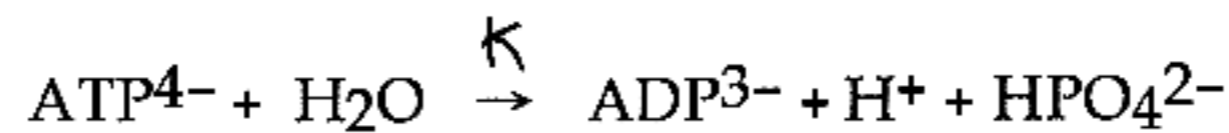


Practice Problems for Enzymes and Enzyme Kinetics

1) What do catalysts do? How do they accomplish this?

- CATALYSTS LOWER THE ACTIVATION ENERGY FOR A REACTION AND THUS INCREASE THE RATE AT WHICH THE RXN REACHES EQ.
- LOWER ΔG^\ddagger (TRANSITION STATE FREE ENERGY) ΔH^\ddagger & ΔS^\ddagger ← SEE NOTES

2) a) What are all of the different ways to express the rate of the following balanced equation for the hydrolysis of adenosine triphosphate (ATP)?

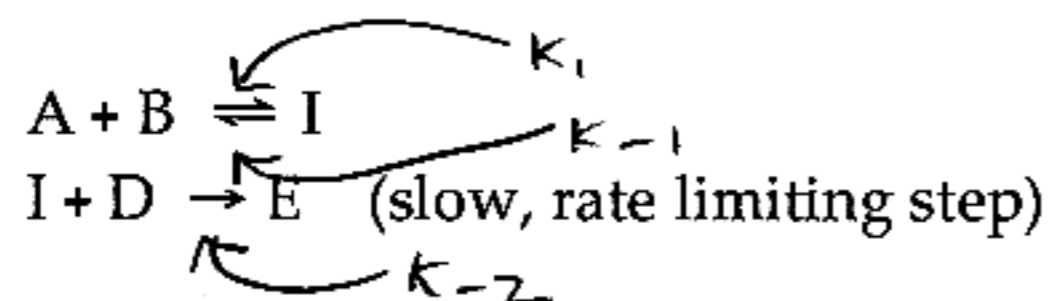


$$-\frac{d[\text{ATP}^{4-}]}{dt} = -\frac{d[\text{H}_2\text{O}]}{dt} = \frac{d[\text{ADP}^{3-}]}{dt} = \frac{d[\text{H}^+]}{dt} = \frac{d[\text{HPO}_4^{2-}]}{dt}$$

b) What is a valid way to express the rate law for this reaction

ANY OF THE ABOVE RATE EXPRESIONS, SAY $\frac{d[\text{H}^+]}{dt} = k[\text{ATP}^{4-}]^n [\text{H}_2\text{O}]^m$

3) Suppose you have the following reaction scheme:



$n + m = \text{TOTAL RXN ORDER}$ — MUST BE DETERMINED EXPERIMENTALLY

a) What is $\frac{d[\text{I}]}{dt}$ equal to?

$$\frac{d[\text{I}]}{dt} = k_1[\text{A}][\text{B}] - k_{-1}[\text{I}] - k_2[\text{I}][\text{D}]$$

b) What is the overall rate law for this reaction (hint: use the steady state approximation)?

RATE = $k_2[\text{I}][\text{D}]$ WHAT IS $[\text{I}]$?

AT STEADY STATE, $\frac{d[\text{I}]}{dt} \approx 0$, SO: $0 = k_1[\text{A}][\text{B}] - k_{-1}[\text{I}] - k_2[\text{I}][\text{D}]$

$$k_{-1}[\text{I}] + k_2[\text{I}][\text{D}] = k_1[\text{A}][\text{B}]$$

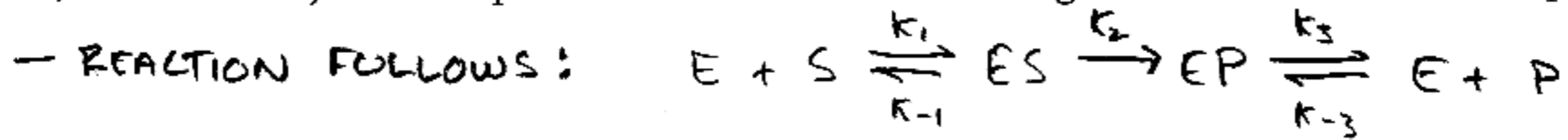
$$[\text{I}](k_{-1} + k_2[\text{D}]) = k_1[\text{A}][\text{B}]$$

