Assignment - 10: Solution:

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

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

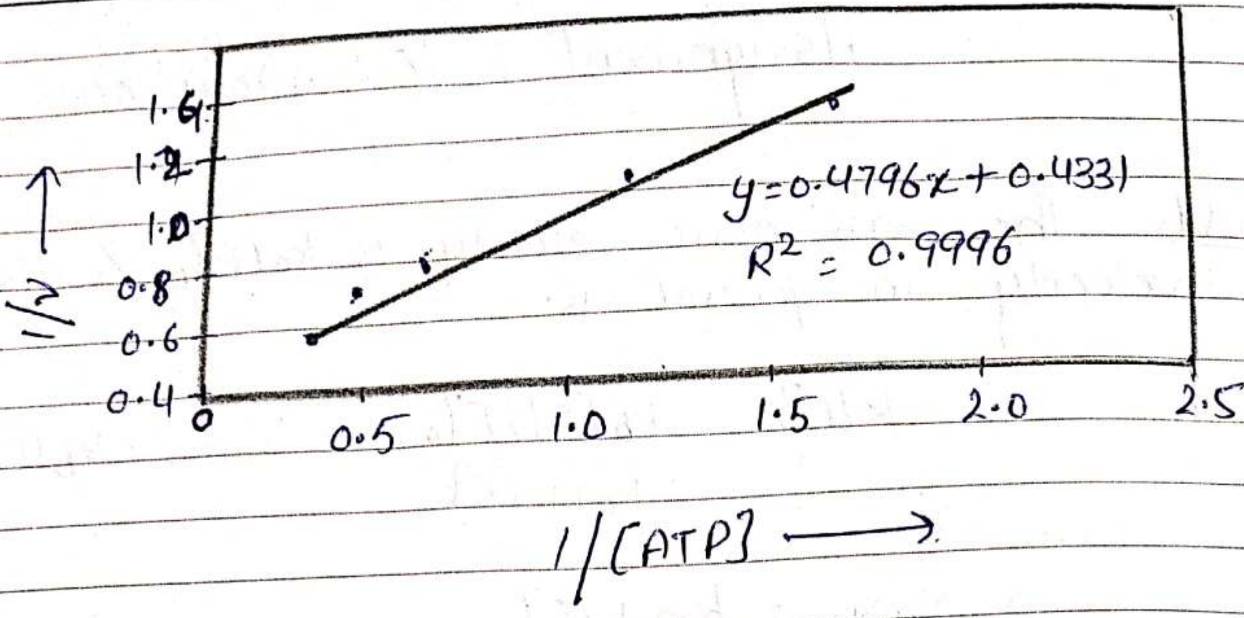
$$\Rightarrow v_{max} = \frac{K_m + [S]}{[S]} v$$

$$v_{max} = \left[\frac{(0.024\,mol\,dm^{-3}) + (0.890\,mol\,dm^{-3})}{(0.089\,mol\,dm^{-3})}\right] \times \left[1.15 \times 10^{-4}\,mol\,dm^{-3}s^{-1}\right]$$

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

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



$1/v_{max}$ = Intercept

v_{max} = 1/Intercept

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

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

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

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

$= \underline{115 s^{-1}}$

$K_{cat} = K_b$

$= 113\ s^{-1}$

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

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

$= \cancel{8}1.11\ \mu mol\ dm^{-3}$

$\varepsilon = K_{cat} / K_m$

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

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

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

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

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

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Given,

$$\text{Slope} = \frac{k_m}{V_{max}} = 300.$$

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

for Lineweaver-Burk Plot.

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

$$k_m \times (2 \times 10^5) = 300.$$

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

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

<u>Ans 4.</u> In case of Competitive Inhibition:

$$\alpha = 1 + [I]/K_1 \quad \& \quad \alpha' = 1$$

$$\Rightarrow \quad v = \frac{v_{max}}{1 + \alpha\, k_m/[S]_0}$$

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

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

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

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

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

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

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

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

$$SES \rightleftharpoons ES + S$$

$$K_1 = [ES][S] / [SES]$$

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_1, Km =

$([E][S]/[ES])$ 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.

