Practicum 1 – group A

Enzymatic activity of nitrophenyl-phosphatase

Quantitative cell analysis & tissue engineering

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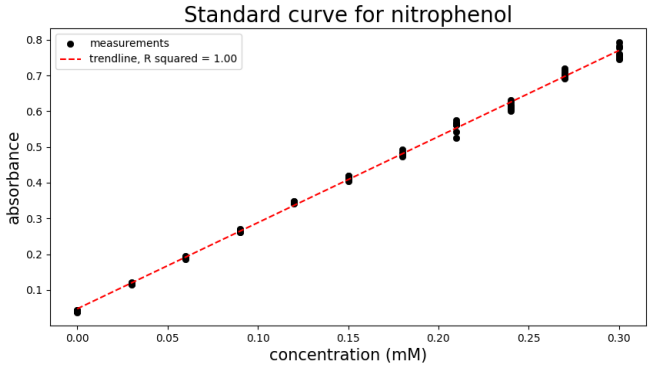
I Standard curve for nitrophenol

1. Make a plot of the absorbance versus concentration and calculate the trendline. ($E = bx + a$ with x = nitrophenol concetration) And calculate the concentration of P01.

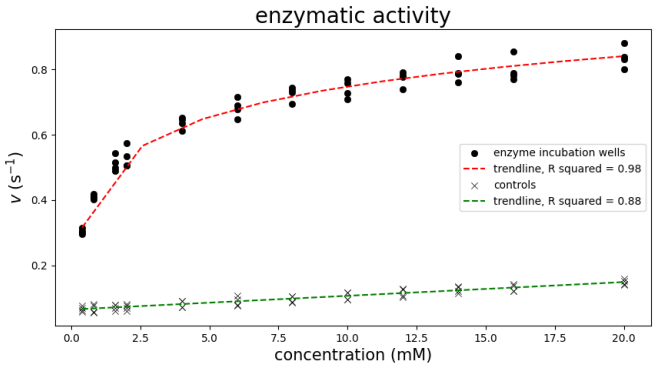


The estimated concentration of PO1 is 0.01743 mM. (We made the mean of all of the results to estimate it's concentration.)

II Enzymatic activity as a function of substrate concentration

1. Plot the enzymatic activity (as absorbance after 30 minutes (Y-axis)) against the substrate concentration (X-axis) and display R^2.

Note: in this expirement the absorbance was measured after 20 minutes.

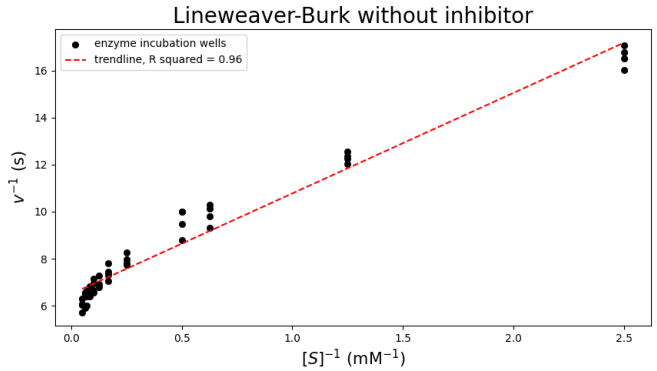


v (enzymatic activity) is calculated as $\frac{\text{absorbance}}{1200\text{s}}$, 1200 s = 20 min in oven, with absorbance a dimensionless quantity, hence v got untis $\frac{1}{\text{s}}$.

2. Lineweaver-Burk plot: $\frac{1}{v}$ (Y-axis) against $\frac{1}{[S]}$ (X-axis) and display R^2. Formula of Michaëlis Menten:

