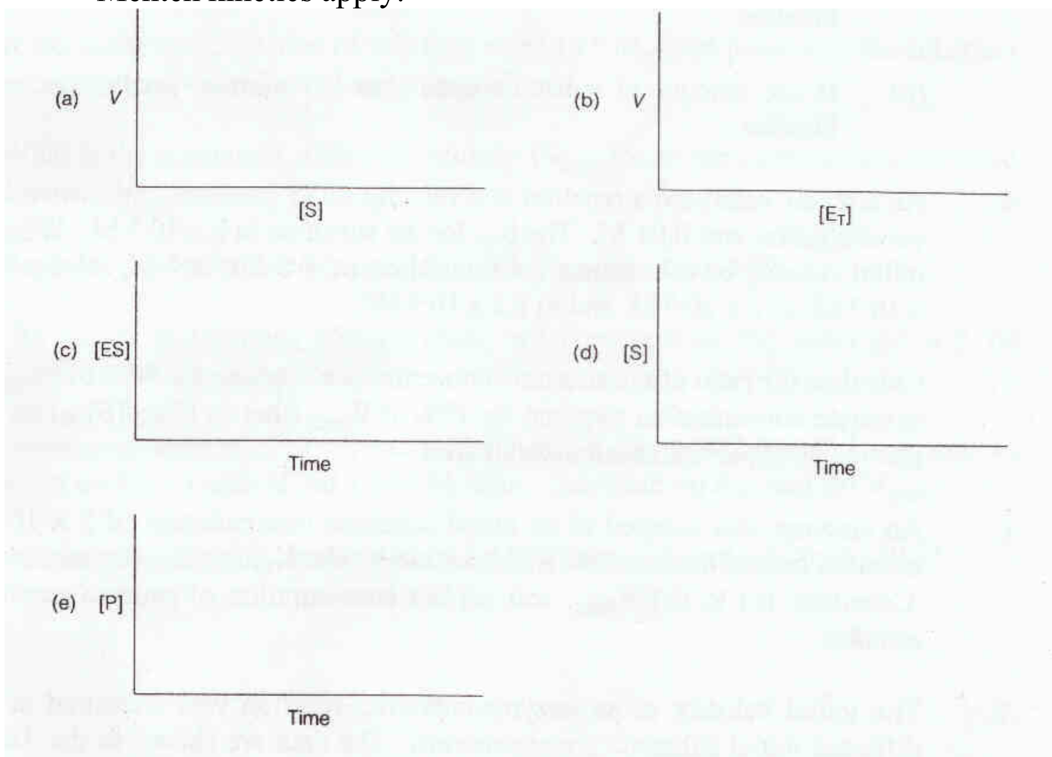
BIOC*4540 Enzymology
Problem Set #2

1. Sketch the appropriate plots on the following axes. Assume that simple Michaelis-Menten kinetics apply.



2. The enzyme-catalyzed hydrolysis of sucrose:

   sucrose + $H_2O \rightarrow$ glucose + fructose

   takes the following time course.

Time (min)	**[sucrose] M**
0	0.5011
30	0.4511
60	0.4038
90	0.3626
130	0.3148
180	0.2674

Determine the first-order rate constant and the half-life of the reaction. Why does this bimolecular reaction follow a first-order rate law? How long will it take to hydrolyze 99% of the sucrose initially present? How long will it take if the amount of sucrose initially present is twice that given in the table (above)?

3. (a) Under a given set of conditions (pH, buffer, temperature, etc.) it was found that a certain enzyme had a K_m of 6 x 10^{-4} M. During a subsequent study, 0.5 μmole of the substrate was dissolved in this buffer, enzyme was added, and the volume was immediately brought to 25 mL. Predict the order of kinetics.

   (b) If the amount of substrate used was 0.5 mmole, predict the order of the kinetics.

4. An enzyme catalyzed a reaction at a velocity of 35 μmoles/L-min when its substrate concentration was 0.01 M. The K_m for the substrate is 2 x 10^{-5} M. What would the initial velocity be at substrate concentrations of a) 3.5 x 10^{-5} M, b) 4 x 10^{-4} M, c) 2 x 10^{-4} M, d) 2 x 10^{-6} M, and e) 1.2 x 10^{-6} M?

