Name: _____

CHEMISTRY 333
HOMEWORK # 2
DUE FRIDAY OCTOBER 23, 2009 (NO LATER THAN 5 PM!)
SHOW ALL OF YOUR WORK AND HAND IN ALL GRAPHS
(35 points)

1. (20 points)
The kinetics of an enzyme were analyzed in both the absence and presence of **Inhibitor A** and **Inhibitor B**. Given the following data, calculate or construct the following for A and B on separate graphs.

a) Plot the data as a Michaelis-Menten saturation curve.
b) Estimate the K_m and V_{max} from these curves both in the presence and absence of inhibitors.
c) Plot the data in the Lineweaver-Burk format
*Make sure to label the both the inhibitor line and the no inhibitor line
d) **Mathematically** determine the K_m and V_{max}.
e) What types of inhibitors are A and B? How can you tell?
f) On each graph draw a line that would indicate an increase in the concentration of inhibitor.

*Make sure to turn in all your plots, corresponding equations and calculations.

	V (mmol/min)	
[S] (mM)	**No Inhibitor**	**A (5mM)**
.2	5.0	3.0
.4	7.5	5.0
.8	10.0	7.5
1.0	10.7	8.3
2.0	12.5	10.7
4.0	13.6	12.5

	V (mmol/min)	
[S] (mM)	**No Inhibitor**	**B (0.1mM)**
.2	5.0	2.0
.4	7.5	3.0
.8	10.0	4.0
1.0	10.7	4.3
2.0	12.5	5.0
4.0	13.6	5.5

2. (15 points) Draw the following disaccharides in Haworth projections. **Label** the anomeric carbon with a * and **circle** any reducing ends.