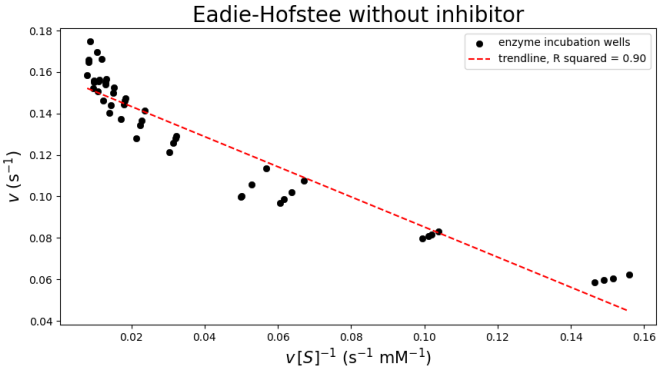
$$v = V_{\max} \frac{[S]}{K_m + [S]}$$

$$\frac{1}{v} = \frac{1}{V_{\max}} \left(\frac{K_m}{[S]} + 1 \right)$$



3. Eadie-Hofstee plot: $\frac{v}{[S]}$ (X-axis) against v (Y-axis) and display R^2.

$$v = -K_m \frac{v}{[S]} + V_{\max}$$

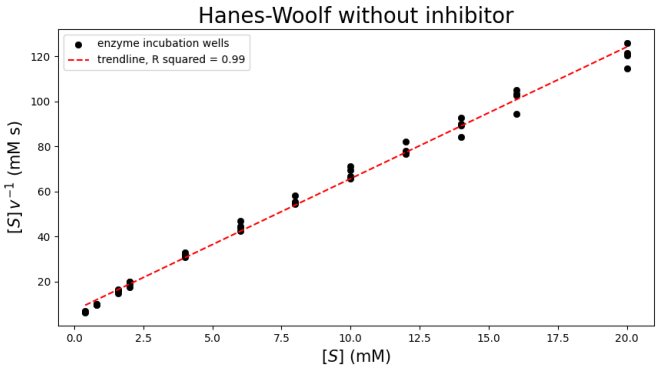


4. Hanes-Woolf plot: $\frac{[S]}{v}$ (Y-axis) against $[S]$ (X-axis) and display R^2.

$$\frac{[S]}{v} = \frac{[S]}{V_{\max}} + \frac{K_m}{V_{\max}}$$

5. Calculate K_m-values (molarity of substrate in incubation mixture) and $V_{\max}$ (formed nitrophenol (mg) per 30 minutes) for every plot. Do you observe differences? Why?

The K_m and $V_{\max}$ value can be derived from the trendline ($y = bx + a$):

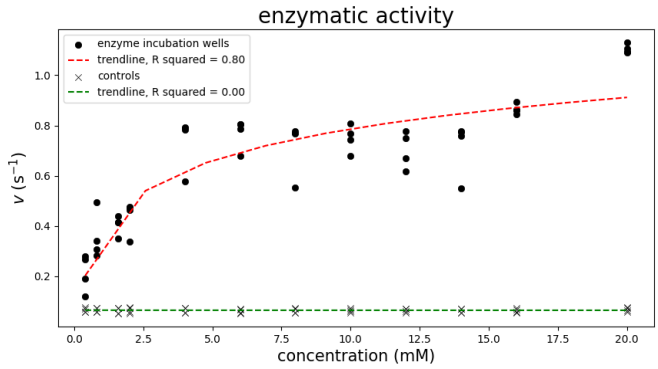


- Lineweaver-Burk plot: $V_{\max} = \frac{1}{a} = 0.15\ \frac{1}{\text{s}}$, $K_m = \frac{b}{a} = 0.66$ mM
- Eadie-Hofstee plot: $V_{\max} = a = 0.16\ \frac{1}{\text{s}}$, $K_m = -b = 0.73$ mM
- Hanes-Woolf plot: $V_{\max} = \frac{1}{b} = 0.17\ \frac{1}{\text{s}}$, $K_m = \frac{a}{b} = 1.22$ mM

III Enzymatic activity in the presence of an inhibitor

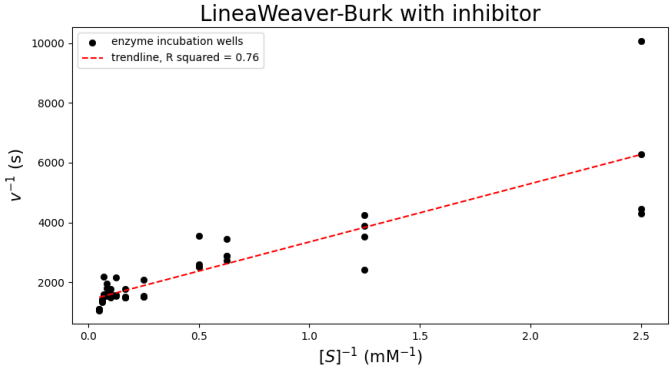
1. Plot the enzymatic activity (as absorbance after 30 minutes (Y-axis)) against the substrate concentration (X-axis). Add a trendline and R^2

Note: in this expirement the absorbance was measured after 20 minutes.

