Enzyme Kinetics Worksheet

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Q1. Include a screenshot from *EXCEL* showing your raw data values for all experiments run (Undiluted enzyme vs substrate, diluted enzyme vs substrate). **(2 pts)**

(we didn't have time to do duplicates)

Enzyme Reaction Velocity Data Collection Sheet

Undiluted Enzyme

Cuvette [Substrate] mM	Velocity (Abs/s)	Velocity (Abs/s)	Ave Velocity	Std Dev
0.490196078	0.08	0.06	0.0700	0.0141
0.245098039	0.069	0.062	0.0655	0.0049
0.12254902	0.034	0.051	0.0425	0.0120
0.06127451	0.068		0.0680	0.0000
0.030637255	0.018		0.0180	0.0000
0.015318627	0.01		0.0100	0.0000
0.007659314	0.002		0.0020	0.0000
0.003829657	0.002		0.0020	0.0000

1:1 Diluted Enzyme

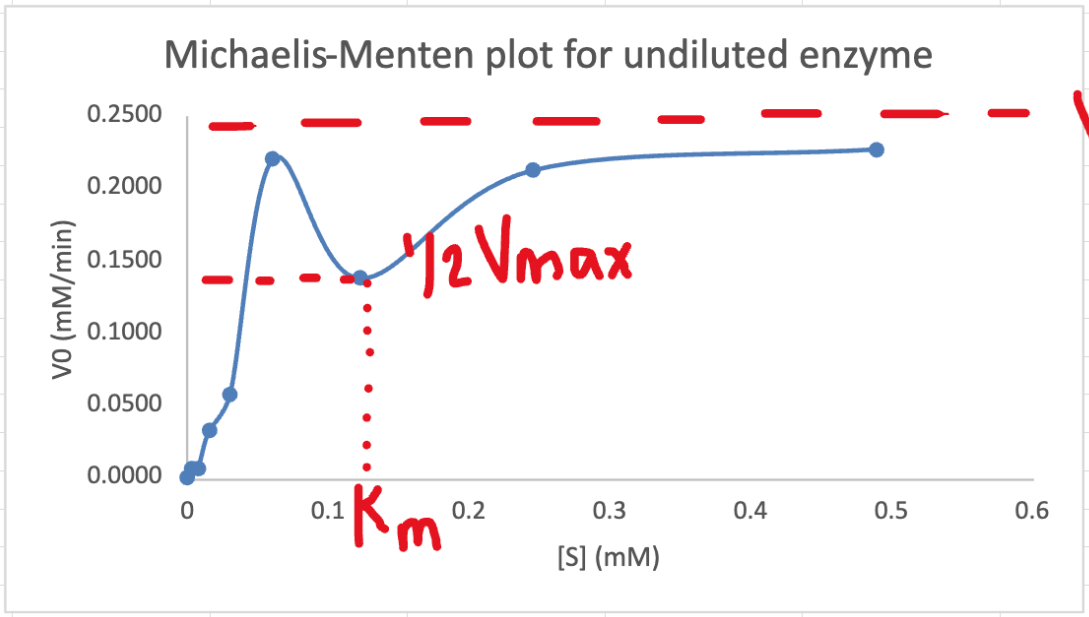
Cuvette [Substrate] mM	Velocity (Abs/s)	Velocity (Abs/s)	Ave Velocity	Std Dev
0.490196078	0.061		0.0610	0.0000
0.245098039	0.027		0.0270	0.0000
0.12254902	0.025		0.0250	0.0000
0.06127451	0.023		0.0230	0.0000
0.030637255	0.016		0.0160	0.0000
0.015318627	0.01		0.0100	0.0000
0.007659314	0.009		0.0090	0.0000
0.003829657	0.005		0.0050	0.0000

Q2. Graphs of undiluted enzyme (generated in EXCEL)