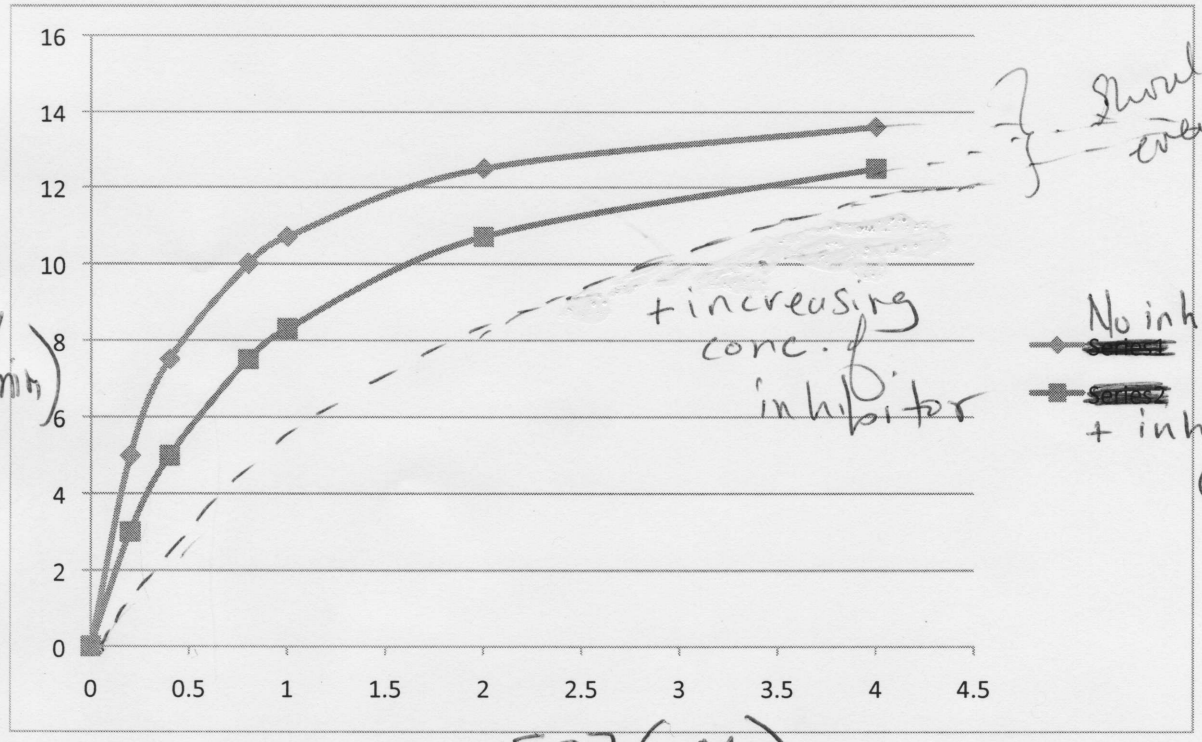
a) galactose β(1,4) mannose

b) glucose β,α(1,1) galactose

c) fructose α(2,5) ribose

Michaelis Menten Inhibitor A

	V (mmol/min)	
[S] (mM)	No Inhibitor	A (5mM)
0	0	0
0.2	5	3
0.4	7.5	5
0.8	10	7.5
1	10.7	8.3
2	12.5	10.7
4	13.6	12.5



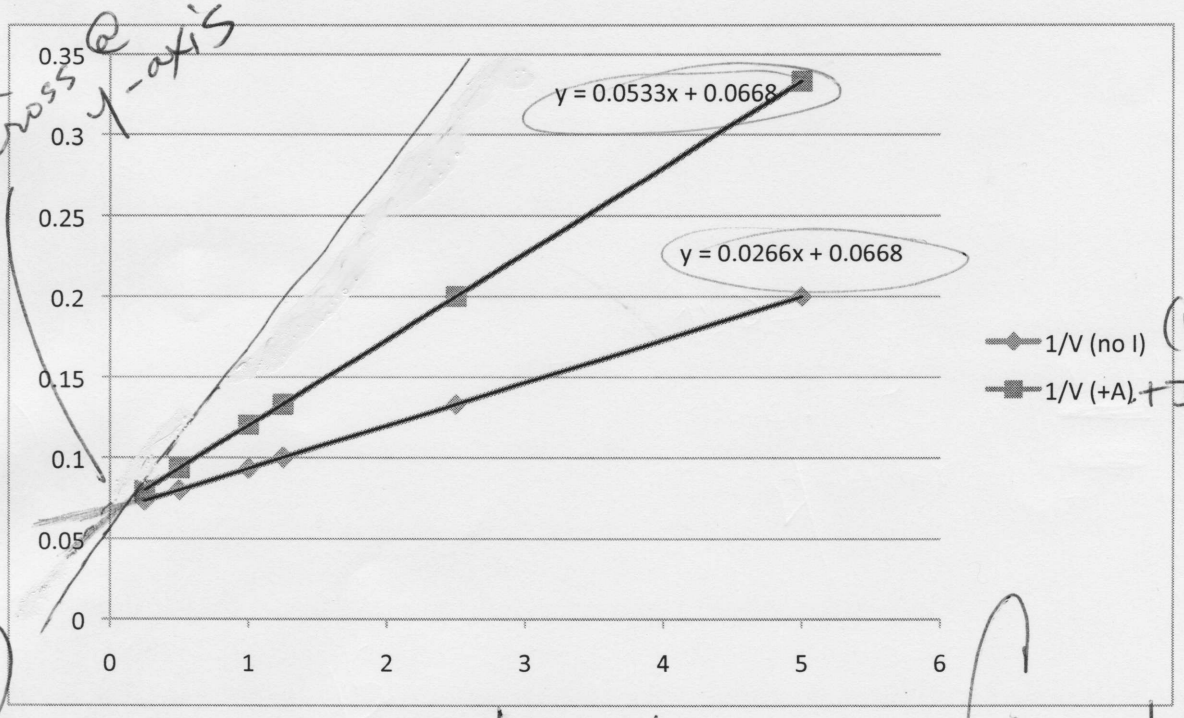
Estimated Km

	without I	with I
	~ 0.4 mM	~ 0.7 mM

Vmax ~ 15 mmol/min ~ 14 mmol/min

Lineweaver Burk: Inhibitor A

	V (mmol/min)				
[S] (mM)	No Inhibitor	A (5mM)	1/[S]	1/V (no I)	1/V (+A)
0.2	5	3	5	0.2	0.333333333
0.4	7.5	5	2.5	0.133333333	0.2
0.8	10	7.5	1.25	0.1	0.133333333
1	10.7	8.3	1	0.093457944	0.120481928
2	12.5	10.7	0.5	0.08	0.093457944
4	13.6	12.5	0.25	0.073529412	0.08



