# LS2102: Biology Laboratory

# LAB REPORT 2

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## Contents

# 1 Aim

To determine the unknown concentration of p-NPP(para-Nitrophenylphosphate) and determine the enzymatic properties of Alkaline Phosphatase enzyme.

# 2 Procedure

## 2.1 Preparation of Standard Curve

1. Solutions of different concentrations (containing buffer, pNP and NaOH), having different amount of pNP were prepared.
2. Absorbance of each solution was taken at 405nm.
3. Graph of Absorbance vs. Concentration of pNP was plotted and fit with a $Ax + B$.
4. The value of slope and intercept from the fit curve was noted.

## 2.2 Estimation of Kinetic Parameters

1. The buffer, enzyme and substrate solutions were prepared according to the table provided.
2. The solutions were kept for incubation at 300K for 15 minutes.
3. Absorbance at 405nm was taken for each solution.
4. The reaction velocities were calculated by obtaining product at the specified absorbance from the standard linear fitting $Ax + b$ done in the previous step.
5. The Michaelis-Menton Curve was plotted with fit $\frac{Ax}{B + x}$
6. The Lineweaver-Burke Curve were plotted with again a standard linear fit
7. From the curve, various kinetic parameters were calculated.

# 3 Data Tables and Graphs

## 3.1 Data Table to Prepare Standard Absorbance vs pNP Curve

| Tube Number | Volume of Buffer (µL) | Volume of pNP (µL) | Volume of NaOH (mL) | Concentration of pNP (µM) | Absorbance At 405 nm | Average Absorbance |
|---|---|---|---|---|---|---|
| Blank | 1000 | 0 | 1 | 0 | 0.0 | 0.0 |
| Blank | | | | | 0.0 | |
| 1 | 985 | 15 | 1 | 3 | 0.024 | 0.021 |
| 2 | | | | | 0.018 | |
| 3 | 975 | 25 | 1 | 5 | 0.047 | 0.044 |
| 4 | | | | | 0.041 | |
| 5 | 950 | 50 | 1 | 10 | 0.075 | 0.0805 |
| 6 | | | | | 0.086 | |
| 7 | 925 | 75 | 1 | 15 | 0.131 | 0.147 |
| 8 | | | | | 0.163 | |
| 9 | 900 | 100 | 1 | 20 | 0.224 | 0.225 |
| 10 | | | | | 0.226 | |
| 11 | 875 | 125 | 1 | 25 | 0.272 | 0.273 |
| 12 | | | | | 0.274 | |
| 13 | 850 | 150 | 1 | 30 | 0.317 | 0.32 |
| 14 | | | | | 0.323 | |
| 15 | 800 | 200 | 1 | 40 | 0.41 | 0.417 |
| 16 | | | | | 0.424 | |

