Enzymes Problem Set 1

A) What concentration of the substrate would be required to obtain an initial rate of $0.5V_{max}$, $0.9\ V_{max}$, $0.95\ V_{max}$?

B) The following initial rate data were obtained for an enzyme catalyzed reaction:

Substrate Concentration (μmol/L)	Measured Initial Rate (μmol/Lmin)
1	0.08
5	0.25
10	0.33
100	0.48
1000	0.50

i) What is the value of K_M and V_{max}?

ii) Calculate the concentration of substrate remaining after 60 minutes if the initial substrate concentration is 20 μmol/L.

C) The following initial rate data were obtained by adding 2.5 μg of a purified enzyme having molecular weight of 42,300 Daltons into 100 mL of water with the indicated concentrations of substrate.

S_0	v
(mmol/L)	(μmol/Ls)
1.00	4.50
2.00	7.90
4.00	11.10
8.00	15.20

Calculate the value of k_{CAT}, K_M and the pseudo first order rate constant.

D) The following initial rate data were obtained by adding 0.35 mg of a purified dimeric enzyme having (total) molecular weight of 82,800 Daltons into 100 mL of water with the indicated concentrations of substrate.

Substrate Conc (mM)	v (mM/min)
1.0	0.55
2.0	0.94
3.0	1.32
5.0	1.87
10.0	2.59

i. Calculate the value of k_{CAT}, K_M and the pseudo first order rate constant.
ii. For a solution having an enzyme concentration of 3.5 mg/L, calculate the the final substrate concentration after 5 minutes of reaction when the initial substrate concentration is 15 mM.
iii. For a solution having an enzyme concentration of 1.0 mg/L, calculate the the final substrate concentration after 5 minutes of reaction when the initial substrate concentration is 15 mM.

E) Tyrosine phenol lyase catalyzes the reversible conversion of tyrosine to phenol, pyruvate and ammonium. The enzyme uses pyridoxal 5-phosphate (PLP) as a cofactor. The enzyme catalyzes similar reactions as well, for example the reversible conversion of L-dopa (3,4-dihydroxy L-phenylalanine) to catechol, pyruvate and ammonium. An assay for the activity of this enzyme involves incubating excess phenol, pyruvate and ammonium with the enzyme sample for a measured length of time. After the termination of the reaction, the L-tyrosine present in the sample is quantified by liquid chromatography.

The following solutions are mixed:

500 μL	1.3 M NH_4Cl/0.05M Na_4EDTA pH adjusted to 8.5
100 μL	80 mM Na_2SO_3 (a reducing agent to stabilize the reaction)
100 μL	800 mM Pyruvate
100 μL	1.0 mM PLP
100 μL	500 mM Phenol

At t=0, you add 100 μL of an enzyme solution known to have a protein concentration of 8.5 μg/mL. After 7.0 minutes, you add 100 μL trichloroacetic acid which terminates the reaction. Using the HPLC, you find that the concentration of L-tyrosine in this final solution is 2.3 mM.

i) What is the activity of the original enzyme solution (in IU/mL)?
ii) What is the specific activity of the original enzyme solution (in IU/mg protein)?

F) DHA kinase catalyzes the conversion of dihydroxyacetone (DHA) to dihydroxyacetone phosphate (DHAP) using ATP as a co-substrate. The enzyme assay relies on a coupled reaction in which the reaction product, DHAP, is subsequently converted into glycerol 3-phosphate by the presence of the enzyme glycerol 3-phosphate dehydrogenase, which uses NADH as a co-substrate. Glycerol is added to the assay to prevent glycerol kinase interference. α,α-dipyridyl is added to the assay to prevent glycerol dehydrogenase interference. NADH absorbs light at a wavelength of 340 nm. Thus, the rate of DHA kinase activity is directly related to the rate at which NADH disappears from the solution.

The following solutions are mixed:

200 μL	200 mM triethanolamine-HCl buffer
400 μL	25 mM α,α-dipyridyl
50 μL	5 U glycerol 3-phosphate dehydrogenase from rabbit muscle
50 μL	2.0 M glycerol
50 μL	2.0 mM NADH
50 μL	20 mM $MgCl_2$
50 μL	20 mM dihydroxyacetone, DHA
50 μL	20 mM ATP

At t=0, you add 100 μL of an enzyme solution known to have a protein concentration of 4.3 μg/mL, and measure the rate of decrease in absorbance of the solution to be 0.52 AU/min. The molar extinction coefficient of NADH is 6.22 AU·L/mmol for the light path length used. "AU" means absorbance unit.

i) Write the reaction of the enzyme and the couple reaction, including the cofactors. Highlight the actual species measured. Why is excess glycerol 3-phosphate dehydrogenase needed?
ii) What is the activity of the original enzyme solution (in IU/mL)?
iii) What is the specific activity of the original enzyme solution (in IU/mg protein)?

G) Iodoacetamide (MW = 185) is a common irreversible inhibitor of enzymes because it binds to reactive cysteine residues in a 1:1 molar ratio.

The enzyme glyceraldehyde 3-phosphate dehydrogenase has a MW of 150,000, and is known to have one reactive cysteine residue at the active site. A 5 mL solution of this enzyme at a concentration of 1.2 mg/mL is completely inactivated by 0.03 mg iodoacetamide. How many subunits does this enzyme contain?

H) Invertase mediates the conversion of sucrose into glucose and fructose. You place 0.66 mg of invertase in a beaker of 500 mL buffer initially containing 20 mM sucrose and no glucose and fructose. The value of K_M is 2.5 mM and the value of k_{cat} is 1550 s^{-1}. The invertase you are using has a molecular weight of 65000.

i) What is the initial reaction rate (mmol sucrose/Lmin)?

ii) What will be the concentration of sucrose (mM) after 5 min?

iii) What will be the reaction rate (mmol sucrose/Lmin) after 5 min?
By what percentage has the reaction rate decreased after 5 min?

iv) What will be the concentration of sucrose (mM) after 10 min?
What will be the concentration of glucose after 10 min?

v) What will be the reaction rate (mmol sucrose/Lmin) after 10 min?
By what percentage has the reaction rate decreased after 10 min?

vi) If in the 500 mL solution you used 1.32 mg of the enzyme (instead of 0.66 mg) what will be the concentration of sucrose after 10 min?