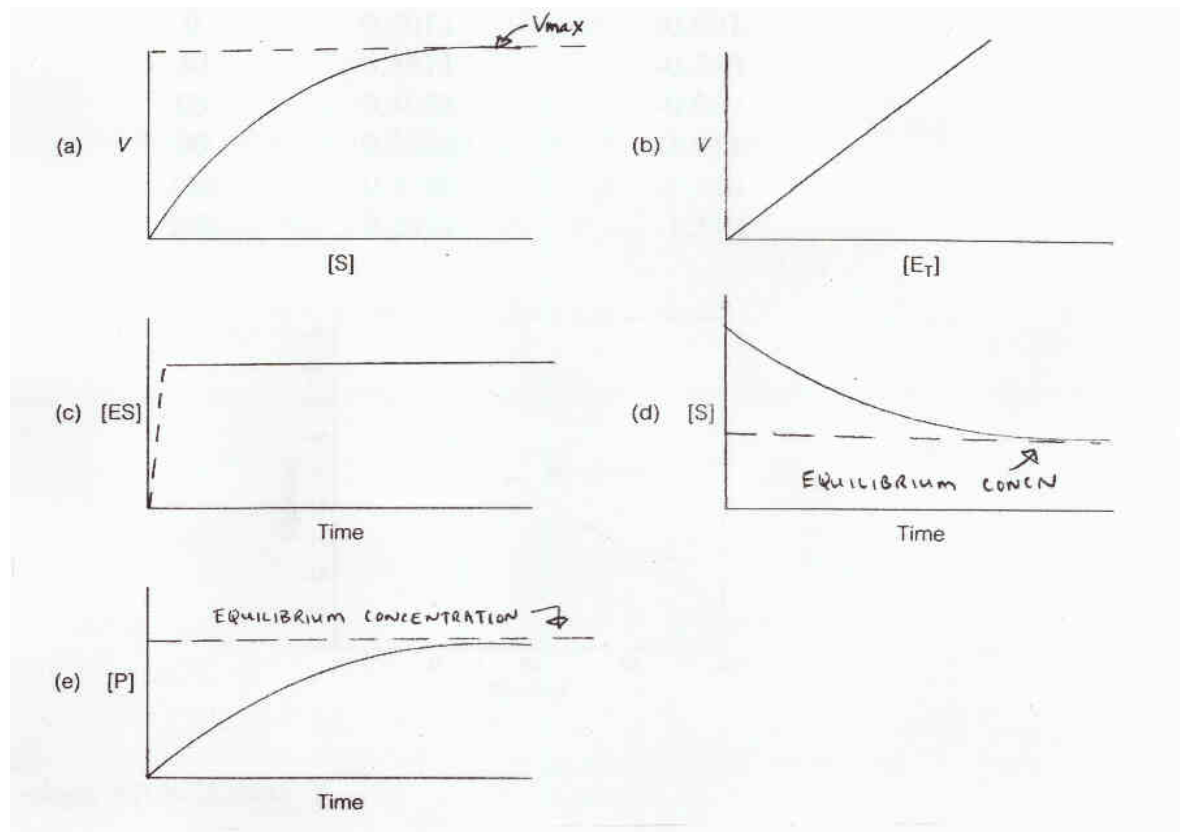


BIOC*4540 Enzymology **Problem Set #2 ANSWERS**

1. Sketch the appropriate plots on the following axes. Assume that simple Michaelis-Menten kinetics apply.



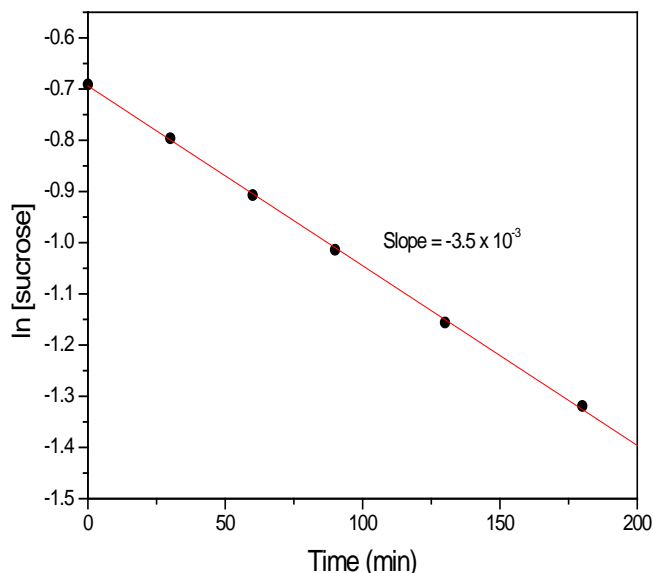
2.

