10.542 – Biochemical Engineering
Spring 2005

Problem Set #2
Solutions should be written and submitted on your own paper. All pages should be stapled together.

1) Shuler & Kargi, Problem 3.2

   b) Show that the reaction will proceed far to the right only if $k_1k_2 \gg k_{-1}k_{-2}$.

2) Shuler & Kargi, Problem 3.5

3) A pesticide inhibits the activity of a particular enzyme, which can therefore be used to assay for the presence of the pesticide in an unknown sample.

   a) In the laboratory, the initial rate data shown in the table below were obtained. What type of inhibitor (competitive, uncompetitive, non-competitive) is the pesticide? Determine the values of V_{max}, K_M, and K_I.

	***v*, M/min x 10^6**	
***S*, M**	**No inhibitor**	**10^{-5} M inhibitor**
3.3×10^{-4}	56	37
5.0×10^{-4}	71	47
6.7×10^{-4}	88	61
1.65×10^{-3}	129	103
2.21×10^{-3}	149	125

   b) After 50 ml of the same enzyme solution in part (a) is mixed with 50 ml of 8×10^{-4} M substrate and 25 ml of a test sample, the initial rate observed is 18 μM/min. What is the pesticide concentration in the test sample assuming no other inhibitors or substrates are present?

4) You run a set of reactions to determine reaction rate as a function of substrate concentration and notice that the rate decreases as your substrate concentration increases. You suspect that either the substrate or the product is inhibiting the reaction. Describe the experiments you would conduct to determine which compound is inhibitory. If the *product* is inhibitory, how would you determine both the type of inhibition and the dissociation constant for the enzyme-inhibitor (or enzyme-substrate-inhibitor) complex, K_I? [Note: You need to describe both the data you would collect as well as how you would use that data to solve this problem.]

(over)

5) An enzyme-catalyzed reaction irreversibly generates protons according to the equation

$$H_2O + E + S^+ \rightarrow E + SOH + H^+$$

The active form of the enzyme is E^-. $[E^{2-}]/[E^-]=1.0$ at pH=10=pK_1 and $[E^-]/[E]=1.0$ at pH=6.0=pK_2.

a) Using Analysis 1 presented in Class (*i.e.*, focusing only on the enzyme), show that the reaction velocity at pH 7.0 is given approximately by

$$v = \frac{V_{\max} K_2 [S]}{(K_M + [S])(K_2 + [H^+])}$$

b) Integrate the previous equation to obtain an expression for substrate concentration [S] as a function of time, the initial pH and initial substrate concentration.