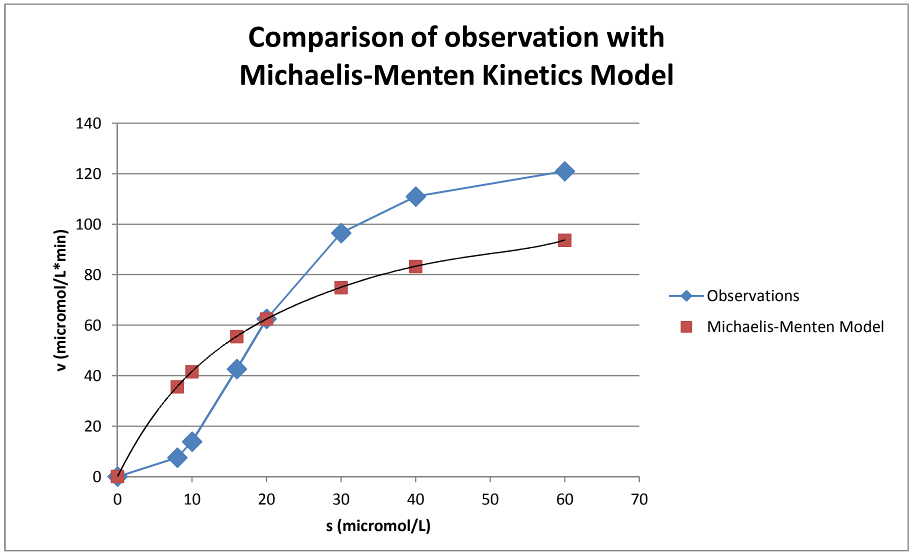
Problem 3.9

s	v	v	Eadie-Hostee Plot	
	Observed	M-M Model		
μmol/L	μmol/Lmin	μmol/Lmin	v/s	v
0	0	0		
8	7.5	35.7	0.938	7.5
10	13.9	41.7	1.390	13.9
16	42.7	55.6	2.669	42.7
20	62.5	62.5	3.125	62.5
30	96.5	75.0	3.217	96.5
40	111	83.3	2.775	111.0
60	121	93.8	2.017	121.0
200	125	113.6	0.625	125.0
500	125	120.2	0.250	125.0

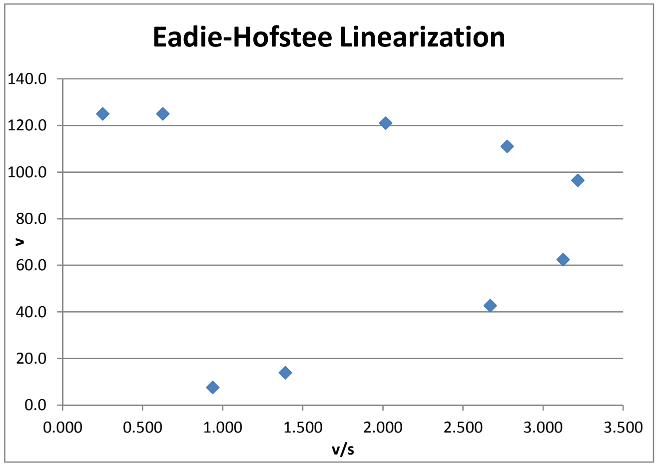


By inspection: V_{MAX} = 125 μmol/Lmin

K_M = 20 μmol/L

Compare the data of the reaction rate versus substate concentration (blue) with the prediction using the parameters obtained by inspection. We see that at high values of S, the observed reaction rate is greater than predicted. However, at low S, the observed reaction rate is less than we predict. The enzyme kinetics **does not follow** Michaelis-Menten kinetics. Instead, this shape is the classic shape for an allosteric enzyme.

The fact that the Michaelis-Menten kinetics model does not adequately describe the data is further illustrated if one attempts to fit the data to any of the linearizations typically used to find V_{MAX} and K_M. For example, using the Eadie-Hofstee linearization, we obtain:



THE MICHAELIS-MENTEN MODEL CLEARLY DOES NOT DESCRIBE THE ENZYME KINETICS! SO, DON'T USE IT!

Many students make comments to me after the completion of this problem. The comments generally are of the following nature: "*How was I supposed to know that the data fit an allosteric enzyme model? You never told us about the allosteric enzyme model. I have never seen this before.* "

This argument, although on one level is quite true, is ultimately a very poor and somewhat immature argument, and misses a much larger point. In your life you will be valued for your ability to solve problems, particularly ones that are new and not obvious. If all you can do is "solve" the Michaelis-Menten equation or solve problems that you have already seen, then you will not be very successful. Indeed, it is very likely that no one will ever hire you to solve the Michaelis-Menten equation. Your value as an engineer and employee will be to provide your insight and abilities to solving a problem that you <u>haven't</u> seen before, using the background you have.