Time (min)	[sucrose] M	$\ln[\text{sucrose}]$
0	0.5011	-0.691
30	0.4511	-0.796
60	0.4038	-0.907
90	0.3626	-1.014
130	0.3148	-1.156
180	0.2674	-1.319

$$k = -\text{slope} = 0.0035 \text{ min}^{-1}$$

$$t_{1/2} = 0.693/k = 0.693/0.0035 \text{ min}^{-1} = 198 \text{ min}$$

The reaction follows *pseudo* first-order kinetics because in aqueous solution the H_2O concentration of 55.5 M remains essentially constant in comparison to the sucrose concentration. The reaction actually is second order.



$$(1-0.99) = 0.01 = (1/2)^n \text{ where } n \text{ is the number of half-lives}$$

$$n (\log 0.5) = \log 0.01$$

$$n = -2.00/-0.301 = 6.64 \text{ half-lives. Thus, the time to hydrolyze 99\% of the sucrose} = t_{0.01} = nt_{1/2} = 6.64 \times 198 = 1315 \text{ min.}$$

The time to react a given fraction of a substance that reacts in a first-order process is independent of the amount initially present. Hence, the time to hydrolyze 99% of $2 \times 0.5011 \text{ M}$ sucrose is also 1315 min.

3. (a) $[\text{S}]_0 = 0.5 \text{ } \mu\text{mole}/25 \text{ mL} = 2 \times 10^{-2} \text{ } \mu\text{mole}/\text{mL} = 2 \times 10^{-5} \text{ M}$
 $K_M = 6 \times 10^{-4} \text{ M}$ therefore $[\text{S}]_0 < K_M$ and thus **first order** kinetics.
- (b) $[\text{S}]_0 = 0.5 \text{ mmole}/25 \text{ mL} = 2 \times 10^{-2} \text{ mmole}/\text{mL} = 2 \times 10^{-2} \text{ M}$
 $K_M = 6 \times 10^{-4} \text{ M}$ therefore $[\text{S}]_0 > K_M$ and thus **zero order** kinetics.
4. (a) We must assume that the initial velocity observed at the $[\text{S}] = 0.01 \text{ M}$ is the V_{max} , i.e., $[\text{S}]_0 \gg K_M$, therefore $v_0 = V_{\text{max}} = 35 \text{ } \mu\text{mole L}^{-1} \text{ min}^{-1}$.
At $[\text{S}]_0 = 3.5 \times 10^{-5} \text{ M}$ then $v_0 = (35 \text{ } \mu\text{mole L}^{-1} \text{ min}^{-1}) (3.5 \times 10^{-5} \text{ M}) / [(2.0 \times 10^{-5} \text{ M}) + (3.5 \times 10^{-5} \text{ M})] = 22.3 \text{ } \mu\text{mole L}^{-1} \text{ min}^{-1}$.
- (b) At $4.0 \times 10^{-4} \text{ M}$ $[\text{S}]_0$, $[\text{S}]_0$ is between K_M and $100 K_M$ (note: at $[\text{S}]_0 \geq 100 K_M$, $v_0 = V_{\text{max}}$)
Thus, v_0 will be between $17.5 \text{ } \mu\text{mole L}^{-1} \text{ min}^{-1}$ ($V_{\text{max}}/2$) and $35 \text{ } \mu\text{mole L}^{-1} \text{ min}^{-1}$ (V_{max})

$$v_0 = (35 \mu\text{mole L}^{-1}\text{min}^{-1}) (4 \times 10^{-4}\text{M}) / [(2.0 \times 10^{-5}\text{M}) + (4 \times 10^{-4}\text{M})] = 33.3 \mu\text{mole L}^{-1}\text{min}^{-1}.$$

(c) At $2 \times 10^{-4}\text{M}$ $[S]_0$ then $[S]_0 = (10) K_M$

$$v_0 = V_{\max}[S]/(K_M + [S]) = V_{\max}(10 K_M)/(K_M + 10 K_M) = V_{\max}(10/11) = 0.91 V_{\max}$$

$$v_0 = 0.91(35 \mu\text{mole L}^{-1}\text{min}^{-1}) = 31.9 \mu\text{mole L}^{-1}\text{min}^{-1}.$$

(d) At $2 \times 10^{-6}\text{M}$ $[S]_0$ then $[S]_0 = 0.1 K_M$

$$v_0 = V_{\max}(0.1 K_M)/(K_M + 0.1 K_M) = V_{\max}(0.1 K_M)/1.1 K_M = 0.091 V_{\max}$$

$$v_0 = 0.091(35 \mu\text{mole L}^{-1}\text{min}^{-1}) = 3.19 \mu\text{mole L}^{-1}\text{min}^{-1}.$$

