

## Practicum 1 – group A

### Enzymatic activity of nitrophenyl-phosphatase

Quantitative cell analysis & tissue engineering

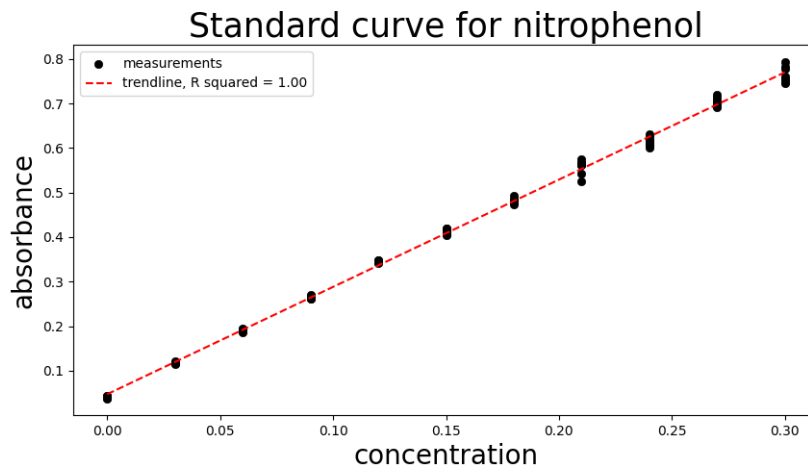


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Date: October 5, 2022

Faculty of  
engineering and  
architecture

#### I Standard curve for nitrophenol

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

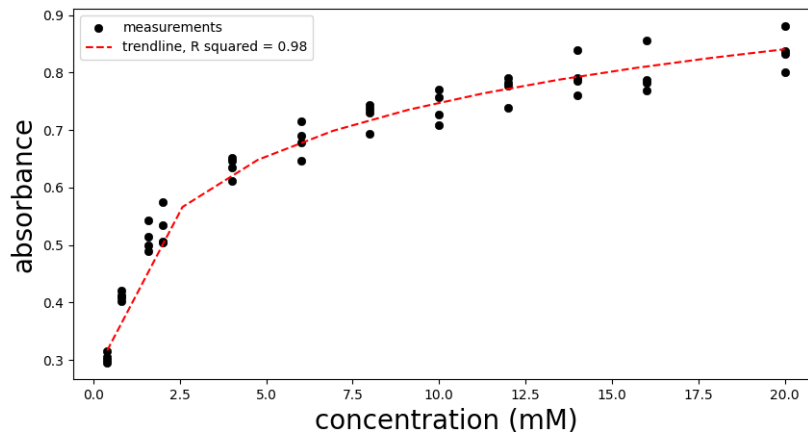


The estimated concentration of PO1 is 0.01743 mM. (We made the mean of all of the results to estimate it's concentration.)

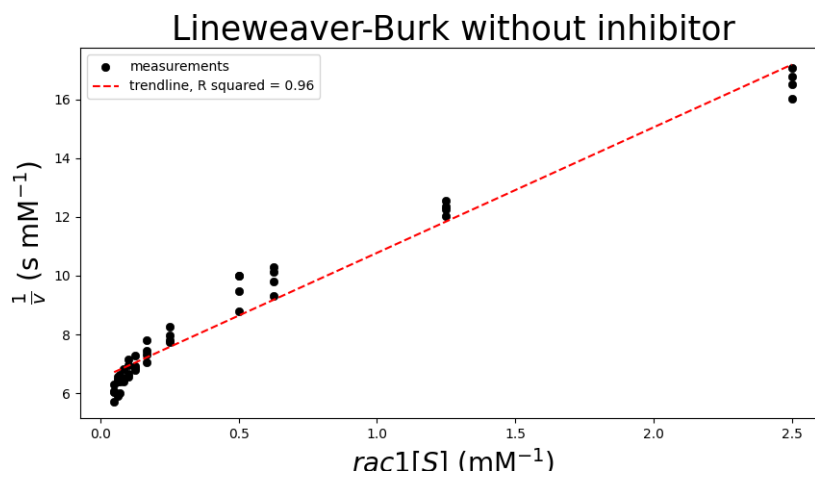
#### II Enzymatic activity as a function of substrate concentration