$$[\text{I}] = \frac{k_1[\text{A}][\text{B}]}{k_{-1} + k_2[\text{D}]}$$

SO: RATE = $k_2[\text{I}][\text{D}]$

$$\text{RATE} = \frac{k_1 k_2 [\text{A}][\text{B}][\text{D}]}{k_{-1} + k_2[\text{D}]}$$

4) State the major assumptions we made when deriving the Michaelis-Menten equation.



- k_2 IS RATE LIMITING, SLOW STEP

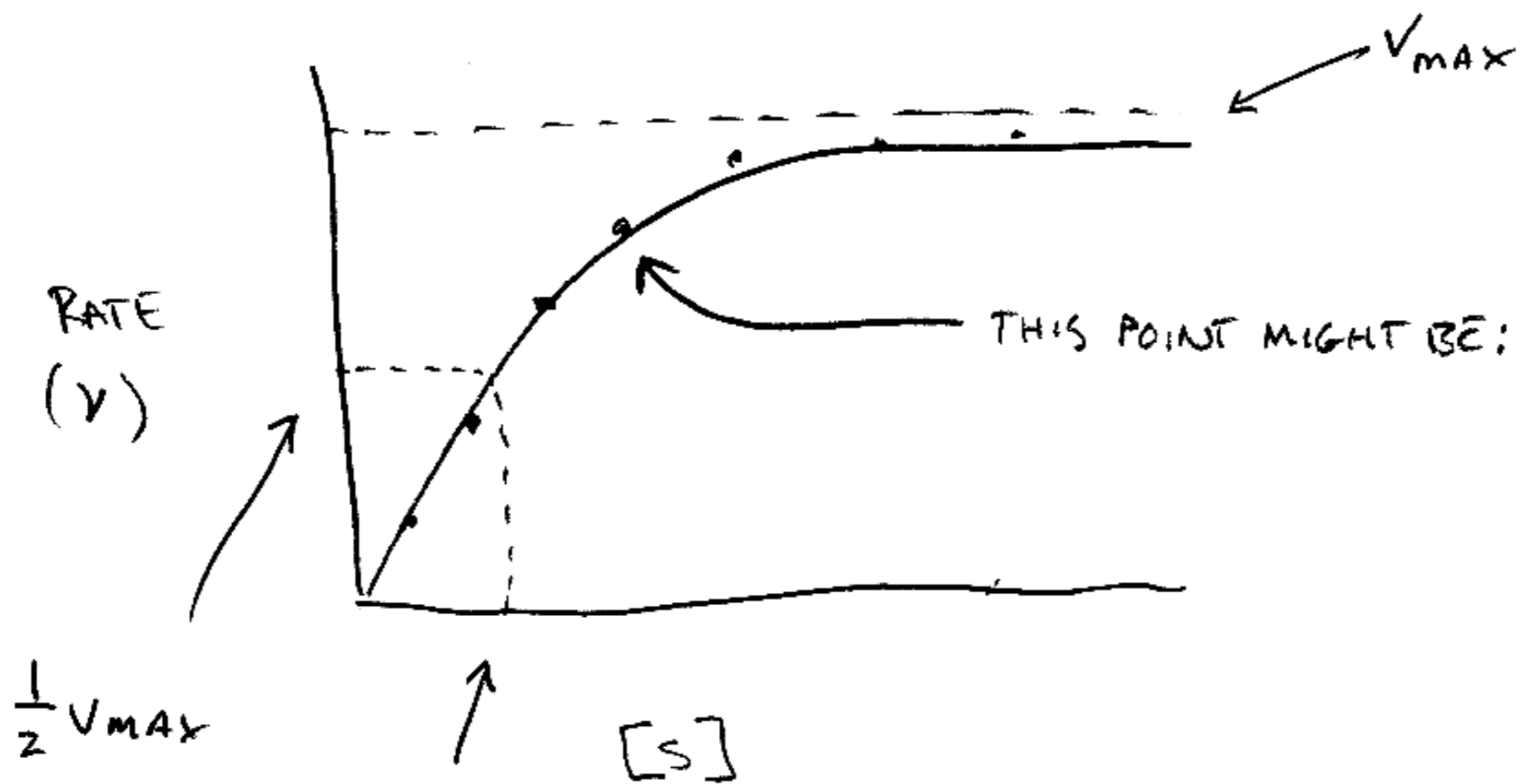
- k_{-2} & k_{-3} ARE NEGLIGIBLE (NOT MUCH 'P' EARLY IN REACTION)

