Purpose:

The purpose of this lab is to study various enzyme kinematic parameters using the enzyme, acid phosphatase and the substrate PNPP (*p*-nitrophenylphosphate). The acid phosphate will turn PNPP, which is colorless, to *p*-nitrophenol (PNP), which is yellow; the concentration of PNP can be determined using a spectrometer. In experiment 1, the rate of product formation will be studied; each team will carry out the reaction using a different concentration of enzyme and the absorbance will be measured. With the information from all the teams, a time curve will be plotted, allowing the determination of the concentration of enzyme that will yield a constant rate of product formation. In experiments 2 and 3, the reaction will be carried out in the presence and absence of an unknown inhibitor and the absorbances will be measured. A Hanes plot can then be made from which the K_M and V_{max} (kinetic parameters) can be determined. Analyzing the effects of the inhibitor on the two mentioned kinetic parameters will allow the identification of the type of inhibitor.

Results and Discussion:

Rate of product formation

R1. Convert the corrected absorbances from step 7 into amounts of PNP. In your calculations, you should assume a molar absorptivity for PNP of 1.88x10^4 M^{-1}cm^{-1} (ε_{405}), a path length for the cuvette of 1 cm and a final assay volume of 2.5 mL (This final volume takes into consideration the 2 mL of KOH that was added for stopping the reaction). From the volume of the incubation mixture (0.5 mL aliquot: before the addition of KOH) and the substrate concentration, calculate the percentage of substrate converted. Show your calculations for a given time. From the concentration of the provided stock solution of enzyme, calculate the concentration of acid phosphatase in your assay. Summarize your results in the following Table 4.

Table 1. Acid phosphatase assay results from 0 to 15 minutes. This table shows the corrected absorbance values, concentration of PNP, amount of PNP and percentage of PNPP converted for each time aliquot of the reaction mixture. The reaction mixture was made with 0.5 mL of a 1.0 M sodium acetate buffer (pH 5.7), 3.6 mL of water, 0.4 mL of 0.5 mg/mL acid phosphatase, and 0.5 mL of 20 mM PNPP, giving a total volume of 5.0 mL. Prior to the addition of the substrate (PNPP), the mixture was mixed and pre-incubated for a few minutes at 37˚C. Following the addition of the substrate, the reaction was mixed and kept at 37˚C; at each time interval mentioned in the table (0 minutes, 2 minutes, etc.), a 0.5 mL aliquot was transferred from the reaction mixture to a prepared test tube containing 2.0 mL of 0.5 M KOH to stop the reaction. The absorbance of each test tube at 405 nm was recorded. The 0 minute test tube absorbance (0.073) was subtracted from each absorbance value to give the corrected absorbance. From these absorbance values and stock concentrations, the concentration of PNP, amount of PNP and percentage of PNPP converted were calculated. It was assumed that the molar absorptivity for PNP is 1.88x10^4 M^{-1}cm^{-1} (ε_{405}), and the path length for the cuvette is 1 cm.

Time (min)	A_{405}	PNP (mM)	PNP (µmol)	Percentage of PNPP converted
0	0	0	0	0
2	0.215	0.01144	0.02860	2.86
4	0.412	0.02192	0.05480	5.48
6	0.596	0.03170	0.07925	7.93
10	1.022	0.05436	0.1359	13.6
15	1.364	0.07255	0.1814	18.1

Sample calculations of A_{405} (corrected) for t = 2 minutes:

Variables:
- A_{405} (0 min) = 0.073
- A_{405} (2 min) = 0.288
- A_{405} corrected = ?

A_{405} corrected = A_{405} (2 min) – A_{405} (0 min)
A_{405} corrected = 0.288 – 0.073
A_{405} corrected = 0.215

The corrected absorbance for the 2 minute test tube is 0.215.

Sample calculations for PNP (mM) for t = 2 minutes:

Variables:
- A_{405} = 0.215
- l = 1 cm
- ε_{405} = *18800 $M^{-1}cm^{-1}$*
- c = ?

$A_{405} = (\varepsilon_{405})(c)(l)$

$$c = \frac{A_{405}}{(\varepsilon_{405})(l)}$$

$$c = \frac{0.215}{(18800\ M^{-1}cm^{-1})(1\ cm)}$$

$c \approx 1.144 \times 10^{-5}$ M
c = 0.01144 mM

The concentration of PNP in the assay is 0.01144 mM (for the 2 minute test tube).