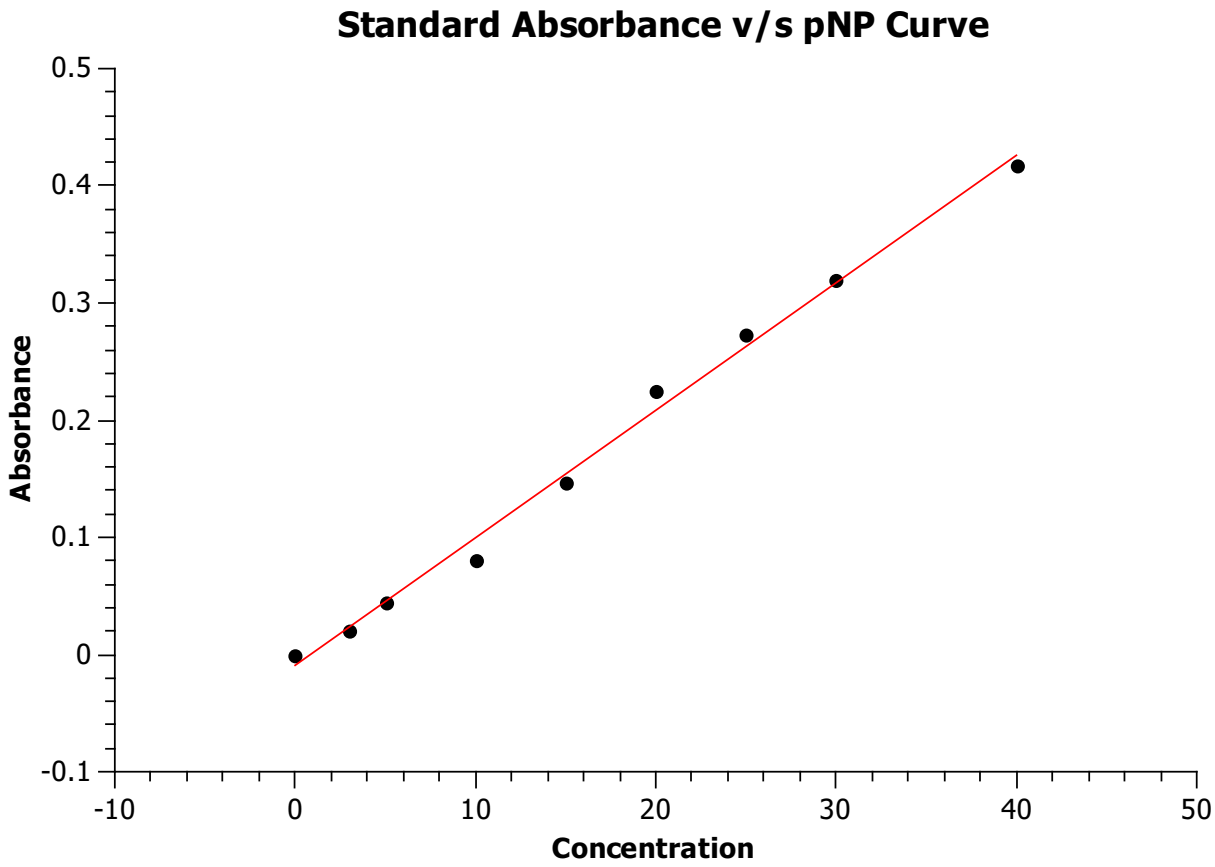
## 3.2 Table 2(Kinetic Parameters)

| Tube No. | Volume of Buffer (µL) | Volume of pNP (µL) | Volume of ALP Enzyme (µL) | Volume of NaOH (mL) | Concentration of pNP (mM) | Absorbance At 405nm | Correction Factor | Corrected Absorbance |
|---|---|---|---|---|---|---|---|---|
| Blank | 1000 | 0 | 25 | 1 | 0 | 0 | 0 | 0 |
| Blank | | | | | | 0 | | 0 |
| 1 | 965 | 10 | 25 | 1 | 0.1 | 0.021 | 0.001 | 0.02 |
| 2 | | | | | | 0.012 | | 0.011 |
| 3 | 950 | 25 | 25 | 1 | 0.25 | 0.038 | 0.004 | 0.034 |
| 4 | | | | | | 0.037 | | 0.033 |
| 5 | 925 | 50 | 25 | 1 | 0.5 | 0.057 | 0.010 | 0.047 |
| 6 | | | | | | 0.061 | | 0.051 |
| 7 | 900 | 75 | 25 | 1 | 0.75 | 0.056 | 0.011 | 0.045 |
| 8 | | | | | | 0.060 | | 0.049 |
| 9 | 875 | 100 | 25 | 1 | 1 | 0.076 | 0.014 | 0.062 |
| 10 | | | | | | 0.080 | | 0.066 |
| 11 | 775 | 200 | 25 | 1 | 2 | 0.117 | 0.029 | 0.088 |
| 12 | | | | | | 0.124 | | 0.095 |
| 13 | 725 | 250 | 25 | 1 | 2.5 | 0.142 | 0.040 | 0.102 |
| 14 | | | | | | 0.138 | | 0.098 |
| 15 | 475 | 500 | 25 | 1 | 5 | 0.209 | 0.074 | 0.135 |
| 16 | | | | | | 0.215 | | 0.141 |
| 17 | 225 | 750 | 25 | 1 | 7.5 | 0.278 | 0.112 | 0.2668 |
| 18 | | | | | | 0.273 | | 0.2618 |
| 19 | 75 | 900 | 25 | 1 | 9 | 0.326 | 0.133 | 0.193 |
| 20 | | | | | | 0.334 | | 0.201 |