(e) At $1.2 \times 10^{-6}\text{M}$ $[S]_0$: $v_0 = (35 \mu\text{mole L}^{-1}\text{min}^{-1}) (1.2 \times 10^{-6}\text{M}) / [(2 \times 10^{-5}\text{M}) + (1.2 \times 10^{-6}\text{M})] = 1.98 \mu\text{mole L}^{-1}\text{min}^{-1}.$

$$5. \quad v_0/V_{\max} = [S]/(K_M + [S]) \quad 0.90/1 = [S]_{90}/(K_M + [S]_{90})$$

$$0.90 K_M + 0.90[S]_{90} = [S]_{90}; 0.90K_M = [S]_{90} - 0.90[S]_{90};$$

$$0.90K_M = 0.10 [S]_{90}; [S]_{90} = 0.90K_M/0.10; [S]_{90} = 9K_M$$

Thus, when the substrate concentration is $9K_M$, the v_0 is 90% of the V_{\max} .

$$v_0/V_{\max} = [S]/(K_M + [S]) \quad 0.10/1 = [S]_{10}/(K_M + [S]_{10})$$

$$0.10K_M + 0.10[S]_{10} = [S]_{10}; 0.10K_M = [S]_{10} - 0.10[S]_{10};$$

$$0.10K_M = 0.90 [S]_{10}; [S]_{10} = 0.10K_M/0.90; [S]_{10} = 0.111K_M$$

Thus, when the substrate concentration is $0.111K_M$ then $v_0 = 10\%$ of V_{\max} .

$$[S]_{90}/[S]_{10} = 9K_M/0.111K_M = 81.1$$

6. (a) $[S]_0 = 2 \times 10^{-5}\text{M} \ll K_M$ therefore reaction is first order w.r.t. substrate concn.

For a first order reaction: $t_{1/2} = 0.693/k$, so $6 \text{ min} = 0.693/k$, $k = 1.16 \times 10^{-1} \text{ min}^{-1}$.

(b) Recall from lecture notes: $k = V_{\max}/K_M$ for a first order reaction so then $V_{\max} = k(K_M)$

$$V_{\max} = (1.16 \times 10^{-1} \text{ min}^{-1})(5 \times 10^{-3}\text{M}) = 5.8 \times 10^{-4} \text{ M min}^{-1}$$

(c) For a first order reaction: $2.303 \log([S]_0/[S]_t) = kt$ (integrated Michaelis-Menten equation).

$$2.303 \log (2 \times 10^{-5} \text{ M}/[S]_t) = 0.116 \text{ min}^{-1}(15 \text{ min})$$

$$2.303 (\log 2 \times 10^{-5} - \log [S]_t) = 1.733 \text{ M}$$

$$\log 2 \times 10^{-5} - \log [S]_t = 0.752 \text{ M}$$

$$(0.301 - 5) - \log [S]_t = 0.752 \text{ M}$$

$$-\log [S]_t = 5.451 \text{ M}$$

$$1/[S]_t = 2.825 \times 10^5 \text{ M}$$

$$[S]_t = 3.54 \times 10^{-6} \text{ M}$$

$$\text{Thus, } [P]_{15} = [S]_0 - [S]_t = (2 \times 10^{-5} \text{ M}) - (3.54 \times 10^{-6} \text{ M}) = 1.646 \times 10^{-5} \text{ M}$$

7. (a) Michaelis-Menten plot. Can use curve-fitting to obtain parameters.

(b) See graph 8(b) Lineweaver-Burk plot, $y = 1.222 \times 10^{-2} + 4.985 \times 10^{-7}X$
Y intercept = $1/V_{\max} = 1.222 \times 10^{-2}$ $V_{\max} = 82 \mu\text{moles L}^{-1}\text{min}^{-1}$
X intercept = $-1/K_M = -1.222 \times 10^{-2}/4.985 \times 10^{-7} = -2.45 \times 10^4$
 $K_M = -(1/-2.45 \times 10^4) = 4.08 \times 10^{-5}\text{M}$