- STEADY STATE IS ACHIEVED: $\frac{d[ES]}{dt} \approx \frac{d[EP]}{dt} \approx 0$

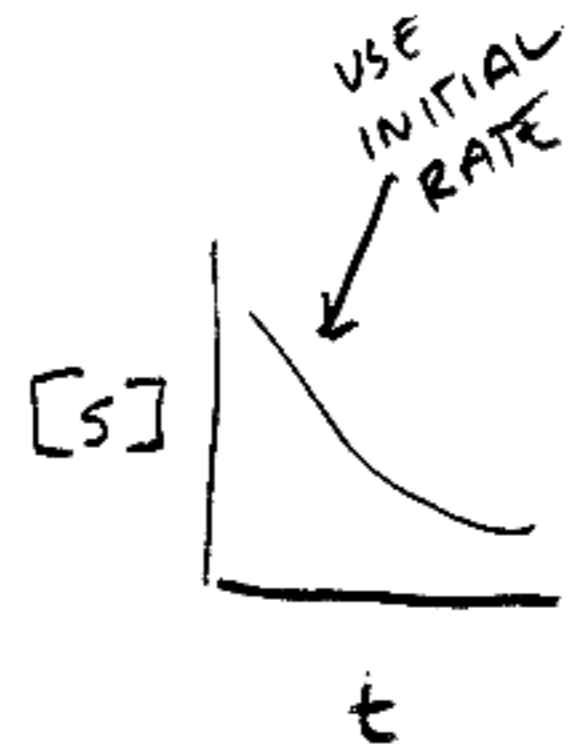
- $[E]_T = [E] + [ES]$ ← $[EP]$ IS NEGLECTED BECAUSE $k_3 \gg k_2$

- RATE = V_{max} WHEN $[ES] = [E]_T$ (SATURATED)

5) Sketch a Michaelis-Menten plot for an enzyme following Michaelis-Menten kinetics and include hypothetical points along the plot. Label both axes, V_{max} and K_m and state how each point on the graph was obtained.



$[S] = K_m$
(AT $\frac{1}{2} V_{max}$)



SLOPE OF INITIAL RATE = $\frac{d[S]}{dt}$

6) a) What equations are derived from the Michaelis-Menten equation to create an Eadie plot and a Lineweaver-Burk plot? Why do we need these equations?