Sample calculations for PNP (μmol) for t = 2 minutes:

Variables:
- v = 0.0025 L
- c = 0.01144 mM
- n = ?

n = cv
n = (0.01144 mM)(0.0025 L)
$n = 2.860 \times 10^{-5}$ mmol
n = 0.02860 μmol

There is 0.02860 μmol of PNP present in the assay (for the 2 minute test tube).

Sample calculations for percentage of PNPP converted for t = 2 minutes:

Concentration of PNPP (M)	μmol of PNPP	% of PNPP converted
Variables: • c_1 = 0.020 M • v_1 = 0.0005 L • v_2 = 0.005 L • c_2 = ? $c_1v_1 = c_2v_2$ $c_2 = \frac{c_1v_1}{v_2}$ $c_2 = \frac{(0.020\ M)(0.0005\ L)}{(0.005\ L)}$ c_2 = 0.002 M The concentration of PNPP in the aliquot was 0.002 M (for the 2 minute test tube).	Variables: • v = 0.0005 L • c = 0.002 M • n_{PNPP} = ? n_{PNPP} = cv n_{PNPP} = (0.002 M)(0.0005 L) $n_{PNPP} = 1.00 \times 10^{-6}$ mol n_{PNPP} = 1.00 μmol The amount of PNPP in the aliquot was 1.00 μmol (for the 2 minute test tube).	Variables: • n_{PNPP} = 1.00 μmol • n_{PNP} = 0.02860 μmol • % converted = ? % converted = $(\frac{n_{PNP}}{n_{PNPP}})$*100 % converted = $(\frac{0.02860\ \mu mol}{1.00\ \mu mol})$*100 % converted = 2.86 % 2.86 % of PNPP was converted to PNP (in the 2 minute test tube).

Calculation of the concentration of acid phosphatase in the assay:

Variables:

- c_1 = 0.5 mg/mL
- v_1 = 0.4 mL
- v_2 = 5 mL
- c_2 = ?

$$c_1v_1 = c_2v_2$$

$$c_2 = \frac{c_1v_1}{v_2}$$

$$c_2 = \frac{(0.5 \text{ mg/mL})(0.4 \text{ mL})}{(5 \text{ mL})}$$

$$c_2 = 0.04 \text{ mg/mL}$$

The concentration of acid phosphatase in the assay was 0.04 mg/mL.

R2. Plot the amount of product (µmol PNP) versus time (minutes) for your results in Table 4. Estimate the initial velocity of the reaction. For how long does the reaction rate remain constant? Indicate the highest level of substrate conversion (in %) that you can observe in the linear range. Can you explain why plots sometime deviates from the straight line (reach a plateau)?

