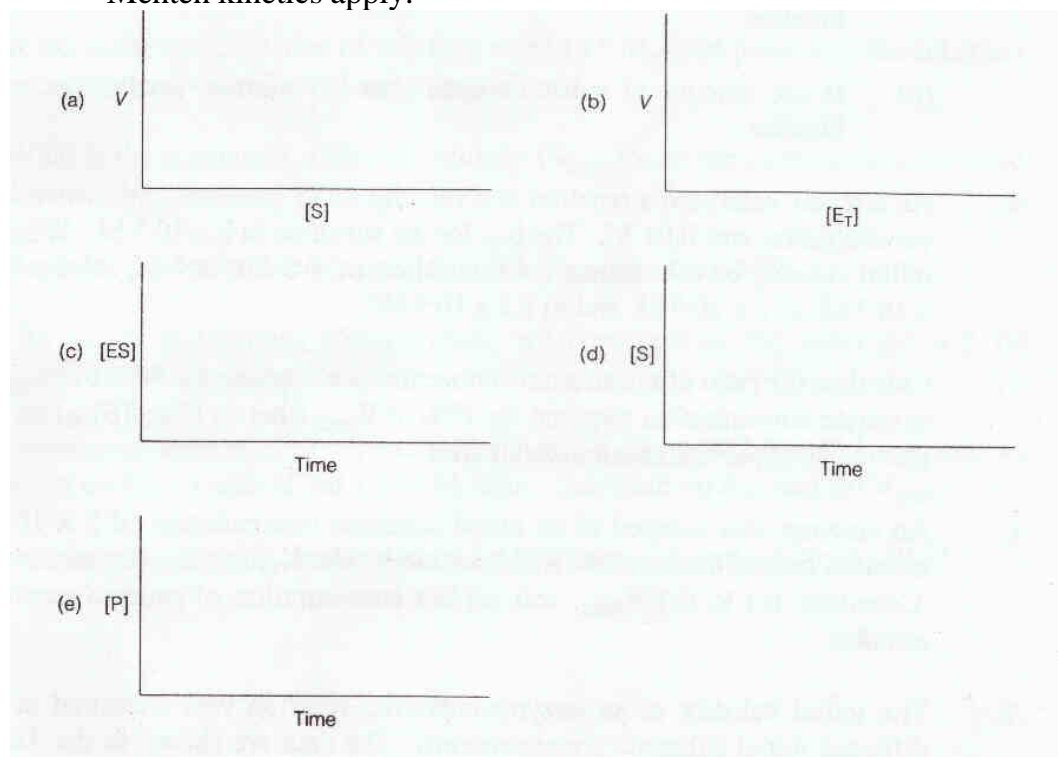


2. **Web-based exercise.** Go to the Protein Data Bank (PDB) site (link found at Course Website, URL = <http://www.rcsb.org/pdb/>) and below the Search menu click on "Searchlite" and in the subsequent search window type "alcohol dehydrogenase" (LADH). You should find that the search retrieved 59 structures. From the list of structures select the file name "1ADC" and click on the red Explore text. (a) What is 5-β-D-Ribosfuanosylpicolinamide Adenine Dinucleotide (Cpad)? (b) Select the summary information. When was the coordinate file deposited at the PDB? What is the resolution in Angstroms for the structure? How many subunits are in the structure? What are the heterogeneous structures/compounds associated with this protein in the structural file? (c) Now click on "Sequence Details" and what are the molecular weights, number of residues, number of alpha helices, and the number of beta strands associated with each subunit? If the subunits are identical then why is there a difference in the secondary structure between the two? (d) Now select "Download/Display File" and choose a PDB file to download (select the uncompressed PDB file). Save the file to an appropriate folder on your computer desktop (note the name of the pdb file). **Optional activity.** Go to the Web site for Rasmol (link also found on the course website homepage) and follow the instructions to download the Raswin for your computer (IBM or Mac) executable file (in self-unzipping format). You can also download the help file from the same page. Install Raswin by double-clicking on the self-extracting file and follow instructions. Once installed run the Rasmol program and open the 1ADC.pdb file. You can now render the enzyme molecule in various formats, rotate it, zoom, measure, and play with it. There is a command line editor at the bottom of the screen.

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



4. The enzyme-catalyzed hydrolysis of sucrose:
sucrose + H₂O → 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)?

5. (a) Under a given set of conditions (pH, buffer, temperature, etc.) it was found that a certain enzyme had a K_m of 6×10^{-4} M. During a subsequent study, 0.5 μ mole 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.
6. An enzyme catalyzed a reaction at a velocity of 35 μ moles/L-min when its substrate concentration was 0.01 M. The K_m for the substrate is 2×10^{-5} M. What would the initial velocity be at substrate concentrations of a) 3.5×10^{-5} M, b) 4×10^{-4} M, c) 2×10^{-4} M, d) 2×10^{-6} M, and e) 1.2×10^{-6} M?
7. 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.
8. An enzyme was assayed at an initial substrate concentration of 2×10^{-5} M. In 6 minutes, half of the substrate had been used. The K_m for the substrate is 5×10^{-3} M. Calculate: (a) k , (b) V_{max} , and (c) the concentration of product produced by 15 minutes.
9. 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. 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	μ moles/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. b) Hanes, c) Eadie-Hofstee, d) Eadie Scatchard, e) direct linear plot.

10. An enzyme was assayed at an initial substrate concentration of 10^{-5} M. The K_m for

the substrate was 2×10^{-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?
11. A Lineweaver-Burk plot of $1/v$ versus $1/[S]$ has a slope of 2.5×10^{-4} min and an intercept on the $1/v$ axis of $3.0 \times 10^{-2} \text{ M}^{-1}\text{min}$. Calculate (a) K_M and (b) V_{\max} .
12. What fraction of V_{\max} is obtained when $K_M/[S]$ has a value of (a) 10; (b) 10^{-1} ?