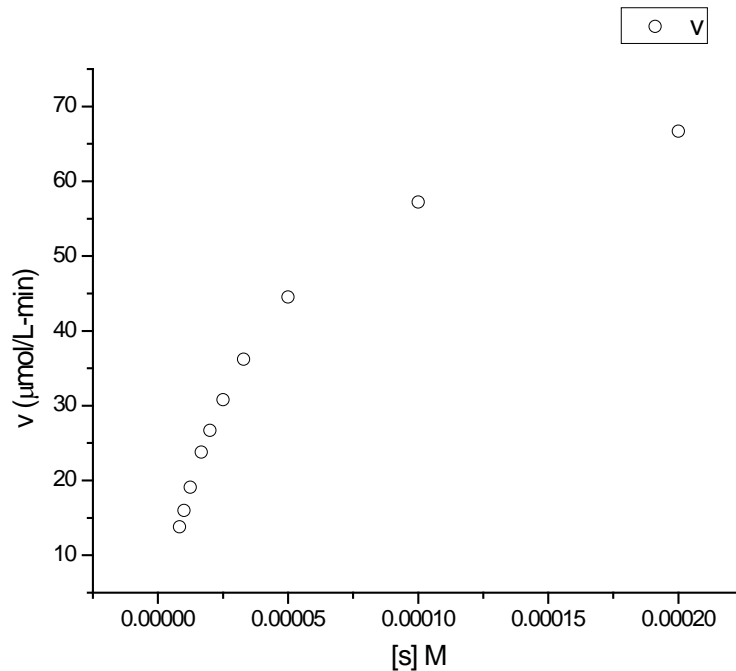
(c) See graph 8(c) Hanes Plot, $y = 4.985 \times 10^{-7} + 1.25 \times 10^{-2} X$
Slope = $1.25 \times 10^{-2} = 1/V_{\max} = 1/1.25 \times 10^{-2} = 80 \mu\text{moles L}^{-1}\text{min}^{-1}$
Y intercept = $K_M/V_{\max} = 4.985 \times 10^{-7}$, $K_M = 80(4.985 \times 10^{-7}) = 3.99 \times 10^{-5} \text{ M}$

(d) See graph 8(d) Eadie-Hofstee Plot, $y = -3.992 \times 10^{-5}X + 80.04$
Slope = $-K_M = -(-3.992 \times 10^{-5}) = 3.99 \times 10^{-5}\text{M}$
Y intercept = $V_{\max} = 80 \mu\text{moles L}^{-1}\text{min}^{-1}$

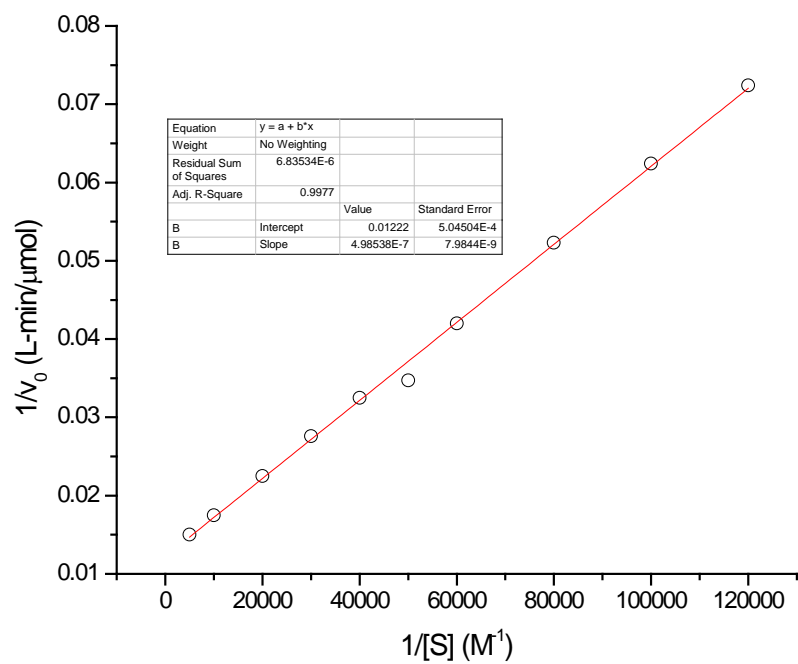
(e) See graph 8(e), $V_{\max} = 78 - 82 \mu\text{moles L}^{-1}\text{min}^{-1}$; $K_M = 3.6 - 4.4 \times 10^{-5} \text{ M}$.

