# Assignment - 10: Solution.

**Ans 1.** The maximum velocity is $k_b[E]_0$ & the velocity in general is:

$$v = k[E]_0 = \frac{k_b[S][E]_0}{K_m + [S]} \quad \text{so } v_{max} = k_b[E]_0$$

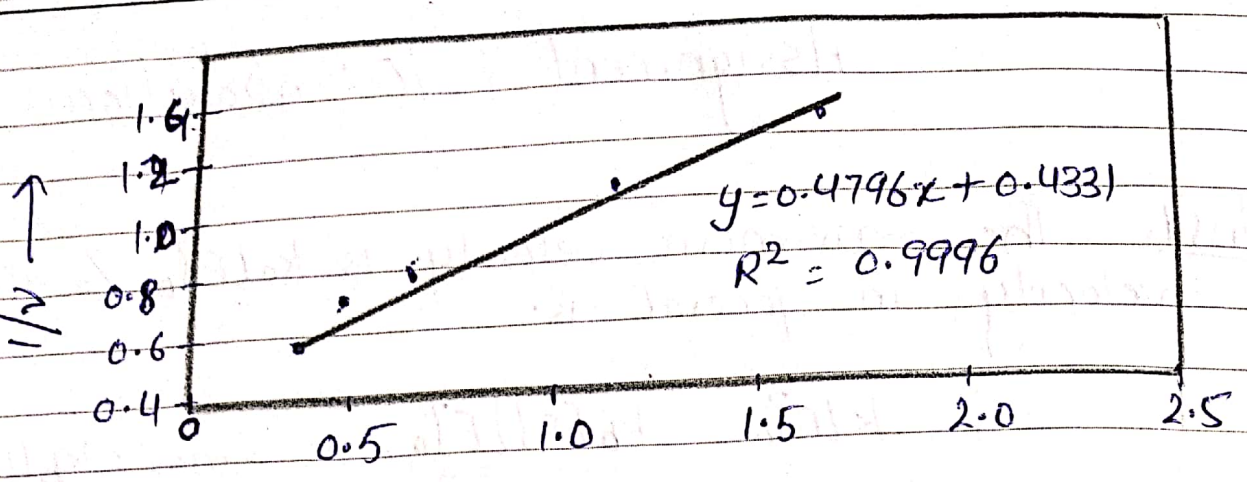
$$\Rightarrow v_{max} = \frac{K_m + [S]}{[S]} v$$

$$v_{max} = \left[\frac{(0.024\ mol\,dm^{-3}) + (0.890\ mol\,dm^{-3})}{(0.889\ mol\,dm^{-3})}\right] \times \left[1.15 \times 10^{-4}\ mol\,dm^{-3}\,s^{-1}\right]$$

$$= 1.181 \times 10^{-4}\ mol\,dm^{-3}\,s^{-1}$$

**Ans 2:** For Lineweaver - Burk Plot ($1/v$ against $1/[S]_0$).

| | | | | | |
|---|---|---|---|---|---|
| $[ATP]\ (\mu mol\,dm^{-3})$ | 0.60 | 0.80 | 1.4 | 2.0 | 3.0 |
| $v\ [\mu mol\,dm^{-3}\,s^{-1}]$ | 0.81 | 0.97 | 1.3 | 1.47 | 1.69 |
| $1/[[ATP]/(\mu mol\,dm^{-3})]$ | 1.67 | 1.25 | 0.714 | 0.50 | 0.33 |
| $1/[v/(\mu mol\,dm^{-3})]$ | 1.23 | 1.03 | 0.769 | 0.680 | 0.592 |




1/[ATP] ⟶

$1/v_{max}$ = Intercept

$v_{max}$ = 1/Intercept.

