

**Biochemistry Laboratory I CHEM4401  
Polyphenoloxidase Laboratory Report (35 pt)**

**Please read section 6.3 in your text (Lehninger 5th Ed.) and the *Lab Report Expectations* section in your laboratory manual**

Bring this supplement to class along with your data from the laboratory and either a **laptop computer (with Microsoft Excel or equivalent) or a USB memory drive.**

1. **Table 1.** Prepare a table of the raw absorbance data collected for samples 1-4 (see below). Be sure to identify the **actual concentration** of DOPA in each sample, NOT the *volume* of DOPA added to each test tube. This can be calculated using the  $C_1V_1 = C_2V_2$  equation.

- Table itself (1 pt)
- Table is numbered and appropriately titled (1 pt)
- Correct DOPA concentrations indicated (1 pt)

Time (seconds)	Abs (sample 1)	Abs (sample 2)	Abs (sample 3)	Abs (sample 4)
	DOPA conc (mM)	DOPA conc (mM)	DOPA conc (mM)	DOPA conc (mM)
0	0	0	0	0
20				
:				
180				

2. **Figure 1.** Plot of Absorbance (y-axis) vs time (x-axis) for each sample on a single graph. Use the “scatter plot” option for each sample. Fit a curve to each set of sample points using a polynomial fit (order =2) **NOT** a linear fit. Use different symbols or colors for each sample. Format each curve by using “hairline” as the curve “weight”.

- Figure itself (1 pt)
- Figure is numbered and appropriate legend is present (1 pt)
- All polynomial curves are present and appropriately identified (2 pt)
- Axes are labeled. Appropriate units used (1 pt)

3. Determine the velocity ( $V_0$ ) of the enzymatic reaction in each sample by converting the change in absorbance per unit time to change in concentration of dopachrome per unit time (Eq. 1). Absorbance readings can be converted to concentration values using the Lambert-Beer equation, as described in part 3 of the *Lab Report Expectations* section of your lab manual. Use an extinction coefficient =  $3.6 \text{ mM}^{-1}\text{cm}^{-1}$  and a path length (b) equal to 1 cm.

The concentration value is then converted to a change in concentration per unit time by dividing it by the amount of time elapsed during the linear portion of the graph (typically the first minute). The change in concentration per unit time is the *velocity* ( $V_0$ ) of the reaction. Calculate the velocity of the reactions for samples 1-4. These values will be entered in table 2. Show your work for one sample. Be sure to use appropriate units (1 pt)

$$\text{(Eq. 1) } V_0 = \frac{\Delta \text{ absorbance}}{\epsilon \cdot b} \cdot \frac{1}{\Delta \text{ time}}$$

5. **Table 2.** Prepare a table with the following columns:

- (i) substrate concentration ([DOPA]) in mM
- (ii) velocity ( $V^o$ ) in  $\text{mM}\cdot\text{min}^{-1}$
- (iii) 1/substrate concentration ( $1/[\text{DOPA}]$ ) in  $\text{mM}^{-1}$
- (iv)  $1/V^o$  (in  $\text{mM}^{-1}\cdot\text{min}$ )

Enter data in table for all 4 samples. As with table 1:

- Table itself (1 pt)
- Table is appropriately numbered and titled (1 pt)
- Correct units are indicated (1 pt).

6. **Figure 2.** Prepare a *Michaelis-Menten* graph using the data from table 2 by plotting plot  $V^o$  (y-axis) vs. the substrate concentration (DOPA) on the x-axis, as outlined in *Determination of  $K_m$  and  $V_{max}$*  in your manual. Fit a polynomial curve to your data points. Use Excel to extend your curve forward and backward by several units to produce a “classic” M-M curve (be sure to use the **force curve through origin option**). Graphically Estimate the maximum velocity ( $V_{max}$ ) and the Michaelis Constant ( $K_m$ ) as outlined in your laboratory manual. Indicate these values in your legend.

- Figure itself (1 pt)
- Figure is numbered and has an appropriate legend (1 pt)
- Axes are labeled with appropriate units (1 pt)
- A polynomial curve, extending both forward and backward through the origin, is fit to the data points (1 pt)
- $V_{max}$  is graphically determined and the value is indicated in the figure legend (1 pt)
- $K_m$  value is graphically determined and the value is indicated in the figure legend (1 pt)

7. **Figure 3.** Prepare a *Lineweaver-Burke* plot using data from table 2 by plotting  $1/V^o$  (y-axis) vs.  $1/\text{substrate concentration (DOPA)}$  (x-axis). Use Excel to create a regression line (line of best fit, NOT a polynomial curve) through your data points. Extend the line forward or backward by several units until it intersects both the y and x axes (do NOT force through origin). Use the plot to produce another estimate of  $V_{max}$  and  $K_m$  as outlined in your laboratory manual.

- Figure itself (1 pt)
- Figure is numbered and has an appropriate legend (1 pt)
- Axes are labeled with appropriate units (1 pt)
- A regression line, intersecting both axes, is fit to the data points (1 pt)
- $V_{max}$  is graphically determined and the value is indicated in figure legend (1 pt)
- $K_m$  value is graphically determined and the value is indicated in figure legend (1 pt)

8. **Table 3.** Use the template below to prepare a table containing the raw absorbance data data for samples 5-7 of the *Determination of pH and Temperature Inhibition* section. Include data for sample 2 for comparison purposes. Table should be numbered, appropriately titled and contain all correct DOPA, pH and temperature values (2.5 pt).

Sample	2	5	6	7
[DOPA] (mM)				
pH				
Temperature				
Time (s)	Absorbance	Absorbance	Absorbance	Absorbance
0	0	0	0	0
20	:	:	:	:
:	:	:	:	:
180	:	:	:	:

9. **Figure 4.** Use the data from table 3 to plot a graph of absorbance (y-axis) vs. time (x-axis) for each of the three different pH samples (2,5,6). Fit a polynomial curve to each set of data points.

- Figure itself (1 pt)
- Figure is numbered and appropriate legend is present (1 pt)
- All curves are present and clearly identified (1.5 pt)
- Axes are labeled with appropriate units (1 pt)

10. **Figure 5.** Use the data from table 3 to plot a graph of absorbance (y-axis) vs time (x-axis) for each of the two different temperature samples (tubes 2 and 7). Again, fit a polynomial curve to each set of data points.

- Figure itself (1 pt)
- Figure is numbered and appropriate legend is present (1 pt)
- All curves are present and clearly identified (1 pt)
- Axes are labeled with appropriate units (1 pt)