## 3.3 Table 3(Kinetic Parameters)

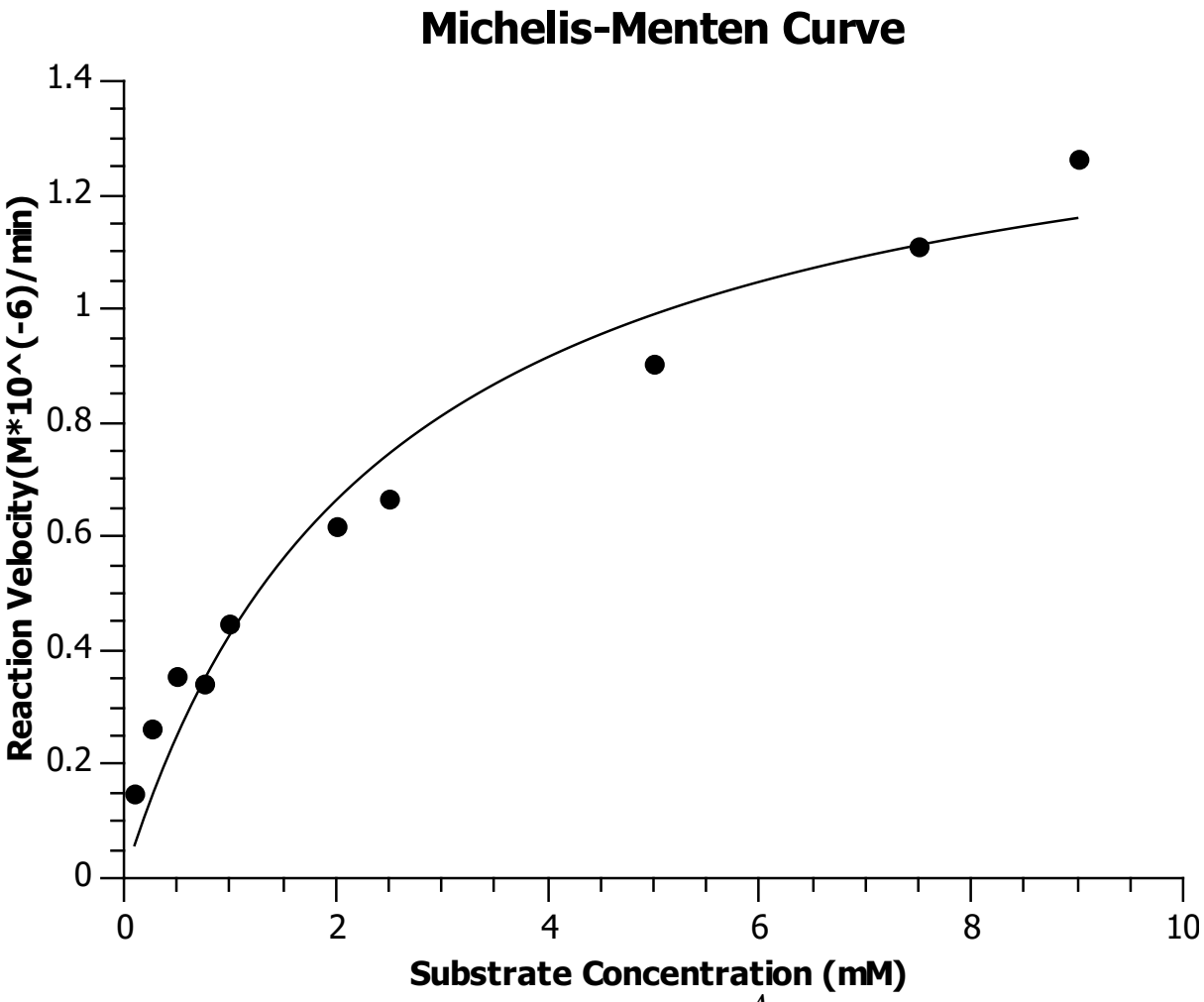
| Tube No. | Concentration of pNP (mM) [S] | 1/[S] ($mM^{-1}$) | Corrected Absorbance | Product from Standard Curve (µM) | V=P/t (µM/min) | Average (µM/min) | 1/V $(µM/min)^{-1}$ | Average $(µM/min)^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.1 | 10 | 0.02 | 2.684 | 0.178933 | 0.151367 | 5.588674 | 6.833109 |
| 2 | | | 0.011 | 1.857 | 0.1238 | | 8.077544 | |
| 3 | 0.25 | 4 | 0.034 | 3.971 | 0.264733 | 0.261667 | 3.777386 | 3.822181 |
| 4 | | | 0.033 | 3.879 | 0.2586 | | 3.866976 | |
| 5 | 0.5 | 2 | 0.047 | 5.165 | 0.344333 | 0.3566 | 2.904163 | 2.807585 |
| 6 | | | 0.051 | 5.533 | 0.368867 | | 2.711007 | |
| 7 | 0.75 | 1.33 | 0.045 | 4.982 | 0.332133 | 0.344367 | 3.010839 | 2.907551 |
| 8 | | | 0.049 | 5.349 | 0.3566 | | 2.804262 | |