$\frac{1}{V}$ $\left(\frac{min}{mmol}\right)$ or $\left(\frac{1}{min/mmol}\right)$

$\frac{1}{[S]}\left(\frac{1}{mM}\right)$

Competitive Inhibitor

K_m Changes

V_{max} stays Same

No Inhibitor: $y = 0.0266x + 0.0668$

$V_{max} = \frac{1}{0.0668} = 15.0\frac{mmol}{min} = V_{max}$

$\frac{K_m}{V_{max}} = 0.0266 = \frac{K_m}{15} = 0.4mM = K_m$

+ inhibitor $y = 0.0533 + 0.0668$

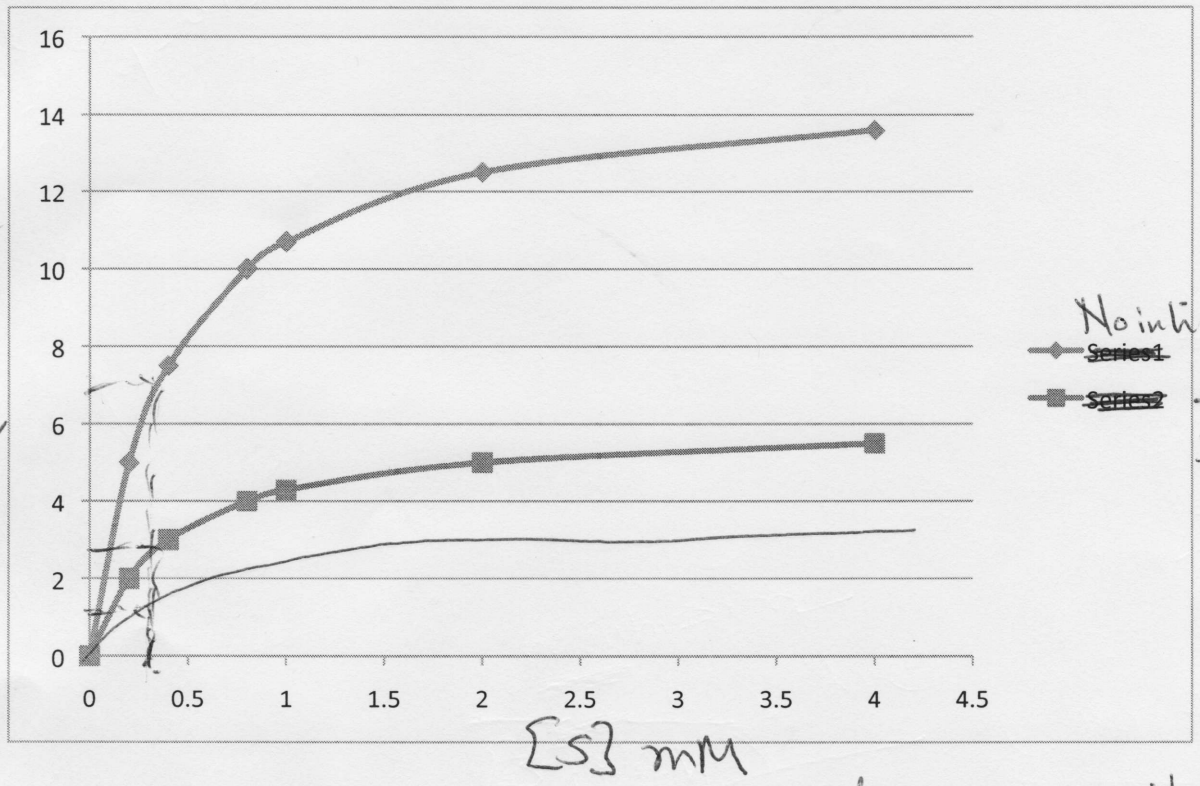
$V_{max} = \frac{1}{0.0668} = 15.0\frac{mmol}{min}$

$\frac{K_m}{V_{max}} = 0.0533 = \frac{K_m}{15} = 0.0533$ $K_m = 0.8mM$

Inhibitor B

Michaelis-Menten

	V (mmol/min)	
[S] (mM)	No Inhibitor	B (0.1mM)
0	0	0
0.2	5	2
0.4	7.5	3
0.8	10	4
1	10.7	4.3
2	12.5	5
4	13.6	5.5