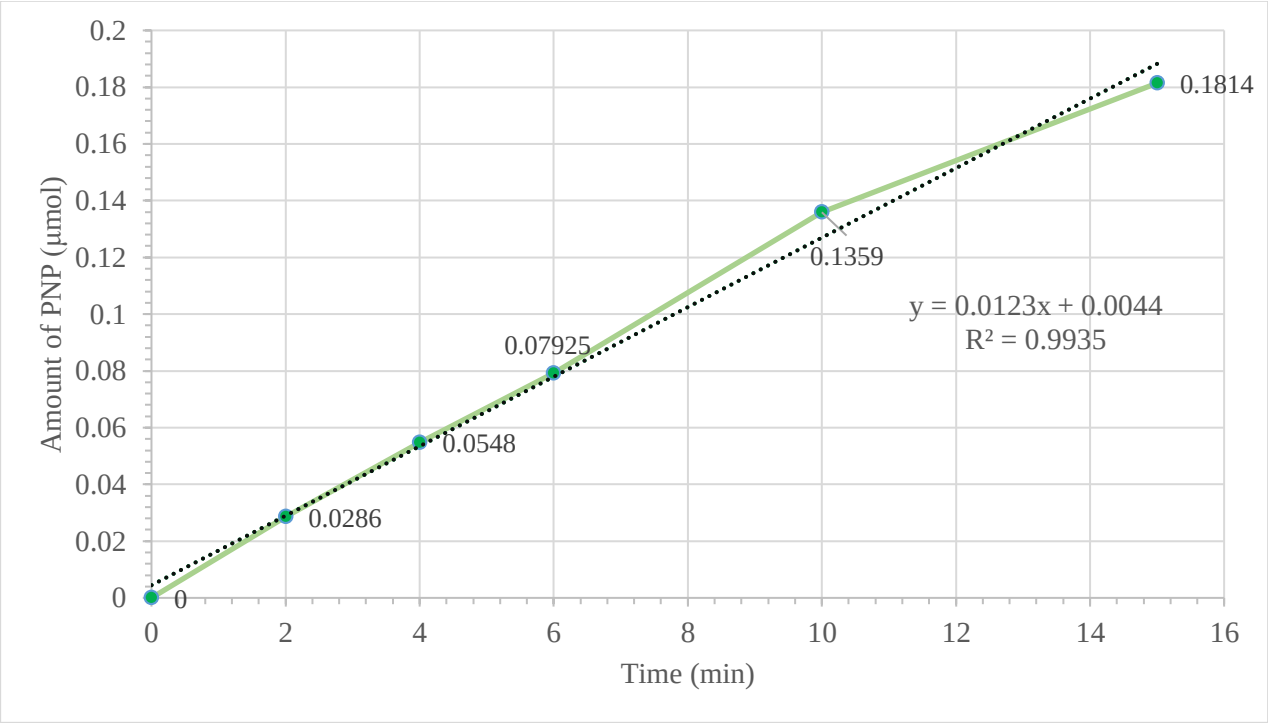

Figure 1. Amount of PNP (µmol) versus time (minutes) for an acid phosphatase assay. The data is from the acid phosphatase assay results as shown in **Table 1**, specifically the time and amount of PNP (µmol). The assay was performed using a reaction mixture from which aliquots were removed at certain time intervals. The reaction mixture was made with 0.5 mL of a 1.0 M sodium acetate buffer (pH 5.7), 3.6 mL of water, 0.4 mL of 0.5 mg/mL acid phosphatase, and 0.5 mL of 20 mM PNPP, giving a total volume of 5.0 mL. Prior to the addition of the substrate (PNPP), the mixture was mixed and pre-incubated for a few minutes at 37˚C. Following the addition of the substrate, the reaction was mixed and kept at 37˚C; at each time interval (0 minutes, 2 minutes, 4 minutes, 6 minutes, 10 minutes and 15 minutes), a 0.5 mL aliquot was transferred from the reaction mixture to a prepared test tube containing 2.0 mL of 0.5 M KOH to stop the reaction. The absorbance of each test tube at 405 nm was recorded from which the amount of PNP (µmol) was ultimately calculated. The amount of PNP (µmol) versus time (minutes) was plotted to visualize the rate of product formation.

Looking at the slope of the linear regression trendline, one can estimate the initial velocity to be about 0.0123 μmol/min. The rate of reaction remains relatively constant from 0 to 6 minutes after which it begins to plateau slightly. Within this linear range between 0 minutes and 6 minutes, the highest level of substrate conversion is 7.93 %. Plots sometimes deviate from straight lines (reach a plateau) because the all the enzymes become occupied with substrates and there is no longer any substrate conversion; at the region of the plateau, the amount of substrate conversion has reached the maximum threshold so from that point on there is a constant amount of product (in this case the product is PNP).

Determination of K_M and V_{max}

R3. Use the [PNPP] values calculated for Table 3. Convert the corrected absorbances from step 19 for both experiments 2 (control) and 3 (inhibitor) into amounts of PNP as above. Calculate the corresponding reaction rates (v). Show all your calculations for one substrate concentration for the control (exp. 2). Summarize your results in two tables (Table 5/6) with the following format:

Table 2. Substrate dependence. This table shows the calculated initial concentration of PNPP, corrected absorbance at 405 nm, concentration and amount of PNP, and reaction rates of both the control and inhibitor experiments. The control experiment was performed by using two sets of 7 test tubes; the incubation test tubes and the zero test tubes. In both sets of tubes, 50 μL of 1.0 M sodium acetate buffer (pH 5.7) and the corresponding amounts of water and PNPP were added; the concentrations of the PNPP in each tube was calculated using stock concentrations and the total assay volume. The incubation test tubes were pre-incubated at 37˚C for 2 minutes while the zero test tubes were left at room temperature throughout the experiment. 2.0 mL of 0.5 M KOH was added to the zero test tubes and after mixing, 50 μL of 0.5 mg/mL acid phosphatase was added. 50 μL of 0.5 mg/mL acid phosphatase was added to each incubation tube at different times. After 10 minutes of incubation for all the tubes, 2.0 mL of 0.5 M KOH was added to stop the reaction. The absorbance values at 405 nm for both sets of tubes was recorded. The zero test tubes were used to correct the absorbance values for incubation test tubes and these corrected absorbance values were used to calculate the concentration of PNP, amount of PNP and reaction rate. The same procedure was repeated for the inhibition experiment except, 50 μL of a 9.0 mM unknown inhibitor (inhibitor #10) was added after the PNPP, the volume of water added was decreased by 50 μL and the zero test tubes were not done again (the zero test tubes from the control experiment were used to correct the absorbance values for the inhibition incubation tubes). It was assumed that the molar absorptivity for PNP is 1.88×10^4 $M^{-1}cm^{-1}$ (ε_{405}), and the path length for the cuvette is 1 cm.

[PNPP]* (M)	Control (experiment 2)				Inhibitor (experiment 3)			
	A_{405}	[PNP] (M)	PNP (μmol)	v (μmol/min)	A_{405}	[PNP] (M)	PNP (μmol)	v (μmol/min)
0.00005	0.176	9.36×10^{-6}	0.0234	0.00234	0.03	1.60×10^{-6}	0.004	0.0004
0.0001	0.359	1.91×10^{-5}	0.0478	0.00478	0.069	3.67×10^{-6}	0.00918	0.000918
0.0002	0.456	2.43×10^{-5}	0.0608	0.00608	0.153	8.14×10^{-6}	0.0204	0.00204
0.0005	0.63	3.35×10^{-5}	0.0838	0.00838	0.295	1.57×10^{-5}	0.0393	0.00393
0.00075	0.789	4.20×10^{-5}	0.105	0.0105	0.359	1.91×10^{-5}	0.0478	0.00478
0.001	0.836	4.45×10^{-5}	0.111	0.0111	0.47	2.50×10^{-5}	0.0625	0.00625
0.002	1.043	5.55×10^{-5}	0.139	0.0139	0.603	3.21×10^{-5}	0.0803	0.00803

* Initial concentration of substrate

Sample calculation for [PNPP]* (initial concentration) for test tube #1:

Variables:

- $c_1 = 0.0005$ M
- $v_1 = 0.00005$ L
- $v_2 = 0.0005$ L
- $c_2 = ?$

$c_1v_1 = c_2v_2$

$c_2 = \frac{c_1v_1}{v_2}$

$c_2 = \frac{(0.0005\ \text{M})(0.00005\ \text{L})}{(0.0005\ \text{L})}$

$c_2 = 0.00005$ M

The initial concentration of PNPP was 0.00005 M in test tube 1.

Sample calculations of A_{405} (corrected) for test tube #1 of the control experiment:

Variables:

- A_{405} (zero) = 0.012
- A_{405} (incubation) = 0.188
- A_{405} corrected = ?

A_{405} corrected = A_{405} (incubation) – A_{405} (zero)

A_{405} corrected = 0.188 – 0.012

A_{405} corrected = 0.176

The corrected absorbance for the test tube #1 of the control experiment is 0.176.

Sample calculations for [PNP] (M) for test tube #1 of the control experiment:

Variables:

- $A_{405} = 0.176$
- $l = 1$ cm
- $\varepsilon_{405} = 18800\ M^{-1}cm^{-1}$
- $c = ?$

$A_{405} = (\varepsilon_{405})(c)(l)$

$c = \frac{A_{405}}{(\varepsilon_{405})(l)}$

$c = \frac{0.176}{(18800\ \text{M}^{-1}\text{cm}^{-1})(1\ \text{cm})}$

$c \approx 9.36 \times 10^{-6}$ M

The concentration of PNP in test tube #1 is 9.36×10^{-6} M (for the control experiment).