5. Calculate the ratio of the substrate concentration required for 90% of V_{max} to the substrate concentration required for 10% of V_{max} (that is, $[S]_{90}/[S]_{10}$) for an enzyme that obeys hyperbolic saturation kinetics.

6. An enzyme was assayed at an initial substrate concentration of 2 x 10^{-5} M. In 6 minutes, half of the substrate had been used. The K_m for the substrate is 5 x 10^{-3} M. Calculate: (a) k, (b) V_{max}, and (c) the concentration of product produced by 15 minutes.

7. The initial velocity of an enzyme-catalyzed reaction was measured at a series of different initial substrate concentrations. The data are shown in the Table below. (a) Plot the data according to the Michaelis-Menten equation. (b) Determine K_m and V_{max} graphically by the Lineweaver-Burk method. (Plot 1/v versus 1/[S]).

[S]	v	$\frac{1}{[S]}$	$\frac{1}{v}$
M	μmoles/liter-min		
8.35×10^{-6}	13.8	12×10^{4}	7.24×10^{-2}
1.00×10^{-5}	16.0	10×10^{4}	6.25×10^{-2}
1.25×10^{-5}	19.1	8×10^{4}	5.23×10^{-2}
1.67×10^{-5}	23.8	6×10^{4}	4.20×10^{-2}
2.0×10^{-5}	26.7	5×10^{4}	3.75×10^{-2}
2.5×10^{-5}	30.8	4×10^{4}	3.25×10^{-2}
3.3×10^{-5}	36.2	3×10^{4}	2.76×10^{-2}
5.0×10^{-5}	44.5	2×10^{4}	2.25×10^{-2}
1.0×10^{-4}	57.2	1×10^{4}	1.75×10^{-2}
2.0×10^{-4}	66.7	0.5×10^{4}	1.50×10^{-2}

Now, plot the data according to the following graphical methods and determine the K_m and V_{max} graphically. c) Hanes, d) Eadie-Hofstee, and e) Direct Linear plot.

8. An enzyme was assayed at an initial substrate concentration of 10^{-5} M. The K_m for the substrate was 2 x 10^{-3} M. At the end of 1 minute, 2% of the substrate had been converted to product.

(a) What percent of the substrate will be converted to product at the end of 3 minutes? What will be the product and substrate concentrations after 3 minutes?

(b) If the initial concentration of substrate were 10^{-6} M, what percent of the substrate will be converted to product after 3 minutes?

(c) What is the maximum attainable velocity (V_{max}) with the enzyme concentration used?

(d) At about what substrate concentration will V_{max} be observed?

(e) At this S (saturating) concentration, what percent of the substrate will be converted to product in 3 minutes?

9. A Lineweaver-Burk plot of 1/v versus 1/[S] has a slope of 2.5 x 10^{-4} min and an intercept on the 1/v axis of 3.0 x 10^{-2} M^{-1}min. Calculate (a) K_M and (b) V_{max}.

10. What fraction of V_{max} is obtained when K_M/[S] has a value of (a) 10; (b) 10^{-1}?

11. The enzyme hexokinase catalyzes the disappearance of its glucose substrate according to first order kinetics, the rate constants at various temperatures being as follows:

Temperature (°C)	**Rate constants (s^{-1})**
15.0	4.18 x 10^{-6}
20.0	7.62 x 10^{-6}
25.0	1.37 x 10^{-5}
30.0	2.41 x 10^{-5}
37.0	5.15 x 10^{-5}

Analyze the data according to the Arrhenius relationship and calculate the activation energy (E_a) and the frequency factor (A) for the hexokinase reaction.

12. The effect of pH on the activity of an enzyme was examined. At its active site, the enzyme has an ionisable group that must be negatively charged for its substrate binding and catalysis to take place. The ionisable group has a pK_a of 6.0. The substrate is positively charged throughout the pH range of the experiment.

$$\begin{array}{l} E^- + S^+ \leftrightarrow E\text{—}S^+ \rightarrow E^- + P^+ \\ + \\ H^+ \\ \updownarrow \\ EH \end{array}$$

(a) Draw the v_o-versus-pH curve when the substrate concentration is much greater than the enzyme K_M.
(b) Draw the v_o-versus-pH curve when the substrate concentration is much less than the enzyme K_M.
(c) At which pH will the velocity equal one-half of the maximal velocity attainable under these conditions.