V mmol/min

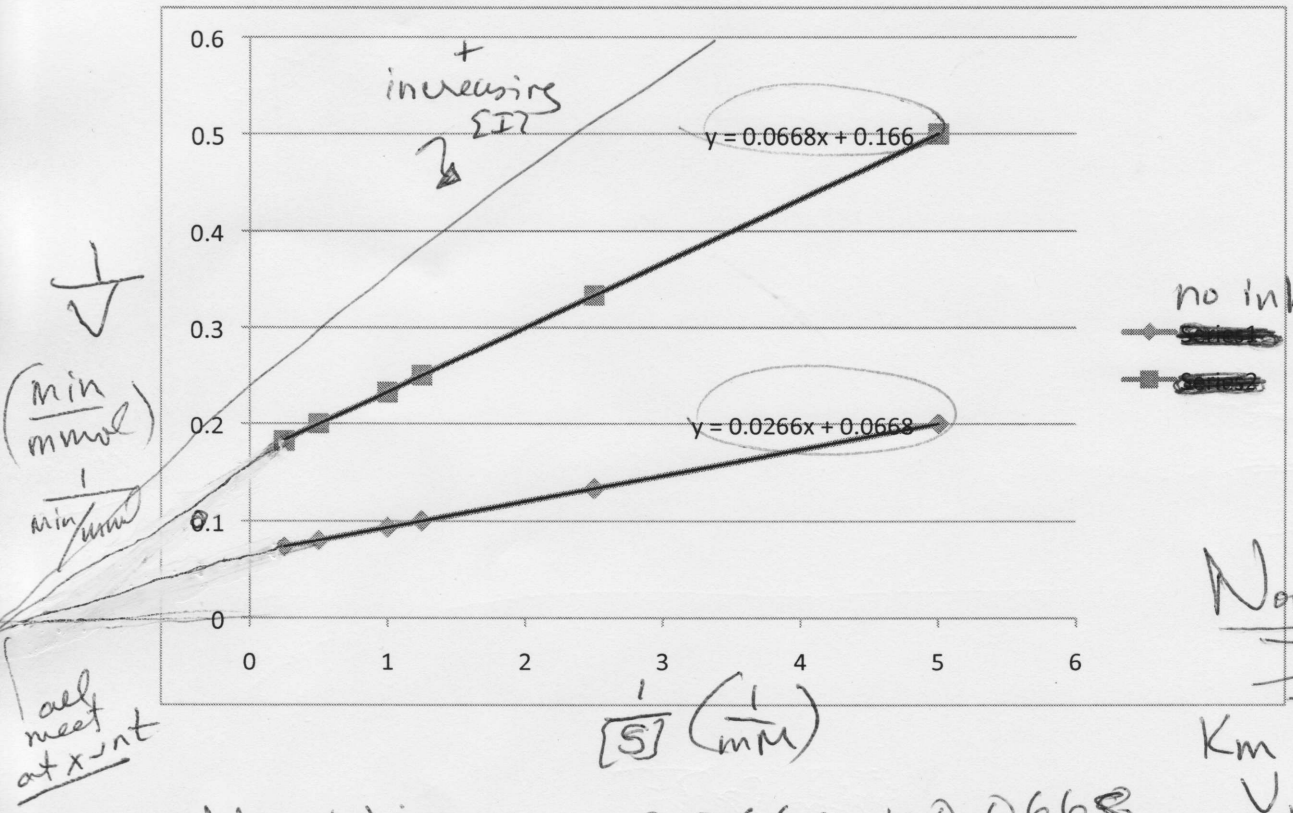
Estimated K_m

	without I	with I
	~0.3-0.4mM	0.3-0.4mM

V_{max} ~ 14 mmol/min ~ 6 mmol/min

Lineweaver-Burk Inhibitor B

	V (mmol/min)				
[S] (mM)	No Inhibitor	B (0.1mM)	1/[S]	1/V (no I)	1/V (+B)
0.2	5	2	5	0.2	0.5
0.4	7.5	3	2.5	0.133333333	0.333333333
0.8	10	4	1.25	0.1	0.25
1	10.7	4.3	1	0.093457944	0.23255814
2	12.5	5	0.5	0.08	0.2
4	13.6	5.5	0.25	0.073529412	0.181818182