= $1/(0.433\ \mu mol\ dm^{-3} s^{-1})$

= $2.31\ \mu mol\ dm^{-3} s^{-1}$

$k_b = v_{max}/[E]_0$

$= (2.31\ \mu mol\ dm^{-3} s^{-1})/(0.020\ \mu mol\ dm^{-3})$

$= 115\ s^{-1}$

$k_{cat} = k_b$

$= \underline{113\ s^{-1}}$

$K_m = V_{max} \times \text{slope}.$

$= (2.31\ \mu mol\ dm^{-3} s^{-1}) \times (0.480\ s)$

$= \underline{1.11\ \mu mol\ dm^{-3}}$

$\varepsilon = k_{cat} / K_m$

$= (115\ s^{-1}) / (1.11\ \mu mol\ dm^{-3})$

$= \underline{104\ dm^3\ \mu mol^{-1} s^{-1}}.$

**Ans 3:** $\text{Rate} = \dfrac{V_{max}[S]}{K_m + [S]}$

$\dfrac{1}{\text{rate}} = \dfrac{K_m + [S]}{V_{max}[S]}.$

$\Rightarrow \dfrac{1}{\text{rate}} = \dfrac{K_m}{V_{max}[S]} + \dfrac{1}{V_{max}}$

Given,

$$\text{Slope} = \frac{K_m}{V_{max}} = 300$$

$$\text{Intercept} = \frac{1}{V_{max}} = 2 \times 10^5$$

} for lineweaver-Burk Plot.

$$\Rightarrow K_m \times \frac{1}{V_{max}} = 300.$$

$$K_m \times (2 \times 10^5) = 300$$

$$K_m = \frac{300}{2 \times 10^5}$$

$$\underline{K_m = 1.5 \times 10^{-3}}$$

Ans 4. In case of Competitive - Inhibition:

$$\alpha = 1 + [I]/K_I \quad \& \quad \alpha' = 1$$

$$\Rightarrow v = \frac{V_{max}}{1 + \alpha K_m/[S]_0}$$

By setting the ratio $v([I]=0)/v[I]$ equal to $1/0.25 = 4.00$ and solving for $\alpha$, we can subsequently solve for the inhibitor concentration that reduces the catalytic rate by 75%.

$$\frac{v([I]=0)}{v([I])} = \frac{1+\alpha K_m/[S]_0}{1+K_m/[S]_0} = 4$$

$$\alpha = \frac{1.333\,(1+K_m/[S]_0)-1}{K_m/[S]_0}$$

$$\alpha = \frac{4.00\,(1+7.5/1.0)-1}{7.5/1.0} = 4.40$$

$$[I] = (\alpha-1)K_I$$

$$= (4.40-1) \times (0.56\ \text{mmol dm}^{-3})$$

$$= \underline{1.90\ \text{mmol dm}^{-3}}$$

Ans 5: (a) We add to the Michaelis-Menten mechanism the inhibition by the substrate:

$$SES \rightleftharpoons ES + S$$

$$K_I = [ES][S]/[SES]$$

Where the inhibited enzyme, SES, forms when S binds to ES and, thereby, prevents the formation of product. This inhibition might possibly occurs when S is at a very high concentration. Enzyme mass balance is written in terms of [ES], $K_I$, $K_m$ = ([E][S]/[ES]) and [S]. For practical purposes the free substrate concentration is replaced by $[S]_0$ because the substrate is typically in large excess relative to the enzyme.

$$[E]_0 = [E] + [ES] + [SES]$$

$$= \frac{K_m[ES]}{[S]} + [ES] + \frac{[ES][S]}{K_I}$$

$$= \left[1 + \frac{K_m}{[S]} + \frac{[S]}{K_I}\right][ES]$$

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Thus,

$$[ES] = \frac{[E]_0}{\left(1 + \frac{K_m}{[S]} + \frac{[S]}{K_1}\right)} \quad (7)$$

and the expression for the rate of product formation becomes

$$v = k_b [ES].$$

$$= \frac{v_{max}}{\left(1 + \frac{K_m}{[S]} + \frac{[S]}{K_1}\right)}$$

where $v_{max} = k_b [E_0]$

The denominator term $[S]_0/K_1$ reflects a reduced reaction rate caused by inhibition as the concentration of S becomes very large.

(b) To examine the effect that substrate inhibition has on the double reciprocal, Lineweaver - Burk plot of $1/v$ against $1/[S]_0$ take the inverse of the above rate expression and compare it to the uninhibited expression.