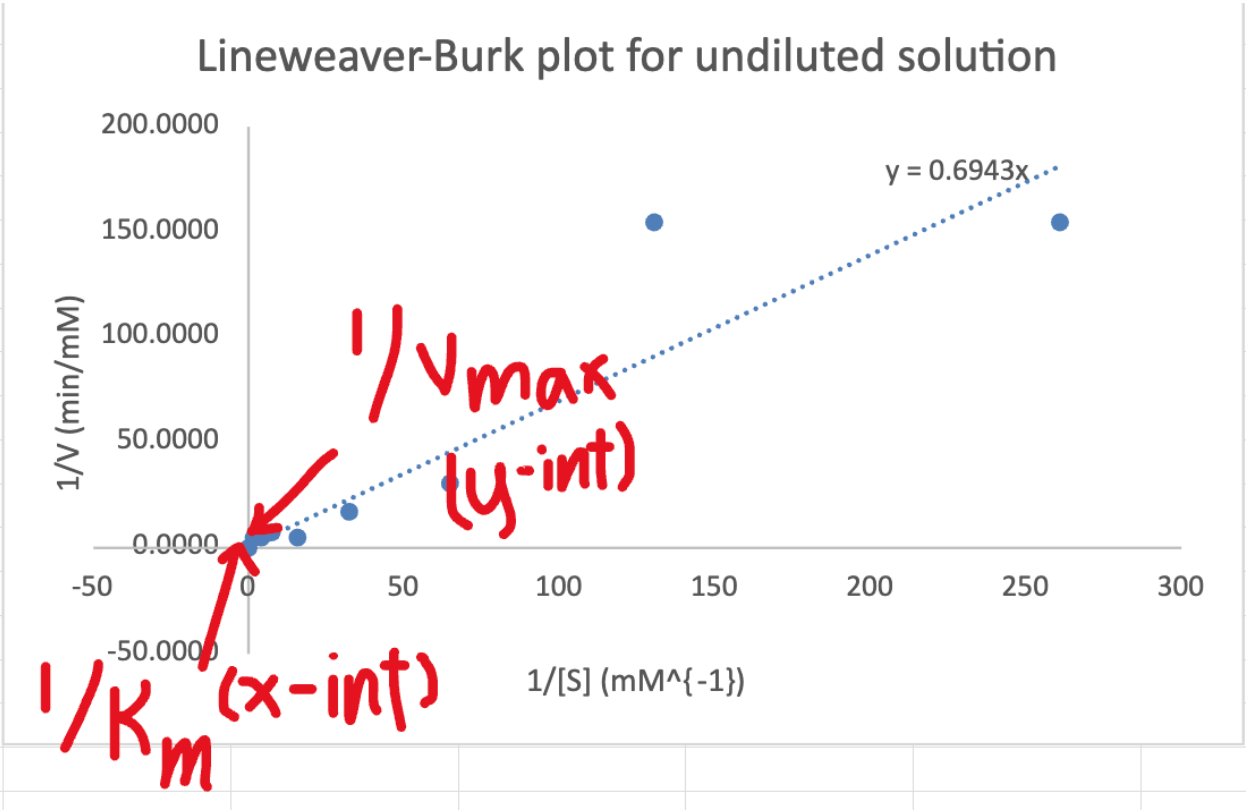
Using the absorbance data (v_o) collected in lab for the undiluted enzyme and the extinction coefficient for PNP (**18.5 mM^{-1} cm^{-1}**), convert the velocity absorbance data v_o, to **mM of product formed per minute**. Use the data to make the two plots listed below. For all plots mark the approximate Km and Vmax on the graph, (the value is not needed just where it would be located, i.e. the x-axis, the slope, y-axis etc). **You should provide the graphs listed with all your axes appropriately labeled with the correct units.**