| 9 | 1 | 1 | 0.062 | 6.544 | 0.436267 | 0.448533 | 2.292176 | 2.231157 |
| 10 | | | 0.066 | 6.912 | 0.4608 | | 2.170139 | |
| 11 | 2 | 0.5 | 0.088 | 8.934 | 0.5956 | 0.617033 | 1.678979 | 1.622616 |
| 12 | | | 0.095 | 9.577 | 0.638467 | | 1.566252 | |
| 13 | 2.5 | 0.4 | 0.102 | 10.22 | 0.681333 | 0.6691 | 1.46771 | 1.495045 |
| 14 | | | 0.098 | 9.853 | 0.656867 | | 1.522379 | |
| 15 | 5 | 0.2 | 0.135 | 13.253 | 0.883533 | 0.901933 | 1.131819 | 1.109191 |
| 16 | | | 0.141 | 13.805 | 0.920333 | | 1.086563 | |
| 17 | 7.5 | 0.13 | 0.2668 | 16.367 | 1.091133 | 1.109133 | 0.06109 | 0.060115 |
| 18 | | | 0.2618 | 16.907 | 1.127133 | | 0.05914 | |
| 19 | 9 | 0.11 | 0.193 | 18.584 | 1.238933 | 1.263433 | 0.807146 | 0.791792 |
| 20 | | | 0.201 | 19.319 | 1.287933 | | 0.776438 | |