Sample calculations for PNP (μmol) for test tube #1 of the control experiment:

Variables:

- $v = 0.0025$ L
- $c = 9.36 \times 10^{-6}$ M
- $n = ?$

$n = cv$

$n = (9.36 \times 10^{-6}\ \text{M})(0.0025\ \text{L})$

$n = 2.34 \times 10^{-8}$ mol

$n = 0.0234$ μmol

There is 0.0234 μmol of PNP present in test tube #1 (for the control experiment).

Sample calculations for the reaction rate (v) for test tube #1 of the control experiment:

Variables:

- $n = 0.0234$ μmol
- $t = 10$ minutes
- $v = ?$

$v = \frac{n}{t}$

$v = \frac{0.0234\ \mu\text{mol}}{10\ \text{minutes}}$

$v = 0.00234$ μmol/minute

The reaction rate (v) for test tube #1 is 0.00234 μmol/min (for the control experiment).

R4. From Tables 5/6 complete Table 7 for the analysis of results by the Hanes method, a linear form of the kinetic equation. Show all of your calculations for one substrate concentration for the control.

Table 3. Data for the use of the Hanes equation. This table shows the substrate concentrations, reaction rates and substrate concentration to reaction rate ratio for both the control and inhibitor experiments. The substrate concentrations, [S], and reaction rates, V, were taken from **Table 4**. The control experiment was performed by using two sets of 7 test tubes; the incubation test tubes and the zero test tubes. In both sets of tubes, 50 µL of 1.0 M sodium acetate buffer (pH 5.7) and the corresponding amounts of water and PNPP were added; the concentrations of the PNPP in each tube was calculated and is shown in the following table. The incubation test tubes were pre-incubated at 37˚C for 2 minutes while the zero test tubes were left at room temperature throughout the experiment. 2.0 mL of 0.5 M KOH was added to the zero test tubes and after mixing, 50 µL of 0.5 mg/mL acid phosphatase was added. 50 µL of 0.5 mg/mL acid phosphatase was added to each incubation tube at different times. After 10 minutes of incubation for all the tubes, 2.0 mL of 0.5 M KOH was added to stop the reaction. The absorbance values at 405 nm for both sets of tubes was recorded. The same procedure was repeated for the inhibition experiment except, 50 µL of a 9.0 mM unknown inhibitor (inhibitor #10) was added after the PNPP, the volume of water added was decreased by 50 µL and the zero test tubes were not done again. The absorbance values for both the control and inhibition incubation tubes were corrected using the zero tubes from the control experiment; these absorbance values were then used to calculate the product concentration, product amount and reaction rate. The substrate concentration was calculated using stock concentrations and the total assay volume. From the substrate concentration and reaction rate, the ratio of substrate concentration to reaction rate was calculated.

[S]* (M)	Control		Inhibitor	
	V (µmol/min)	[S]/V (min/µl)	V (µmol/min)	[S]/V (min/µl)
0.00005	0.00234	0.0214	0.0004	0.125
0.0001	0.00478	0.0209	0.000918	0.109
0.0002	0.00608	0.0329	0.00204	0.0980
0.0005	0.00838	0.0600	0.00393	0.127
0.00075	0.0105	0.0714	0.00478	0.157
0.001	0.0111	0.0901	0.00625	0.160
0.002	0.0139	0.144	0.00803	0.249

* Initial concentration of PNPP

Sample calculation for [S]/V (min/µl) for test tube #1 of the control experiment:

Variables:

- [S] = 0.00005 mol/L
- V = 0.00234 µmol/min
  = 2.34 x 10^{-9} mol/min
- $[S]/_V$ = ?

$$[S]/_V = [S]*\frac{1}{V}$$

$$[S]/_V = (0.00005\ \text{mol/L})*\frac{1}{(2.34 \times 10^{-9}\ \text{mol/min})}$$

$$[S]/_V \approx 21400\ \text{min/L}$$

$$[S]/_V = 0.0214\ \text{min/µl}$$

The $[S]/_V$ for test tube #1 of the control experiment is 0.0214 min/µl.

R5. From Table 7, construct the Hanes plot for the enzyme in the presence and absence of inhibitor. (Prepare one graphic with the two Hanes plots. Draw for each plot the best-fit straight line, also called the linear-regression trend line).