The appropriate way to tackle this problem is:

1) Identify that the Michaelis-Menten equation is not appropriate to model this system's behavior.
2) Use your resources (book, internet, colleagues) to propose models that might be appropriate for this system.
3) Test the proposed models to determine if they do describe the system. That is, solve parameters for the new model.
4) Understand why one model works and why one model does not. Understand the implications.

Allosteric Model

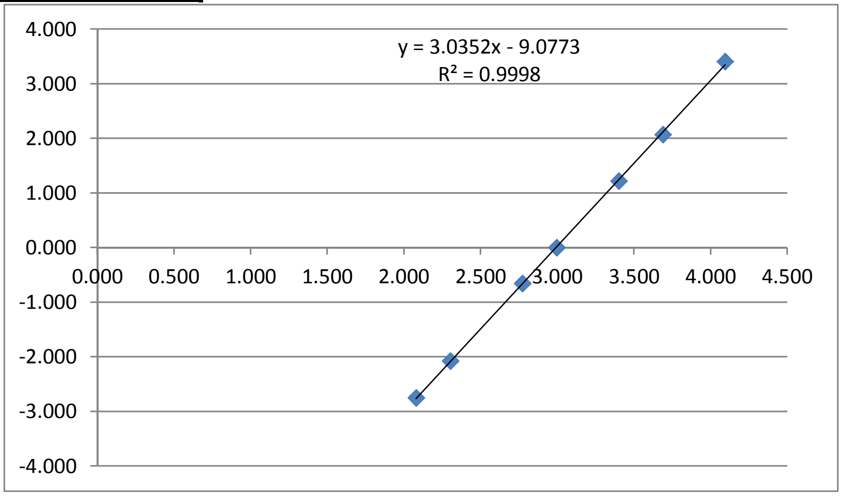
v μmol/Lmin	s μmol/L	$v/(V_{MAX} - v)$	ln(S)	ln ()
0	0	0		
7.5	8	0.063829787	2.079	-2.752
13.9	10	0.125112511	2.303	-2.079
42.7	16	0.518833536	2.773	-0.656
62.5	20	1	2.996	0.000
96.5	30	3.385964912	3.401	1.220
111	40	7.928571429	3.689	2.070
121	60	30.25	4.094	3.409
125	200			
125	500			

N = 3

Intercept = -9.0773

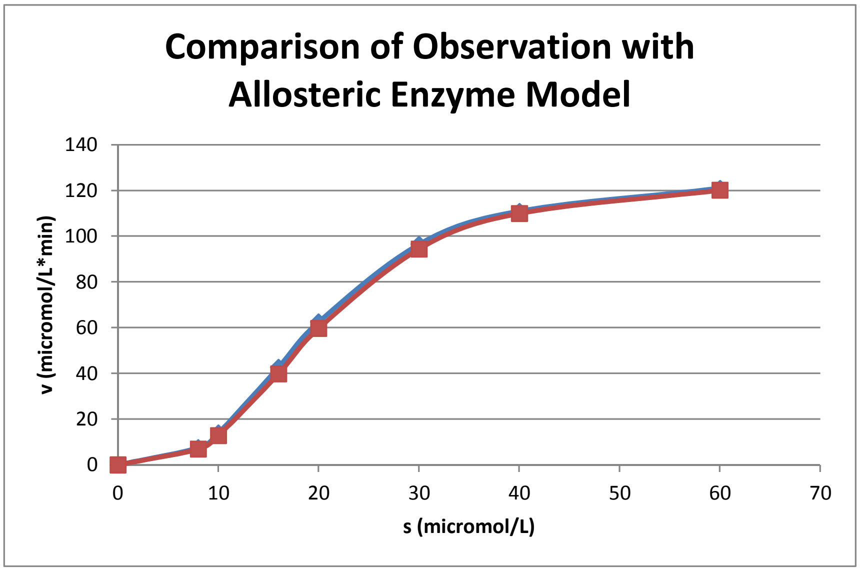
K_M" 8754

So, allosteric model is $$V = \frac{125S^3}{8754 + S^3}$$



We should not stop there! We should compare the observed data with the prediction that this new model gives us.

s μmol/L	v (observed) μmol/Lmin	v (predicted) μmol/Lmin
0	0	0.0
8	7.5	6.9
10	13.9	12.8
16	42.7	39.8
20	62.5	59.7
30	96.5	94.4
40	111	110.0
60	121	120.1
200	125	124.9
500	125	125.0



The allosteric model with N=3 clearly does a superior job of predicting kinetics compared with Michaelis-Menten model.