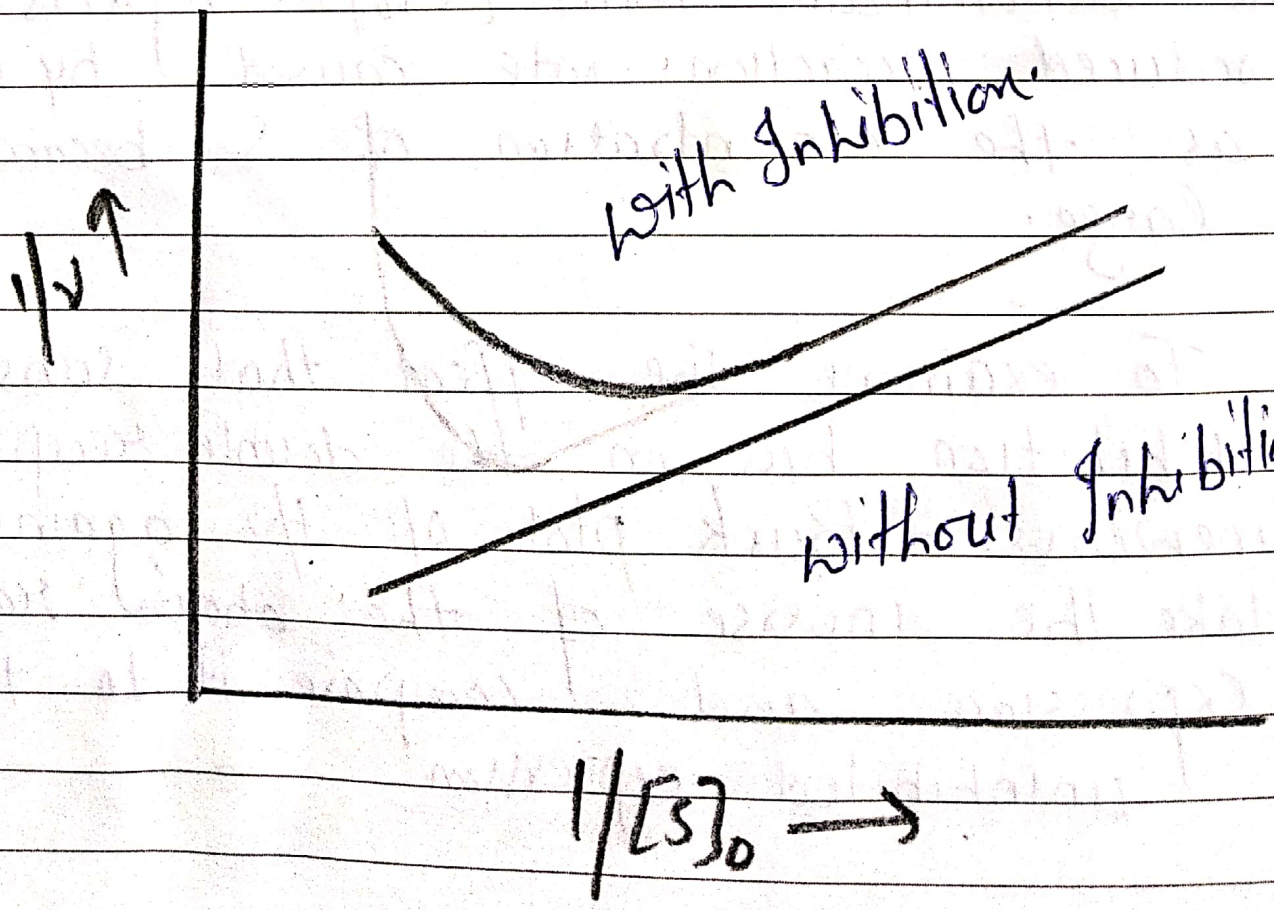
$$\frac{1}{v} = \frac{1}{v_{max}} + \left(\frac{K_m}{v_{max}}\right)\frac{1}{[S]_0}$$

The inverse of the inhibited rate law is:

$$\frac{1}{v} = \frac{1}{v_{max}} + \left(\frac{K_m}{v_{max}}\right)\frac{1}{[S]_0} + \left(\frac{[S]_0^2}{v_{max} K_I}\right)\frac{1}{[S_0]}$$

$$= \frac{1}{v_{max}} + \left(\frac{K_m}{v_{max}} + \frac{[S]_0^2}{v_{max} K_I}\right)\frac{1}{[S]_0}$$

The uninhibited & inhibited line shapes are as follows:



Comparing the two expressions, we see that the two curves match at high value of $1/[S]_0$. However, as the concentration of $[S]_0$ increases ($1/[S]_0$ decreases) the $1/v$ curve with inhibition curves upward because the rxn. rate is decreasing.