Non-competitive Inhibitor

Km stays same

Vmax changes

No inhibitor: $y = 0.266x + 0.0668$

$$V_{max} = \frac{1}{0.0668} = 15 \frac{mmol}{min}$$

$$\frac{K_m}{V_{max}} = 0.0266 = \frac{K_m}{15} = 0.0266 = K_m = 0.4 mM$$

+ inhibitor $y = 0.0668x + 0.166$

$$max = \frac{1}{0.166} = 6.0 mmol/min$$

$$\frac{K_m}{V_{max}} = 0.0668 = \frac{K_m}{6.0} = 0.0668 = K_m = 0.4 mM$$

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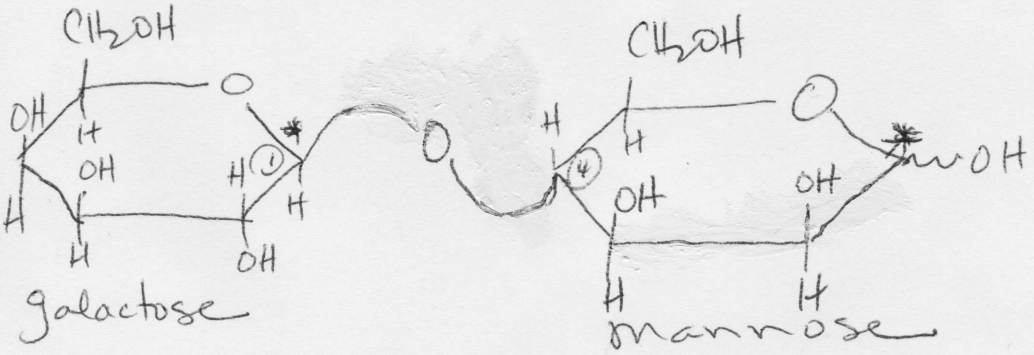
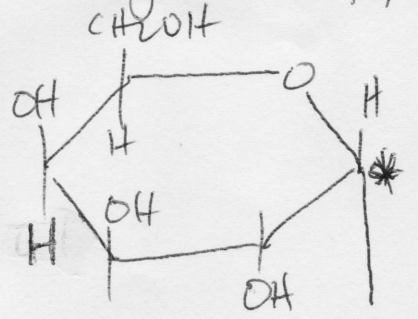
a. galactose β(1,4) mannose

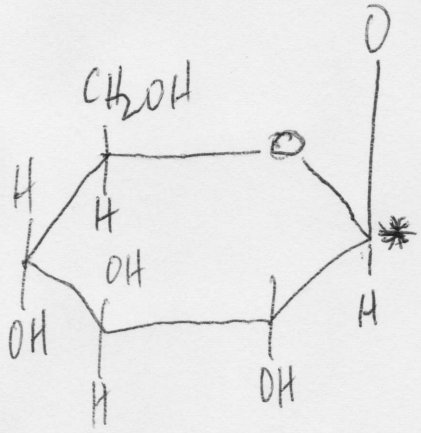


either up or down on mannose

b. glucose β,α (1,1) galactose

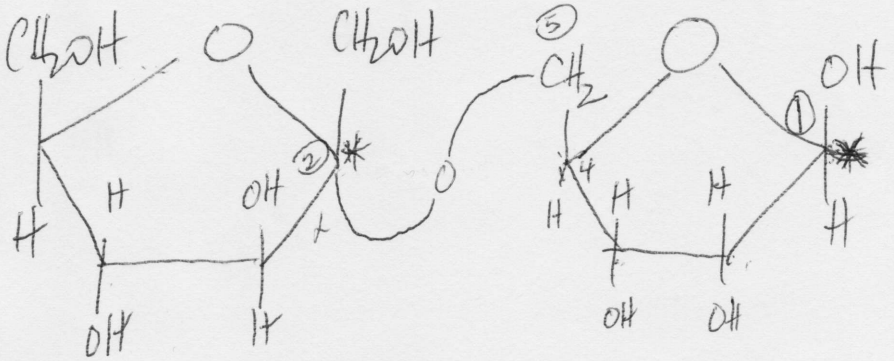


galactose



glucose

c



either α or β on ribose

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