Graphs 7 (a-d)

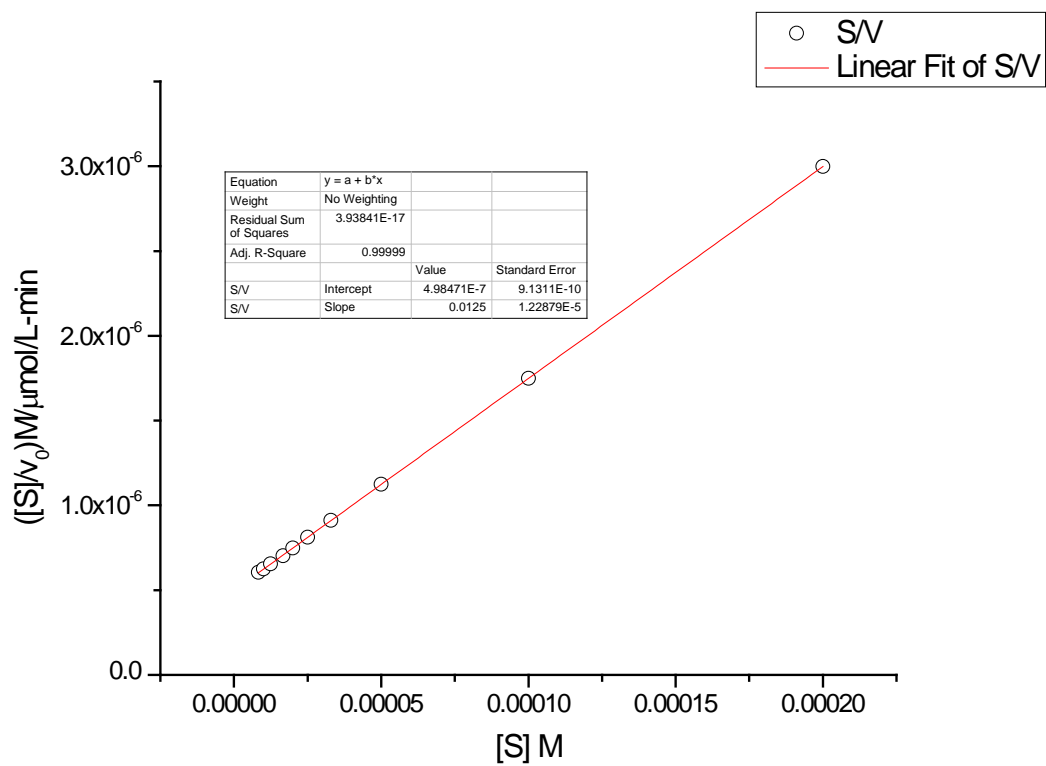
(a) Michaelis-Menten Plot



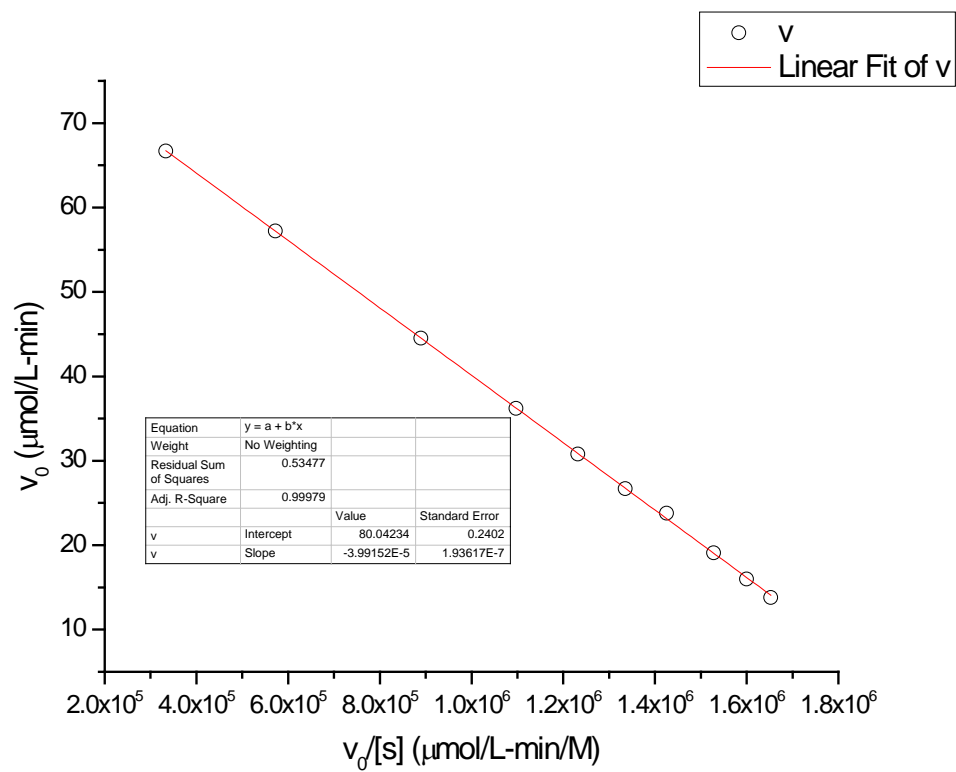
(b) Line-weaver Burk Plot



(c) Hanes Plot



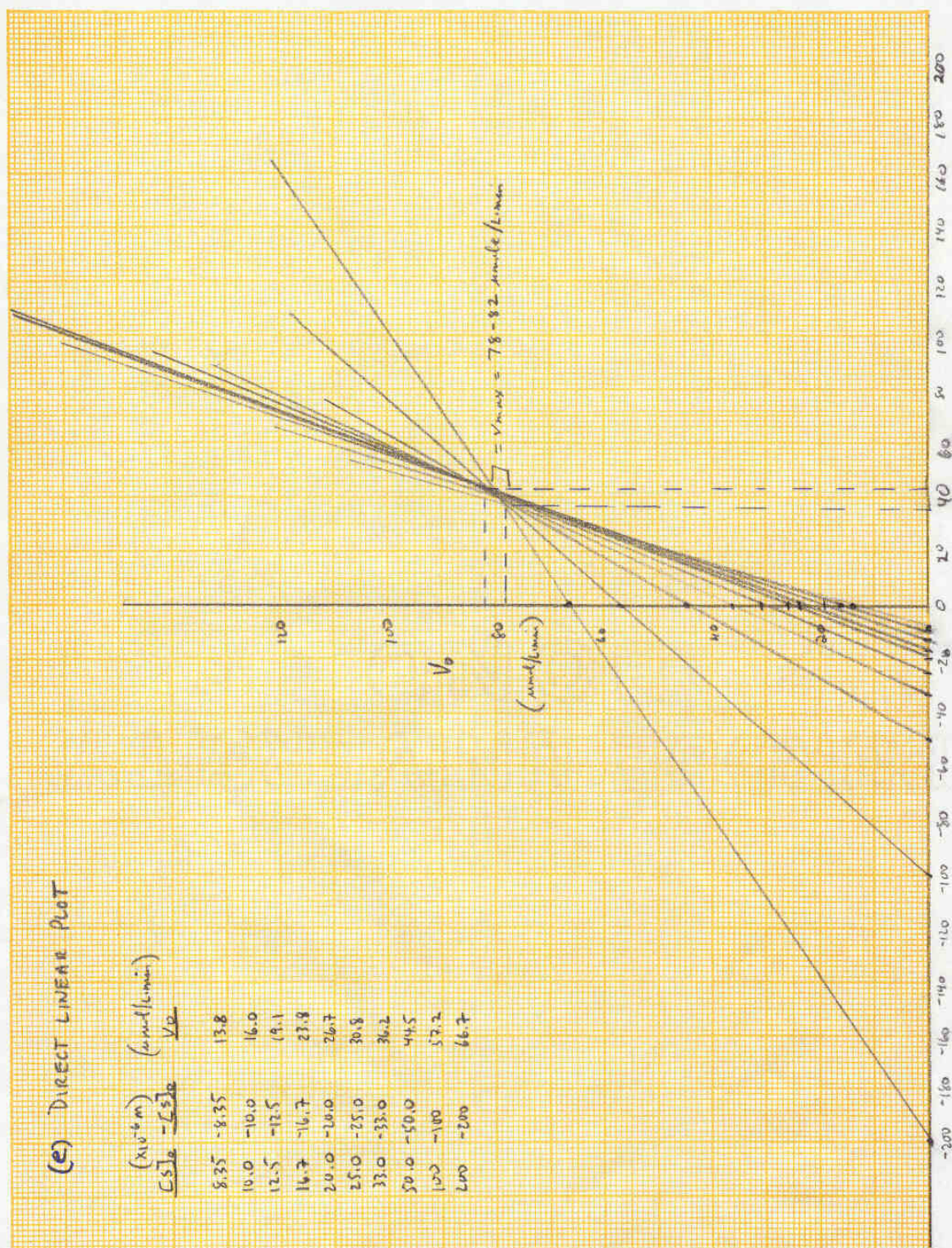
(d) Eadie-Hofstee Plot



(e) DIRECT LINEAR PLOT

$\frac{(x_{10}^6 m)}{CS_{10} - CS_{10}}$ $\frac{(mm/L/min)}{V_0}$

8.35	-8.35	13.8
10.0	-10.0	16.0
12.5	-12.5	19.1
16.7	-16.7	23.8
20.0	-20.0	26.7
25.0	-25.0	30.8
33.0	-33.0	36.2
44.5	-44.5	44.5
57.2	-57.2	57.2
69.7	-69.7	69.7



