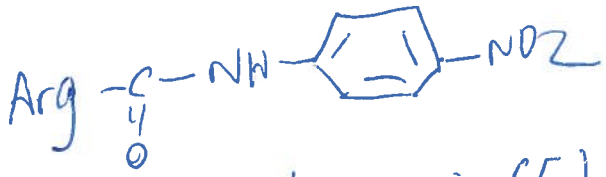
Exp. 4

i) establishing the initial rate V_0 of a

$E + S \rightleftharpoons ES \rightarrow P + E$ reaction

i)  = S = BA

[E] will be varied

↓ Trypsin (E).

parts AB

Arg +  4 nitroaniline

$\lambda = 410nm$

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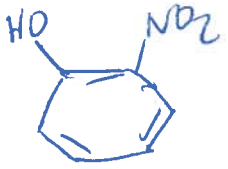
ii) β Galactose — O — 2 nitrophenyl = [S] (varied)

= ONPG

↓ β galactosidase

Part C

β galactose + HO — 2 nitrophenyl 

$\lambda = 420nm$

A) i_o vs. [E]

(2)

p. 71 (#3)

Assay #	(μL) dH₂O	BA reagent	T (0.200 mg/mL)	D.F.
1	50	950	0	(0) 1
2	40	950	10	1 in 5 100
3	30	"	20	2 in 3 50
4	20	"	30	3 in 4 33.33
5	10	"	40	4 in 5 25
6	0	"	50	5 in 6 20

(prepare one at a time, do not add the enzyme until spectroph. is blanked)

for each: measure abs. every 15 s for 3 min

$V_o = K[E]$

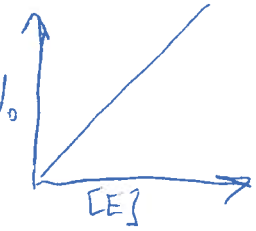
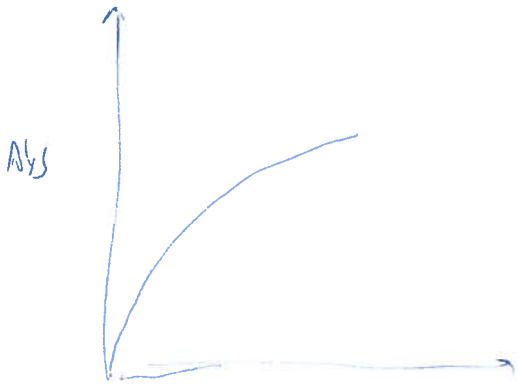


Figure 5.2 textbook.

B) longer assay: 10 min

measure abs. each 10 sec for 2 min
each 15 sec for 3 min
each 30 sec for 5 min



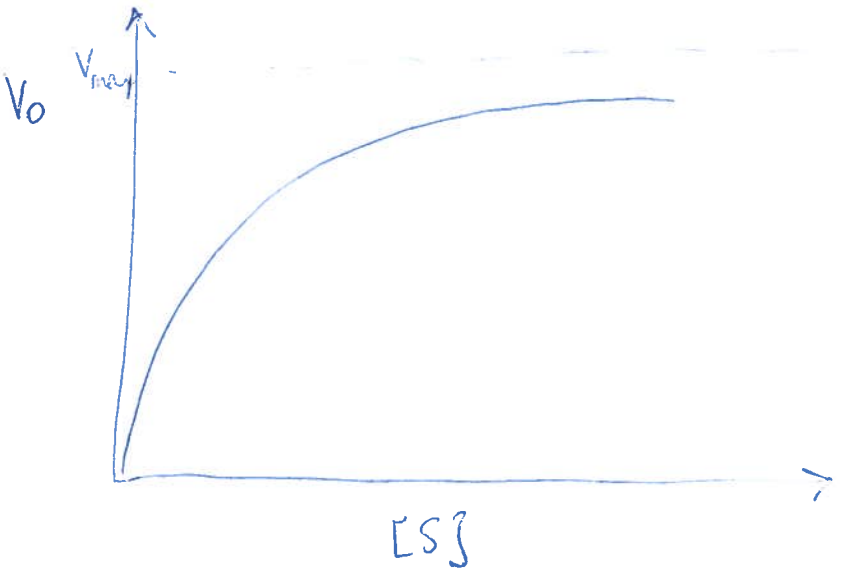
(Figure 5.3 textbook)

C) p. 73

Assay #	(μL) [S] ONPG (5mM)		β galactosidase buffer	+150uL Bgal-
for 1000uL 1	12.5	(80)	937.5	"
2	25	(50)	925	
3	50	(20)	900	
4	100	(10)	850	
5	200	(5)	750	
6	400	(2.5)	550	
7	600	(1.66)	350	

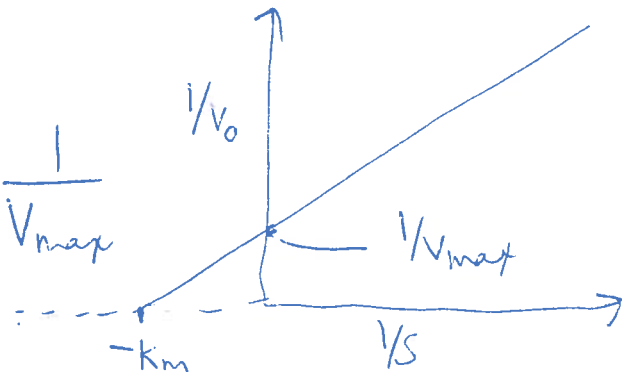
each assay run for 2 minutes

add after blanking



$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{V_o} = \frac{K_m + [S]}{V_{max}[S]} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$



i) when $\frac{1}{[S]} = 0$ $\quad \frac{1}{V_0} = \frac{1}{V_{max}} \Rightarrow V_0 = V_{max}$

ii) when $1/V_0 = 0$

$$\frac{K_m}{V_{max}[S]} = -\frac{1}{V_{max}} \Rightarrow \frac{K_m}{[S]} = -1$$

$$[S] = -K_m$$