



0.50 mL WITH 4.1 $\mu\text{g/L}$ ENZYME
 0.25 mL 2-PROPANOL
 0.25 mL NAD

$$\text{ACTIVITY} = \frac{0.47 \text{ AU/min}}{6.22 \text{ AU L}/\mu\text{mol}} \times \frac{1000 \mu\text{mol}}{\text{mmol}} \times \frac{1.0 \text{ mL}}{0.5 \text{ mL}} \times \frac{\text{L}}{1000 \text{ mL}}$$

$$= 0.15 \text{ IU/mL}$$

9pts

② $\frac{0.15 \text{ IU}}{\text{mL}} \times \frac{\text{L}}{4.1 \mu\text{g}} \times \frac{1000 \text{ mL}}{\text{L}} = \frac{37 \text{ IU}}{\mu\text{g}}$

6pts

- ③ SEVERAL ANSWERS ARE POSSIBLE DEPENDING ON JUSTIFICATION...
 THE ENZYME ASSAY SHOULD IDEALLY MEASURE Y_{MAX} TO
~~BE~~ BE "REASONABLY" ACCURATE THE SUBSTRATE
 CONCENTRATION SHOULD BE HIGH ENOUGH TO
 MEASURE 95% OF Y_{MAX} .

0pts

$$\text{OR } 0.95 = \frac{S}{K_m + S} \Rightarrow S \approx 19 K_m$$

MOREOVER, THE ASSAY CALLS FOR A 4X DILUTION
 OF 2-PROPANOL (FROM 0.25 mL TO 1.0 mL), SO
 THAT THE ORIGINAL 2-PROPANOL SOLUTION SHOULD
 HAVE A CONCENTRATION OF $S \approx 4 \times 19 K_m$
 OR $4 \times 19 \times 4 \text{ mM} = 304 \text{ mM}$

CONCLUSION

A 300 mM 2-PROPANOL SOLUTION WILL GIVE ABOUT A 5% ERROR.

<u>S</u>	<u>v</u>	<u>v/S</u>
15	7.6	0.51
30	11.8	0.39
90	18.2	0.20

PLOT v ("y") VERSUS v/S ("x")

1) $K_M = \underline{35 \text{ mM}}$

6 pts

2) $V_{\text{MAX}} = \underline{25.3 \text{ mmol/Ls}}$

6 pts

3) $k_{\text{CAT}} = \frac{(25.3 \text{ mmol/Ls}) \left(\frac{\text{mol}}{1000 \text{ mmol}} \right)}{(1.33 \text{ mg/L}) \left(\frac{\text{g}}{1000 \text{ mg}} \right) \left(\frac{\text{mol}}{42800 \text{ g}} \right) (2 \text{ mol ACT. SITE/mol ENZ})}$

$k_{\text{CAT}} = \underline{407,000 \text{ s}^{-1}}$

6 pts

4) $S_0 - S + K_M \ln(S_0/S) = V_{\text{MAX}} t$

$[E] = \frac{5 \mu\text{g}}{0.1 \text{ L}} \times \frac{\text{mg}}{1000 \mu\text{g}} = 0.05 \frac{\text{mg}}{\text{L}}$

$V_{\text{MAX}} = \left(\frac{0.05 \text{ mg}}{\text{L}} \right) \left(\frac{\text{mmol}}{42800 \text{ mg}} \right) \left(\frac{2 \text{ mol ACT SITE}}{\text{mol ENZ}} \right) (407,000 \text{ s}^{-1})$
 $= 0.951 \text{ mmol/Ls}$

$$S_0 = \frac{7 \text{ mmol}}{0.100 \text{ L}} = \frac{70 \text{ mmol}}{\text{L}}$$

$$S = \frac{35 \text{ mmol}}{\text{L}}$$

So...

$$70 - 35 + 35 \ln(70/35) = 0.951 t$$

$$\underline{t = 62 \text{ sec}}$$

6 pts