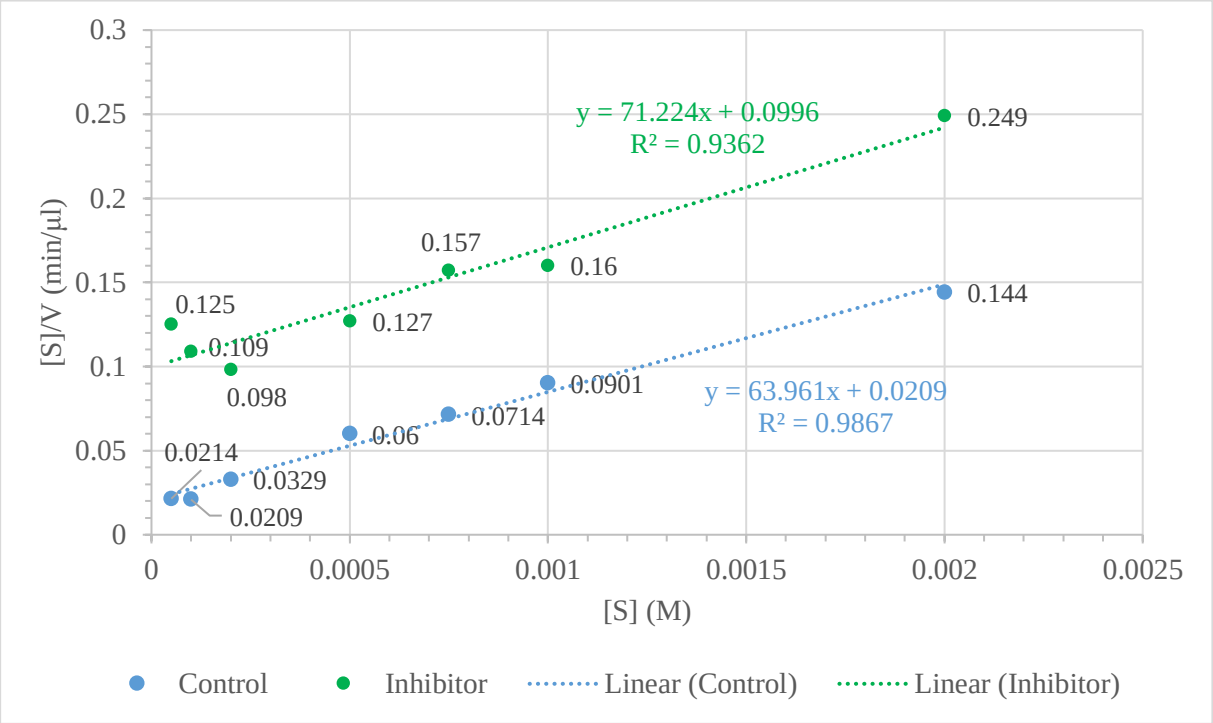

Figure 2. A Hanes plot for the enzyme acid phosphatase (0.5 mg/mL) in the presence and absence of an unknown inhibitor. The substrate concentration and substrate concentration to reaction rate ratio from **Table 3** for both the control and inhibition experiments were plotted in order to determine the K_M and V_{max}. The control experiment was performed by using two sets of 7 test tubes; the incubation test tubes and the zero test tubes. In both sets of tubes, 50 µL of 1.0 M sodium acetate buffer (pH 5.7) and the corresponding amounts of water and PNPP were added; the concentrations of the PNPP in each tube was calculated and is shown in the following table. The incubation test tubes were pre-incubated at 37˚C for 2 minutes while the zero test tubes were left at room temperature throughout the experiment. 2.0 mL of 0.5 M KOH was added to the zero test tubes and after mixing, 50 µL of 0.5 mg/mL acid phosphatase was added. 50 µL of 0.5 mg/mL acid phosphatase was added to each incubation tube at different times. After 10 minutes of incubation for all the tubes, 2.0 mL of 0.5 M KOH was added to stop the reaction. The absorbance values at 405 nm for both sets of tubes was recorded. The same procedure was repeated for the inhibition experiment except, 50 µL of a 9.0 mM unknown inhibitor (inhibitor #10) was added after the PNPP, the volume of water added was decreased by 50 µL and the zero test tubes were not done again. The absorbance values for both the control and inhibition incubation tubes were corrected using the zero tubes from the control experiment; these absorbance values were then used to calculate the product concentration, product amount and reaction rate. The substrate concentration was calculated using stock concentrations and the total assay volume. From the substrate concentration and reaction rate, the ratio of substrate concentration to reaction rate was calculated. The line of best fit for the presence and absence of the inhibitor are y = 71.224x + 0.0996 and y = 63.961x + 0.0209, respectively. In a Hanes plot, the slope corresponds to $1/V_{max}$, the x-intercept corresponds to $-K_M$ and the y-intercept corresponds to K_M/V_{max}.

