

# BL/CH401 -- Enzyme Kinetics Problems

For each Problem Make 2 Graphs:

A. PLOT  $V_o$  versus  $[S]$  to show that the enzyme obeys Michaelis-Menten Equation.

B. PLOT  $1/V_o$  versus  $1/[S]$  to determine  $K_m$  and  $V_{max}$ .

1. The following kinetic data were collected:

[Substrate]	Initial Velocity (ie. $V_o$ )
mM	$\mu\text{moles}/\text{min}$
0.25	1.7
0.5	3.0
1.0	5.0
2.0	7.3
3.0	8.6
5.0	10.1
10.0	11.6

2. The kinetics of an enzyme were analyzed in the absence and presence of inhibitors A and B.

[S]	$V_o$ (nmoles/min)		
	No Inhibitor	5 mM A	0.1 mM B
mM			
1.0	43	30	26
2.0	68	50	41
5.0	105	86	64
10.0	128	113	77
20.0	144	134	88

A. What type of inhibitors are A and B: Competitive or Non-Competitive?

(Use your graphs to answer this question)

B. In addition to calculating  $K_m$  and  $V_{max}$  in the absence of inhibitors, calculate the  $K_i$  constants for the inhibitors A and B.

Equations for calculating inhibition constants (ie  $K_i$  values).

Competitive Inhibition:  $K_m' = K_m (1 + [I]/K_i)$

Non-Competitive Inhibition:  $V_{max}' = V_{max}/(1 + [I]/K_i)$

To calculate the  $K_i$  for inhibitors A and B, use one of these 2 equations and the kinetic constants for the uninhibited reaction (ie  $K_m$  and  $V_{max}$ ), along the  $K_m'$  and  $V_{max}'$  obtained in the presence of each inhibitor. For the inhibitors, use the concentration of inhibitor  $s$  in the calculation of  $K_i$ ; for inhibitor A,  $[I] = 5 \text{ mM}$ ; for inhibitor B,  $[I] = 0.1 \text{ mM}$ .

3. For more practice, do problem 4(a), shown below.

Problem 4(a) is taken from Voet & Voet, last year's textbook.

4. The following table indicates the rates at which a substrate reacts as catalyzed by an enzyme that follows the Michaelis - Menten mechanism: (1) in the absence of inhibitor; (2) and (3) in the presence of 10 mM concentration, respectively, of each of two inhibitors.

[S] (mM)	$V_o$ (micromol/sec)		
	(1)	(2)	(3)
1	2.5	1.17	0.77
2	4.0	2.10	1.25
5	6.3	4.00	2.00
10	7.6	5.7	2.50
20	9.0	7.2	2.86

(a) Determine  $K_M$  and  $V_{max}$  for the enzyme.

For each inhibitor determine the type of inhibition and  $K_i$

More practice problems will be given in the form of problems from previous exams.

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