$$\text{RATE (v)} = \frac{V_{\text{MAX}} [S]}{K_m + [S]}$$

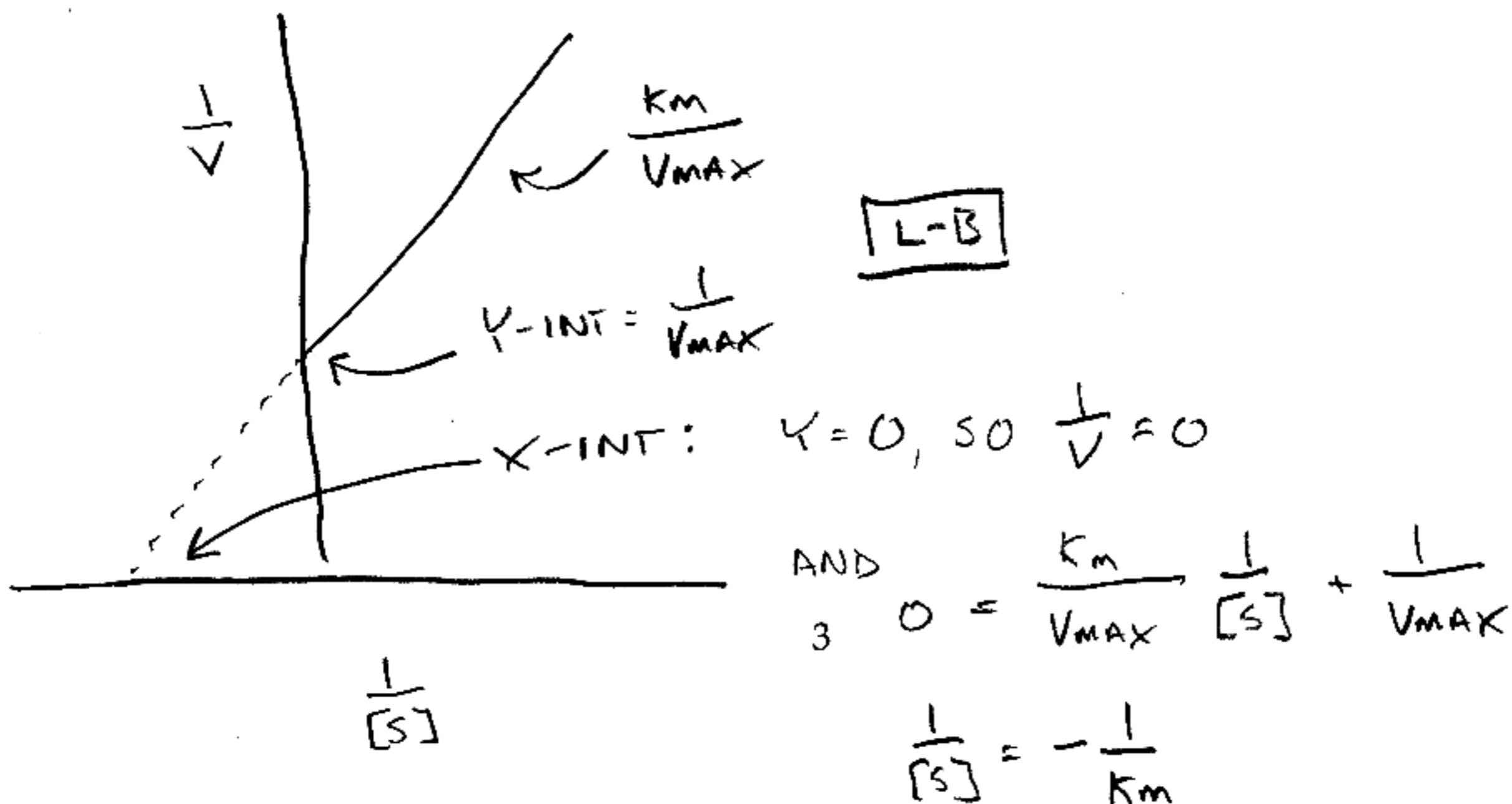
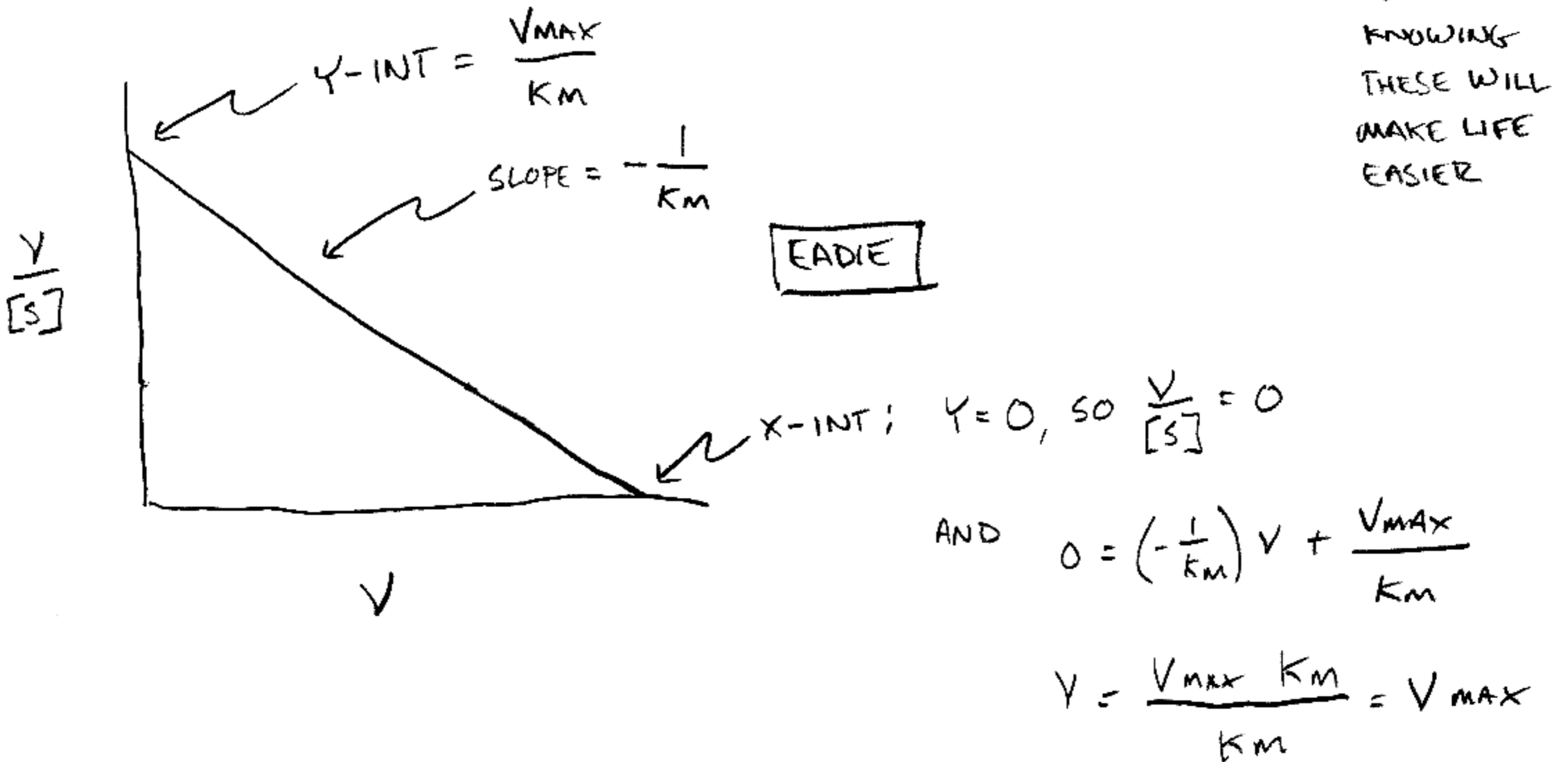
LB $\rightarrow \frac{1}{v} = \left(\frac{K_m}{V_{\text{MAX}}}\right) \frac{1}{[S]} + \frac{1}{V_{\text{MAX}}}$