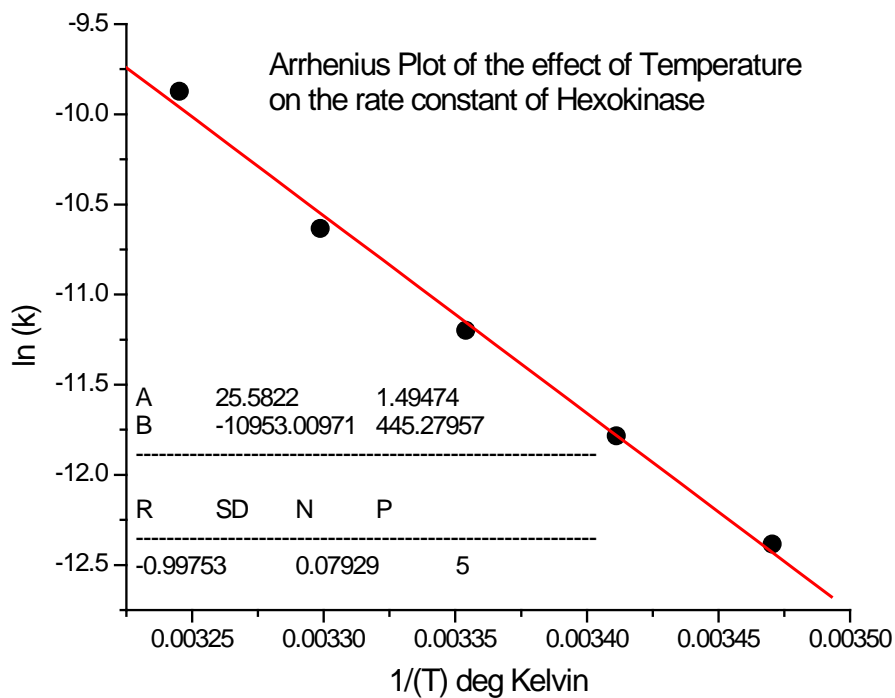
$W_1 \times 10^{-6} [S]$

$W_2 \times 10^{-6} [S]$

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8. (a) $[S]_0 = 1 \times 10^{-5} \text{M} \ll K_M (2 \times 10^{-3} \text{M})$ therefore first order and the reaction will be directly proportional to $[S]$. Since $[S]$ decreases with time the velocity will also decrease with time. For first order reactions, a constant proportion (not amount) is converted to product per unit time.
- $$2.303 \log [S]_0/[S]_t = kt$$
- Firstly, calculate k knowing that at the end of 1 minute 98% of the original substrate remains.
- $$2.303 \log(100/98) = k(1 \text{min})$$
- $$2.303 \log (1.02) = k \text{ min}$$
- $$k = 1.98 \times 10^{-2} \text{ min}^{-1}$$
- Next, calculate $[S]_t$ at $t = 3 \text{ min}$
- $$2.303 \log(100/[S]_t) = 1.98 \times 10^{-2} \text{ min}^{-1}(3 \text{ min})$$
- $$2.303(\log 100 - \log [S]_t) = 5.94 \times 10^{-2}$$
- $$4.606 - 2.303 \log [S]_t = 5.94 \times 10^{-2}$$
- $$-2.303 \log [S]_t = -4.547$$
- $$\log [S]_t = 1.974$$
- $$[S]_t = 94.2\% \text{ of original } [S]$$
- $$[S]_t = 1 \times 10^{-5}(0.942) = 9.42 \times 10^{-6} \text{ M.}$$
- Therefore, the $[P]$ will be $100 - 94.2 = 5.8\% = 1 \times 10^{-5} \text{M} (0.058) = 5.8 \times 10^{-7} \text{ M.}$
- (b) If $[S] = 10^{-6} \text{ M}$ the reaction is still first order. The proportion of substrate converted to product would still be 5.6% by 3 min. However, the absolute amount of substrate consumed (and product formed) would obviously be less than in part (a) above.
- (c) V_{\max} can be estimated since we know K_M (hence the reaction order)
- At $[S]_0 = 1 \times 10^{-5} \text{ M}$ the velocity for the first minute is : $v_0 = k[S]$
- $$v_0 = 0.02 \text{ min}^{-1}(1 \times 10^{-5} \text{M}) = 2 \times 10^{-7} \text{M min}^{-1}$$
- $$v/V_{\max} = [S]/(K_M + [S])$$
- $$2 \times 10^{-7} \text{M min}^{-1}/V_{\max} = 1 \times 10^{-5} \text{M}/(2 \times 10^{-3} \text{M}) + (1 \times 10^{-5} \text{M})$$
- $$V_{\max} = [(2 \times 10^{-3} + 1 \times 10^{-5})(2 \times 10^{-7})/(1 \times 10^{-5})] \text{ M min}^{-1} = 4.02 \times 10^{-5} \text{ M min}^{-1}$$
- (d) V_{\max} will be observed at about $100 K_M$ (rule of thumb estimate) therefore $100(K_M) = 100(2 \times 10^{-3} \text{M}) = 2 \times 10^{-1} \text{M} = 0.2 \text{ M}$
- (e) At $0.2 \text{M } [S]_0$ the reaction will be zero order
- $$[P] = V_{\max}(t) = (4.02 \times 10^{-5} \text{ M min}^{-1})(3 \text{ min}) = 12.06 \times 10^{-5} \text{ M.}$$
- $$12.06 \times 10^{-5} \text{ M}/2 \times 10^{-1} \text{M} (100) = 6.03 \times 10^{-2} \% \cong 0.06\%.$$
9. Lineweaver-Burk equation: $(1/v_0) = K_M/V_{\max}(1/[S]_0) + 1/V_{\max}$
- $$\text{Slope} = K_M/V_{\max} = 2.5 \times 10^{-4} \text{ min}$$
- $$\text{Y intercept} = 1/V_{\max} = 3.0 \times 10^{-2} \text{ M}^{-1} \text{min}$$
- $$V_{\max} = 33.33 \text{ M min}^{-1} \text{ then}$$
- $$K_M = (33.33 \text{ M min}^{-1})(2.5 \times 10^{-4} \text{ min}) = 8.33 \times 10^{-3} \text{M.}$$
10. (a) $v_0 = V_{\max}[S]/(K_M + [S])$ $v_0/V_{\max} = [S]/(K_M + [S])$ $v_0/V_{\max} = 1/(10 + 1) = 1/11 = 0.091$
- (b) $v_0/V_{\max} = 10/(1 + 10) = 10/11 = 0.909.$