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

$$= \frac{K_m[ES]}{[S]} + [ES] + \frac{[ES][S]}{K_1}$$

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

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Thus,

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

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and the expression for the rate of product formation becomes

$$v = k_b [ES].$$

$$= \frac{v_{max}}{\left(1 + \frac{K_m}{[S]} + \frac{[S]}{K_1}\right)} \quad \text{where } v_{max} = k_b [E_0]$$

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.

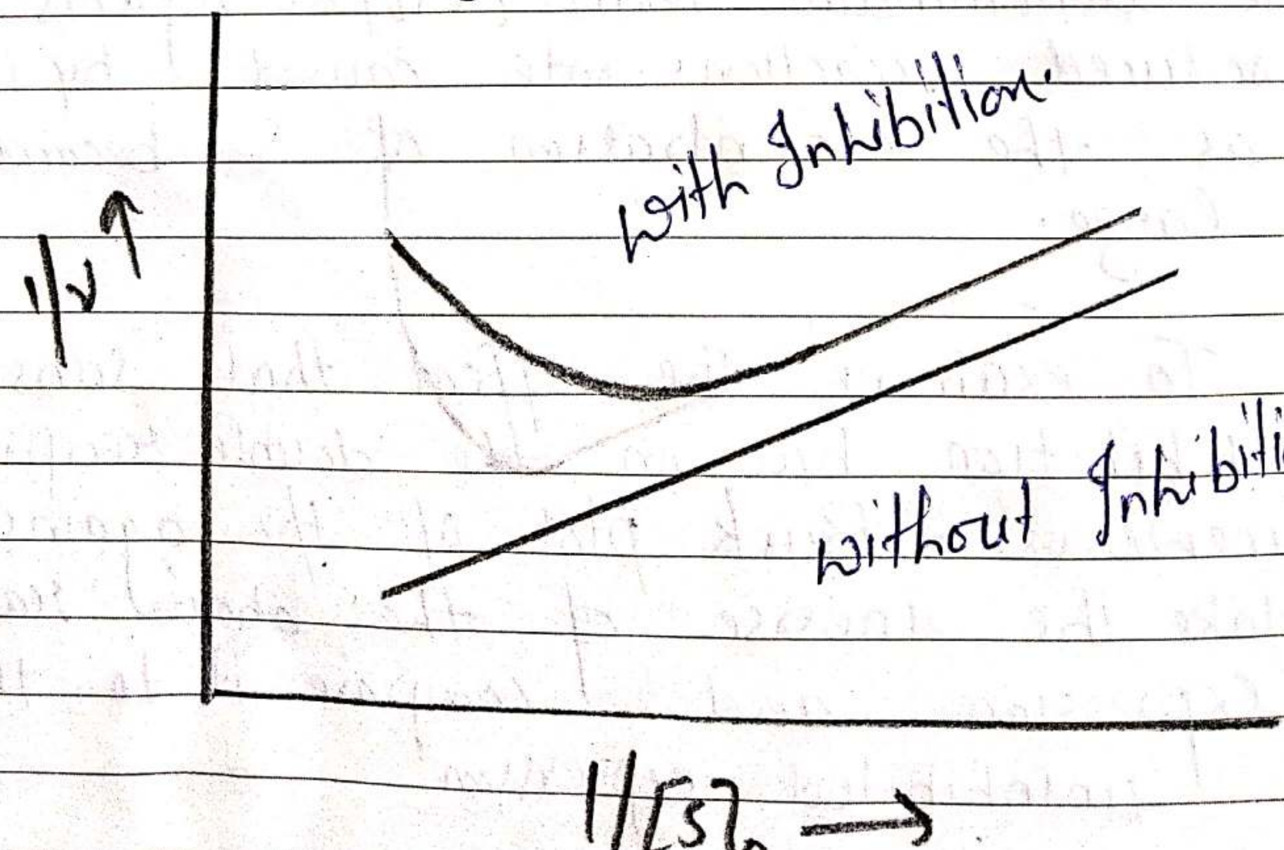
$$\frac{1}{v} = \frac{1}{v_{max}} + \left(\frac{K_m}{v_{max}}\right)\frac{1}{[S]_0}.$$

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:



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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.