1. Plot the enzymatic activity (as absorbance after 30 minutes (Y-axis)) against the substrate concentration (X-axis) and display  $R^2$ .
2. Lineweaver-Burk plot:  $\frac{1}{v}$  (Y-axis) against  $\frac{1}{[S]}$  (X-axis) and display  $R^2$ . Formula of Michaëlis Menten:

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

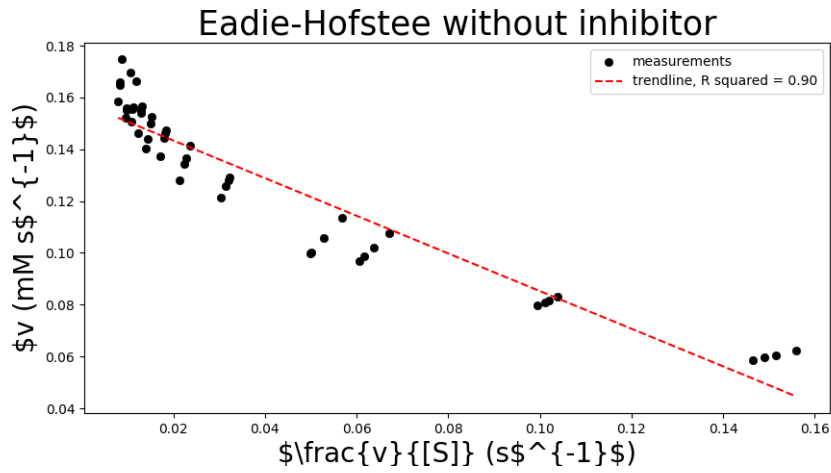


$$\frac{1}{v} = \frac{1}{V_{\max}} \left( \frac{K_m}{[S]} + 1 \right)$$



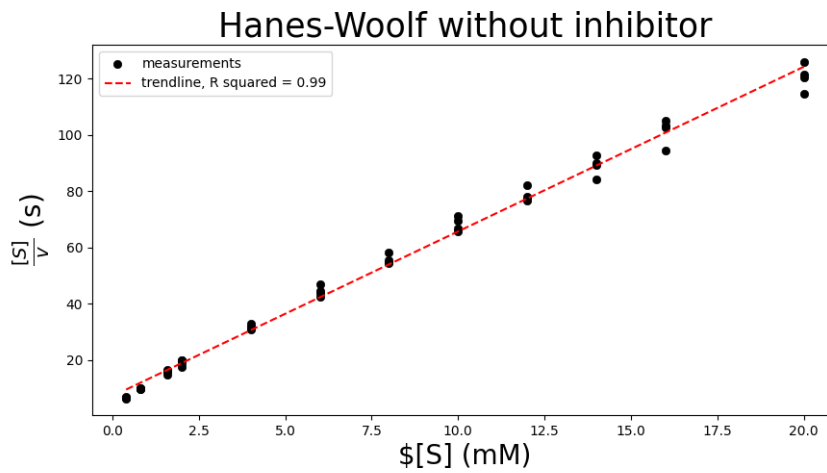
3. Eadie-Hofstee plot:  $\frac{v}{[S]}$  (X-axis) against  $v$  (Y-axis) and display  $R^2$ .

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



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

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

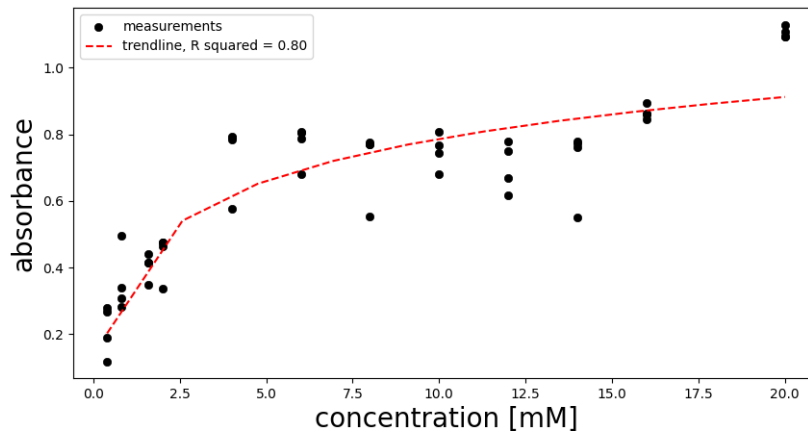


5. Calculate  $K_m$ -value (molarity of substrate in incubation mixture) and  $V_{\max}$  (formed nitrophenol (mg) per 30 minutes) for every plot. Do you observe differences? Why?