R6. From the linear equations of the two best-fit lines found in your Hanes plot, determine the K_M and V_{max} parameters corresponding to each condition. Show your full calculations for the control plot. Summarize your results in Table 8. Indicate the type of inhibitor that you have used. Justify your answer. Remember to use a 25% cut-off for your inhibitor identification (please see Appendix C4 for information on how to calculate the percent difference).

Table 4. Kinetic parameters obtained from experiments 2 and 3. This table shows the calculated values for V_{max} and K_M for both the control and inhibitor experiments. These kinetic parameters were calculated using the line of best fit for both the control and inhibitor plots in the Hanes plot above (**Figure 2**). The line of best fit for the control plot was y = 63.961x + 0.0209 and the line of best fit for the inhibitor plot was y = 71.224x + 0.0996. In a Hanes plot, the slope corresponds to $1/V_{max}$, the x-intercept corresponds to $-K_M$ and the y-intercept corresponds to K_M/V_{max}.

Method	Control		Inhibitor	
	V_{max} (µmol/min or U)	K_M (M)	V_{max} (µmol/min or U)	K_M (M)
Hanes	0.0156	0.000326	0.0140	0.00139

Sample calculations for V_{max} of the control plot:

Variables:
- y = 63.961x + 0.0209
- slope = $^1/_{V_{max}}$
- V_{max} = ?

$^1/_{V_{max}} = 63.961$

$V_{max} = \frac{1}{63.961}$

$V_{max} \approx 0.0156 \frac{mol*µl}{min*L} * \frac{1\ L}{1000000\ µl} * \frac{1000000\ µmol}{1\ mol}$

V_{max} = 0.0156 µmol/min or U

The V_{max} for the control plot is 0.0156 U.

Sample calculations for K_M of the control plot:

Variables:
- y = 63.961x + 0.0209
- y-intercept = $^{K_M}/_{V_{max}}$
- V_{max} = 0.0156 µmol/min
- K_M = ?

$^{K_M}/_{V_{max}} = 0.0209$

$K_M = (0.0209\ min/µl)(V_{max})$

$K_M = (0.0209\ min/µl)(0.0156\ µmol/min)$

$K_M \approx 0.000326\ µmol/\ µl$

K_M = 0.000326 M

The K_M for the control plot is 0.000326 M.

Calculation of the percentage difference of the slope between the control and inhibitor plots:

Variables:
- Control slope = 63.961
- Inhibitor slope = 71.224
- Percentage difference = ?

$\text{Percentage difference} = \frac{\text{Inhibitor slope - Control slope}}{\text{Control slope}} * 100$

$\text{Percentage difference} = \frac{71.224 - 63.961}{63.961} * 100$

Percentage difference ≈ 11.4%

The percentage difference between the slopes for the control and inhibitor plots is 11.4%.

Calculation of the percentage difference of the y-intercept between the control and inhibitor plots:

Variables:

- Control y-int = 0.0209
- Inhibitor y-int = 0.0996
- Percentage difference = ?

$$\text{Percentage difference} = \frac{\text{(Inhibitor y-int) -(Control y-int)}}{\text{(Control y-int)}} * 100$$

$$\text{Percentage difference} = \frac{0.0996 - 0.0209}{0.0209} * 100$$

$$\text{Percentage difference} \approx 377\ \%$$

The percentage difference between the y-intercepts for the control and inhibitor plots is 377%

Since the percentage difference between the slopes for the control and inhibitor plots is 11.4% (less than the 25% cut-off), it can be assumed that the slopes are the same. Meanwhile, since the percentage difference between the y-intercepts for the control and inhibitor plots is 377% (more than the 25% cut-off), it can be assumed that there was an increase in the y-intercept. In a Hanes plot, if the slope stays the same while the y-intercept increases, one can conclude that the inhibitor that was used was a competitive inhibitor. Hence, inhibitor #10 is most likely a competitive inhibitor.