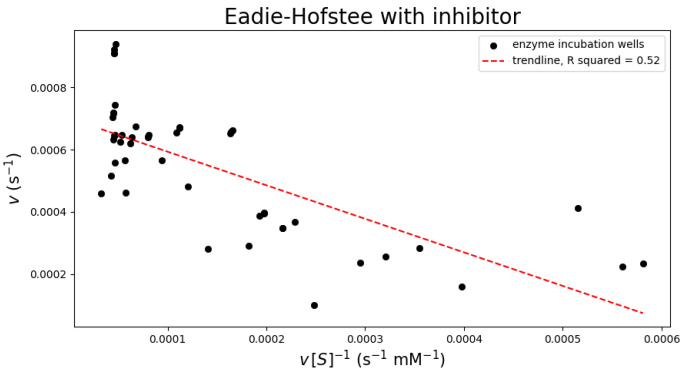


v (enzymatic activity) is calculated as $\frac{\text{absorbance}}{1200\text{s}}$, 1200 s = 20 min in oven, with absorbance a dimensionless quantity, hence v got untis $\frac{1}{\text{s}}$.

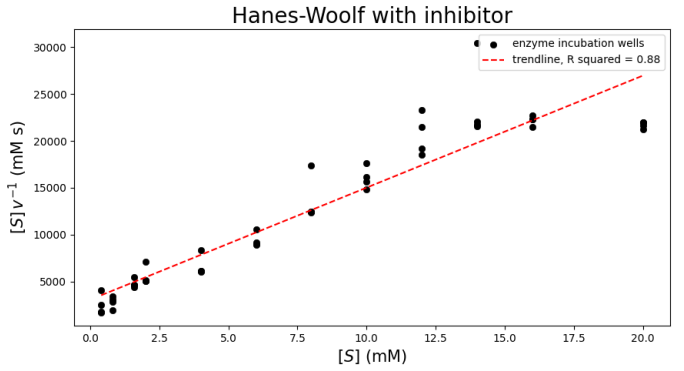
2. Lineweaver-Burk plot: $\frac{1}{v}$ (Y-axis) against $\frac{1}{[S]}$ (X-axis).



3. Eadie-Hofstee plot: $\frac{v}{[S]}$ (X-axis) against v (Y-axis).



4. Hanes-Woolf plot: $\frac{[S]}{v}$ (Y-axis) against $[S]$ (X-axis).

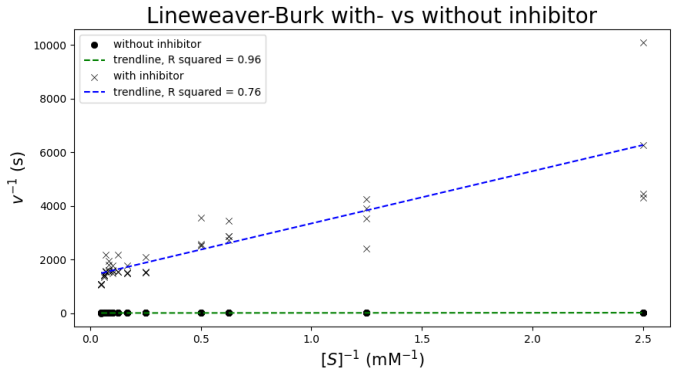


5. Calculate K_m-value (molarity of substrate in incubation mixture) and $V_{\max}$ (formed nitrophenol (mg) per 30 minutes) for every plot.

The K_m and $V_{\max}$ values can be derived from the trendline ($y = bx + a$):

- Lineweaver-Burk plot: $V_{\max} = \frac{1}{a} = 0.000716\ \frac{1}{\text{s}}$, $K_m = \frac{b}{a} = 1.40$ mM
- Eadie-Hofstee plot: $V_{\max} = a = 0.000701\ \frac{1}{\text{s}}$, $K_m = -b = 1.08$ mM
- Hanes-Woolf plot: $V_{\max} = \frac{1}{b} = 0.000836\ \frac{1}{\text{s}}$, $K_m = \frac{a}{b} = 2.55$ mM

6. Is the inhibitor competitive or non-competitive? Motivate your answer.



It is a non-competitive inhibitor: different $V_{\max}$ and not parallel. (Remark: $\frac{\Delta V_{\max}}{V_{\max}} \gg \frac{\Delta K_m}{K_m} \Rightarrow$ assume "constant" K_m values)