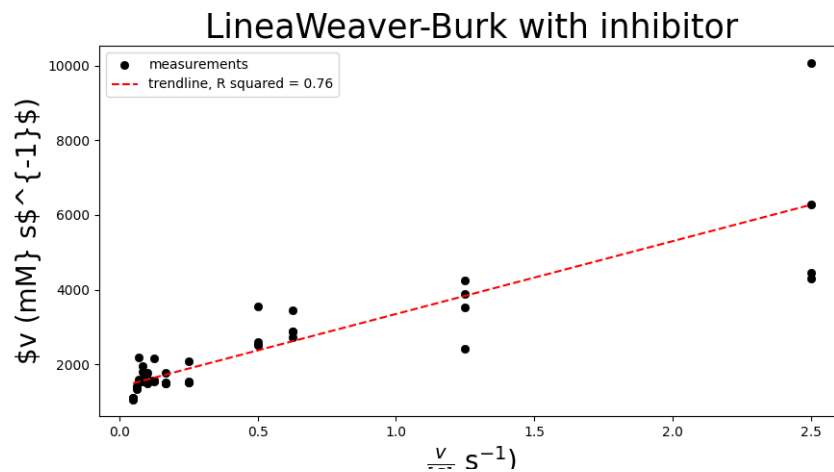
Answer

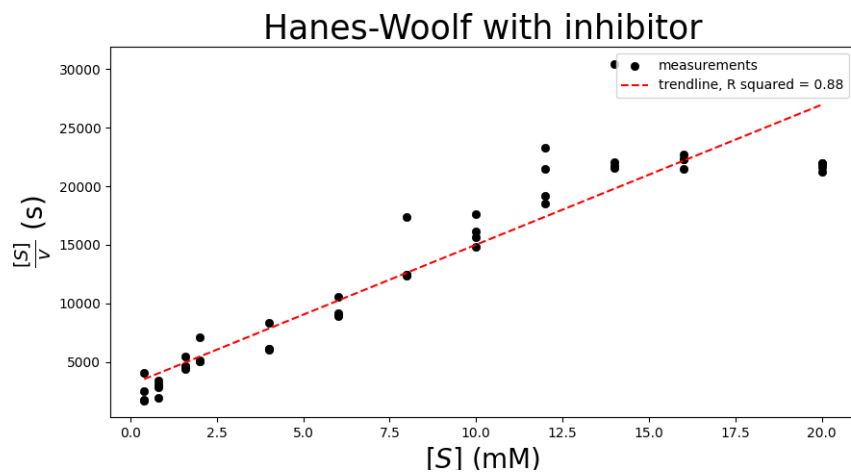
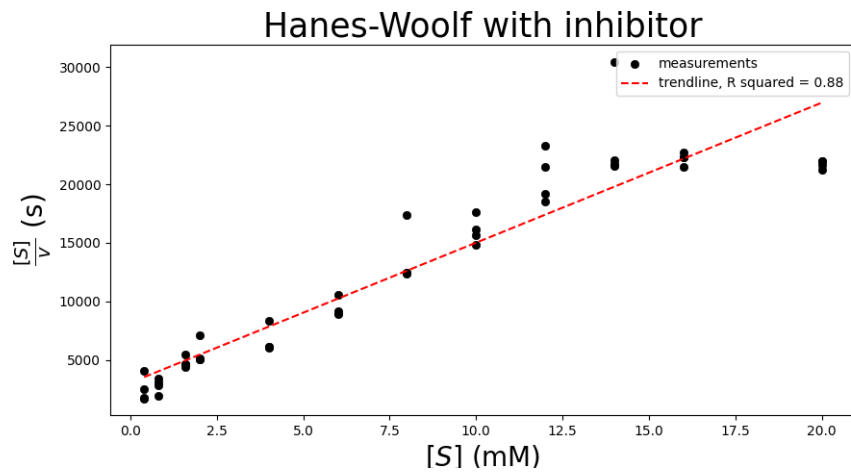
### III Enzymatic activity in the presence of an inhibitor

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



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.

Answer

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

Answer