R7. From the value of V_{max} and K_M for the control and the inhibitor (according to Table 8) and from the definitions of α and α' in Table 1, estimate the constants KI and/or KI' of your inhibitor and summarize your results in Table 9. Show an example of your calculations.

Table 5. Type of Inhibitor. This table shows the inhibitor number, calculated inhibitor concentration, V_{max}, K_M, K_I'and type of inhibitor used in the control and inhibitor experiments. 50 µL of a 9.0 mM unknown inhibitor (inhibitor #10) was used in the inhibitor experiment; this data was used to calculate the inhibitor concentration. From the slopes and y-intercepts of the inhibitor and control plots (**Figure 2**), the percentage differences were calculated to be 11.4 % and 377% for the slopes and y-intercepts respectively; since the slope stayed the same and the y-intercept increased the inhibitor was determined to be competitive. The K_I was calculated using the known effects of a competitive inhibitor on kinetic parameters. It is to be noted that since the inhibitor is competitive, it does not have a K_I' as a competitive inhibitor does not bind to the enzyme substrate complex.

Inhibitor number	[I] (M)	V_{max} (U)	K_M (M)	K_I (M)	K_I' (M)	Type of Inhibitor
-----	0	0.0156	0.000326	-----	-----	-----
10	0.0009	0.0140	0.00139	0.000276	-----	Competitive

Sample calculation for concentration of inhibitor [I] (M):

Variables:

- c_1 = 0.009 M
- v_1 = 0.00005 L
- v_2 = 0.0005 L
- c_2 = ?

$$c_1v_1 = c_2v_2$$

$$c_2 = \frac{c_1v_1}{v_2}$$

$$c_2 = \frac{(0.009\ \text{M})(0.00005\ \text{L})}{(0.0005\ \text{L})}$$

$$c_2 = 0.0009\ \text{M}$$

The concentration of inhibitor is 0.0009 M.

Sample calculation for K_I (M):

Calculation for α	Calculation for K_I
Variables • K_M (control) = 0.000326 M • K_M (inhibitor) = 0.00139 M • α = ? K_M (inhibitor) = α*K_M (control) $\alpha = \frac{K_M\ (\text{inhibitor})}{K_M\ (\text{control})}$ $\alpha = \frac{0.00139\ \text{M}}{0.000326\ \text{M}}$ $\alpha \approx 4.26$ The α for this inhibitor is 4.26.	Variables • [I] = 0.0009 M • α = 4.26 • K_I = ? $\alpha = 1 + \frac{[I]}{K_I}$ $K_I = \frac{[I]}{(\alpha - 1)}$ $K_I = \frac{0.0009\ \text{M}}{(4.26 - 1)}$ $K_I \approx 0.000276$ M The dissociation constant for this inhibitor is 0.000276 M.

R8. From the estimated value of V_{max} for the control (according to Table 9) and the protein concentration of the provided enzyme solution, calculate the specific activity of your phosphatase sample in U/mg. (Hint: the estimated V_{max} is the activity corresponding to the amount of enzyme that you used in the assay for experiment 2).

Calculation for specific activity of phosphatase sample:

Calculation for amount of phosphatase in mg	Calculation for specific activity
Variables: • c_{enzyme} = 0.5 mg/mL • v_{enzyme} = 0.05 mL • m = ? $c_{enzyme} = \frac{m}{v_{enzyme}}$ $m = c_{enzyme}v_{enzyme}$ m = (0.5mg/mL)(0.05 mL) m = 0.025 mg There was 0.025 mg of phosphatase in the assay.	Variables: • V_{max} (control) = 0.0156 U • m = 0.025 mg • Specific activity = ? Specific activity = $\frac{V_{max}\ (\text{control})}{m}$ Specific activity = $\frac{0.0156\ \text{U}}{0.025\ \text{mg}}$ Specific activity = 0.624 U/mg The specific activity of the phosphatase sample is 0.624 U/mg.

References:

- BCH2333 laboratory manual, Experiment 3: Enzymes; pages 45 – 65.