

Kinetics of Tyrosinase
(30 Points)

Name: _____ Partner's Name: _____

Lab Section: _____

1. Complete and attach the following items to this lab report:

a. Data Sheet and Calculation Summary Chart.

Show the corresponding calculations for Run #3 below:

(1) Calculation of Rate of Reaction (V_0) for Run #3, and $1/v_0$:

(2) Calculation of [DL-DOPA] for Run #3, and calculation of $1/[DL-DOPA]$

b. Plots of Absorbance vs. Time.

c. Michaelis-Menten Plot. Clearly represent the estimated values of K_m and V_{max} on the graph.
List below the values estimated from your Michealis-Menten Plot (include proper units):

$K_m =$ _____ $V_{max} =$ _____

d. Lineweaver-Burk Plot. Show all work for calculating values for K_m and V_{max} based on this graph below
(include proper units for ALL values):

(1) Slope of Lineweaver-Burk Plot = _____

Y-intercept of Lineweaver-Burk Plot = _____

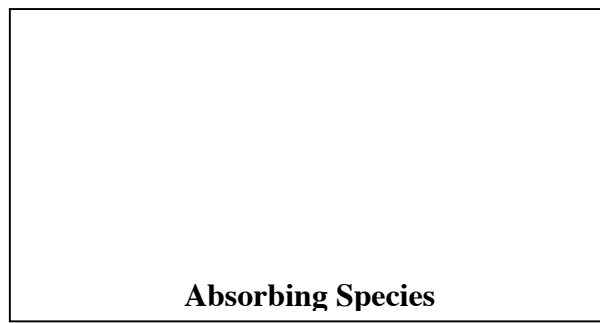
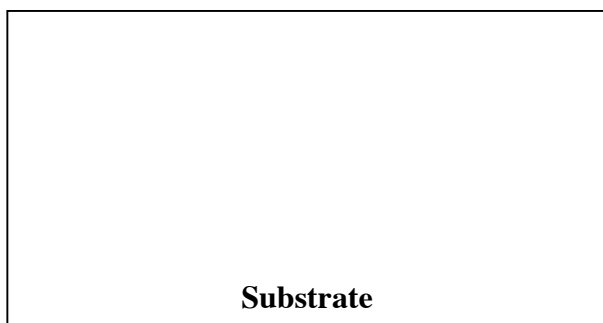
X-intercept of Lineweaver-Burk Plot = _____

(2) $V_{max} =$ _____ (Show all work below)

(3) $K_m =$ _____ (Derive this value from the y-intercept; show all work below)

2. Compare the values of V_{\max} and K_m determined on the two plots. Which do you believe to be more reliable? _____
Explain: _____

3. Draw the structures below for the substrate for the reaction you ran and for the absorbing species whose concentration you measured:



4. Select the **best** answer for the statements below. Assume that you are following all other procedures just as you did in the lab.
- Shaking the solution vigorously after adding the enzyme so that the solution foams (indicative of denatured protein) will probably (raise, lower, not affect) the observed rate of the reaction.

 - Adding a competitive inhibitor into the reaction mix containing your lowest concentration of DL-DOPA will probably (raise, lower, not affect) the observed rate of the reaction. _____
 - Pipeting 4.20mL, rather than 5.20mL, of buffer into the solution of Run #1 will (raise, lower, not affect) the observed rate of the reaction. _____
 - Running the reaction at 15°C, a temperature lower than room temperature (25°C), will probably (raise, lower, not affect) the observed rate of the reaction. _____
 - Running the reaction at 45°C, a temperature high enough to denature the protein, will probably (raise, lower, not affect) the observed rate of the reaction. _____
 - Adding twice the volume of *everything* (including enzyme) in the reaction will (raise, lower, not affect) the observed rate of the reaction. _____
 - Measuring the slope of absorbance vs. time for the entire 60 seconds, rather than using the initial rate, will probably (raise, lower, not affect) the recorded rate of the reaction. _____
 - Waiting 5 minutes after the tyrosinase is added before beginning to take absorbance measurements will probably (raise, lower, not affect) the observed rate of the reaction. _____
 - Adding 0.06mL, rather than 0.6mL, of DL-DOPA into the assay mix will probably (raise, lower, not affect) the observed rate of the reaction. _____
 - Using 0.02mL, rather than 0.20mL, of tyrosinase will probably (raise, lower, not affect) the observed rate of the reaction. _____

5. a. If substrate “A” binds the enzyme with lower affinity than substrate “B”, which substrate exhibits the smaller K_m value? _____
- b. Does a LARGE value of k_{cat} imply strong substrate binding, weak substrate binding, or have no relation to substrate binding? _____
6. Calculating Useful Rate Parameters:
- a. Often, rates are actually reported as “ μ moles of product per min”, which is defined as a “unit” of activity. Convert the value of V_{max} from 1.d. above to these units. Show all work below:

Therefore, $V_{max} =$ _____

b. Turnover Number (k_{cat})

(1) Provide a verbal definition for “turnover number” (k_{cat}): _____

(2) Assuming that each assay mixture contained 5.00 mg of tyrosinase ($M_{wt} = 48,000\text{g/mole}$), what is the *value* of k_{cat} for tyrosinase, using proper units? Show all work below:

Therefore, $k_{cat} =$ _____

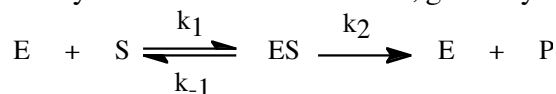
c. Catalytic Efficiency

(1) Provide a verbal definition for “catalytic efficiency”: _____

(2) Calculate the value of the catalytic efficiency of tyrosinase, using proper units. Show all work below:

Therefore, Catalytic Efficiency = _____

(3) When the rate of catalytic turnover (k_2) is extremely fast, the rate-limiting step becomes the rate of collision of substrate and enzyme molecules in solution, given by k_1 below:



The “diffusion-controlled limit” for efficiency, i.e. the highest attainable number limited by the speed by which any two particles can collide at room temperature, is approximately $10^8\text{-}10^9\text{M}^{-1}\text{sec}^{-1}$; an enzyme which exhibits a catalytic efficiency in this range is deemed catalytically perfect. Based on your answers above, is tyrosinase “perfect”? Explain. _____

Calculation Summary Chart

[DL-DOPA] (M)	Slope ($\Delta\text{abs} / \text{time}$) ($\text{s}^{-1}$)	V_o (M s^{-1})	$1/[\text{DL-DOPA}]$ (M^{-1})	$1/v_o$ (s M^{-1})