## 3.4 Graphs



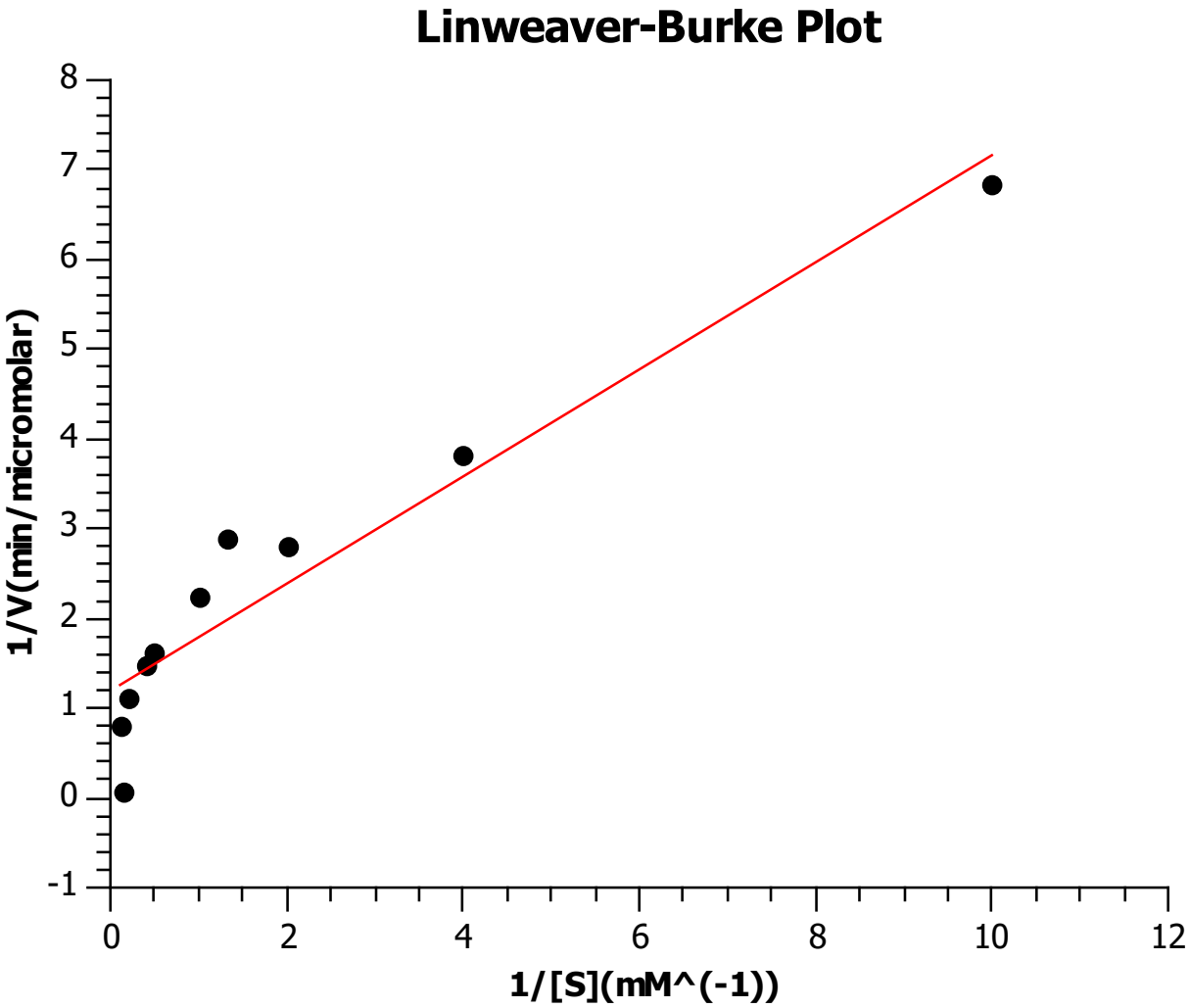

From fitting the line of the curve as per the equation Ax+B we get the values of A,B as:-

B (y-intercept) = -9.2011952191235e-03
A (slope) = 1.0880478087649e-02




From fitting the line of the curve as per the equation $\frac{Ax}{B+x}$ we get the values of A,B as:-

$$B = 2.45$$
$$A = 1.47$$




From fitting the line of the curve as per the equation Ax+B we get the values of A,B as:-

$$\text{B (y-intercept)} = 1.19$$
$$\text{A (slope)} = 0.597$$

# 4 Calculations and Results

From the graph we found out that

$$V_{max} = \frac{1}{B} = 0.840\mu\text{M/min.}$$

$$K_{max} = A * V_{max} = 0.502 \text{ mM}$$

$$[E]_T = \frac{\text{Volume of Enzyme taken}}{\text{Total Volume}} \times \text{Concentration of Stock} = 0.504\mu\text{M}$$

$$k_{cat} = 1.67 \text{ min}^{-1}$$

$$\text{Specific Activity of the enzyme} = \frac{0.840 * 1}{0.00025} = 3,360\mu \ mol \ min^{-1} \ mg^{-1}$$

# 5 Results

Thus with the help of Michelis-Menten Curve and Linweaver-Burke Plot we could figure out the various kinetic parameters of the enzyme alkaline phosphate and the results are given below

$$V_{max} = 0.840 \mu \text{M/min.}$$
$$K_{max} = 0.502 \text{ mM}$$
$$[E]_T = 0.504 \mu \text{M}$$
$$k_{cat} = 1.67 \text{ min}^{-1}$$
$$\text{Specific Activity of the enzyme} = 3{,}360 \ \mu \ mol \ min^{-1} \ mg^{-1}$$