11.



$$\text{slope} = -(-10953.01) = E_a/R$$

$$10953.01 \text{ K} = E_a/8.314 \text{ JK}^{-1}\text{mol}^{-1}$$

$$E_a = 91,063 \text{ J/mol} = 91.06 \text{ kJ/mol}$$

$$\ln(k) = \ln A - E_a/RT$$

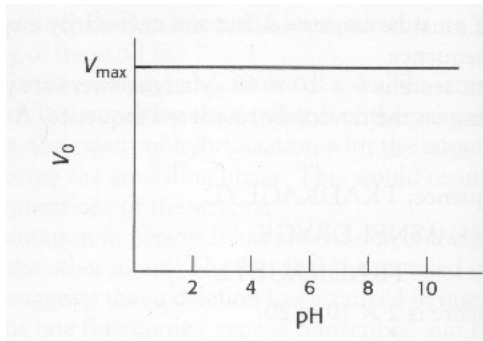
if we use the rate constant for Hexokinase at 15°C then:

$$\ln(4.18 \times 10^{-6}) = \ln A - 91,063/8.314 \times 288.15$$

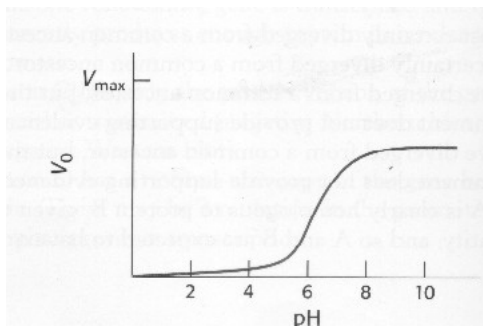
$$-12.385 = \ln A - 38.01; \ln A = -12.385 + 38.01 = 25.625$$

$$A = 1.345 \times 10^{11} \text{ s}^{-1} \text{ (the frequency factor has the same units as the rate constant).}$$

12. (a) When $[S^+]$ is much greater than the value of K_M , pH will have a negligible effect on the enzyme because S^+ will interact with E^- as soon as it becomes available.



(b) When $[S^+]$ is much less than the value of K_M , the plot of v_0 versus pH becomes essentially a titration curve for the ionizable groups, with enzyme activity being the titration marker. At low pH, the high concentration of H^+ will keep the enzyme in the EH form and inactive. As the pH rises, more and more of the enzyme will be in the E^- form and active. At high pH (low H^+), all of the enzyme is E^- .



(c) The midpoint on this curve will be the pK_a of the ionizable groups, which is stated to be pH 6.0.