \frac{2.5}{0.04} = 62.5$

Conclusion:

The calculated catalytic efficiency is 62.5.

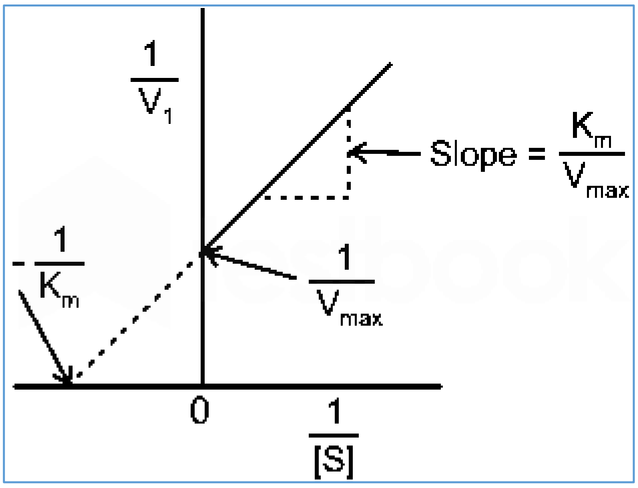
Enzyme Kinetics Question 9 Detailed Solution

Concept:

- For **enzyme-catalyzed reactions** that follow a Michaelis-Menten mechanism, the relationship between the rate of reaction and enzyme-substrate is given by:

$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{k_M}{v_{max}}\frac{1}{[S]}$$

- The expression has the form of a straight-line graph.



- If we **plot pf 1/v against 1/[S]. should give a gradient of K_M/V_{max} and an intercept of V_{max}.**
- The value of the Michaelis Menten constant is therefore given by the ratio of the intercept:

$$\frac{m}{c} = \frac{\frac{K_M}{v_{max}}}{\frac{1}{v_{max}}} = K_M$$

Explanation:-

The **slope of the graph**

= m

= **0.16**

The **intercept of the graph**

= C

= **2.12 mol^{-1} L s**

- We know that the ratio of **slope/intercept** gives the **Michaelis constant value**, So,

$$K_M = \frac{m}{c} = \frac{\frac{K_M}{v_{max}}}{\frac{1}{v_{max}}}$$

- Substituting the given values, we get:

$\frac{m}{c} = \frac{.16}{2.12}$

=0.075

= **0.08**

Conclusion:-

- Hence, the estimated value of the **Michaelis constant** is **0.08 mol L**$_{-1}$

The table present the differences between inhibition types

Comparison of Reversible Inhibition Types

Reversible inhibitors are primarily distinguished by their effects on max(maximum velocity) and (Km binding affinity).

Inhibition Type	**Binding Location**	**Effect on** V_{max} $\boldsymbol{Vmax}$	**Effect on** K_m $\boldsymbol{Km}$	**Can be overcome by more substrate?**
Competitive	Active Site	No change	**Increases**	**Yes** (competes directly)
Non-competitive	Allosteric Site	**Decreases**	No change	**No** (binds elsewhere)
Uncompetitive	Enzyme-Substrate (ES) Complex	**Decreases**	**Decreases**	**No** (inhibitor only binds after substrate)
Mixed	Either E or ES complex	**Decreases**	**Increases or Decreases**	**Partially**