$$Y = m \cdot x + b$$

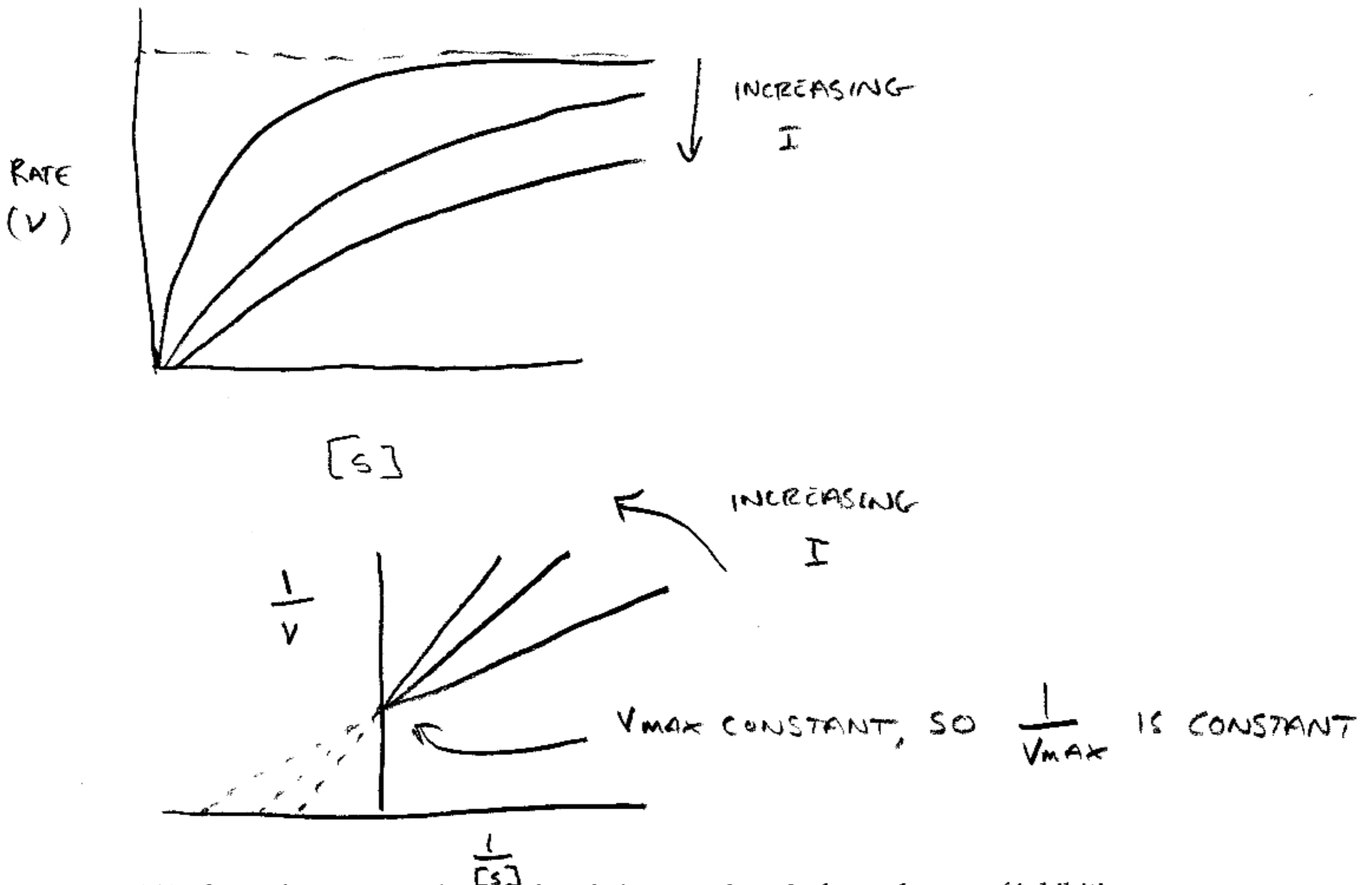
EADIE $\rightarrow \frac{v}{[S]} = \left(-\frac{1}{K_m}\right) v + \frac{V_{\text{MAX}}}{K_m}$

WE CONVERT TO LINEARIZE THE M-M EQUATION

b) Draw a diagram of each of these plots (Eadie and L-B), labelling both axes, the value of the slopes, y-intercepts and the x-intercepts. To find the x-intercept, set the y-value (not the y-intercept) in the equation to zero.



7) Draw MM and LB plots for four enzyme assays in which a competitive inhibitor is being introduced.



8) Explain why V_{max} and K_m values behave as they do for each type of inhibition we discussed.

COMPETITIVE INHIBITION: V_{max} IS UNCHANGED BECAUSE YOU CAN THEORETICALLY REACH V_{max} AT ANY INHIBITOR CONCENTRATION BY ADDING MORE SUBSTRATE. K_m APPEARS TO INCREASE AS YOU CAN SEE FROM THE PLOTS ABOVE.

NON COMPETITIVE INHIBITION: V_{max} CHANGES BECAUSE INHIBITED ENZYME IS OFF. CAN THINK OF IT AS $[E]_T$ IS BEING LOWERED. $V = k_2[ES]$ AT V_{max} , $[ES] = [E]_T$ SO $V_{max} = k_2[E]_T$; AS $[E]_T$ APPEARS LOWER V_{max} IS LOWERED, K_m IS UNCHANGED.