On each graph mark the approximate Km and Vmax

2.1. Basic Michaelis-Menten Hyperbolic curve **(8 pts)**



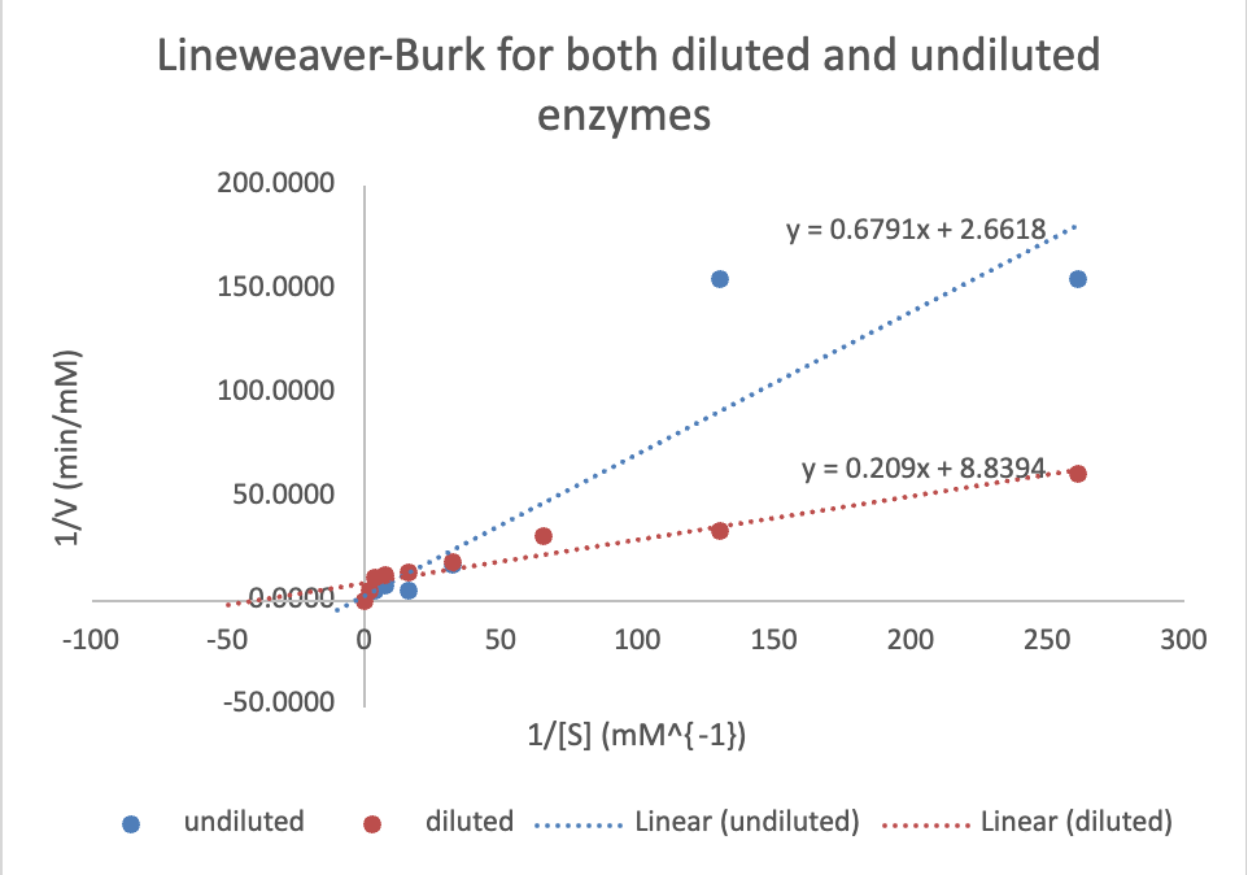
2.2. Double-reciprocal (Lineweaver-Burk) plot **(8 pts)**



***-1/K_m**

Q3. Graph Undiluted and Diluted kinetics superimposed (generated in EXCEL)

Include a double-reciprocal (Lineweaver-Burk) plot (Undiluted & Diluted on the same graph). **You should provide the graphs listed with <u>all your axes appropriately labeled with the correct units.</u> (4 pts)**



What are the Km and Vmax values when the enzyme is undiluted and when it is diluted 1:1? Consider what should ideally occur with your Km and Vmax values. If they deviate significantly from the expected scenario, **please** provide an explanation for why you think this deviation might be happening. **(4 pts)**

The Vmax for the undiluted solution was 0.376 mM/min according to the Lineweaver-Burk plot. Comparatively, the Vmax of the diluted solution was 0.113 mM/min. In addition, the Km, calculated by taking the negative reciprocal of the Km, is 0.255 mM for the undiluted solution and 0.024 mM for the diluted solution. This data trend is for the most part as expected since the Vmax relates to the enzyme concentration. Thus, lowering the enzyme concentration by diluting it should result in a lower Vmax for the diluted solution. In theory, the diluted solution should have a Vmax that is approximately half of the Vmax of the undiluted solution, but the Vmax of the diluted solution is less than half, which is likely due to experimental error. Further, the Km is a constant that is dependent on the enzyme itself, but not its concentration. Thus, the Km for both solutions should be the same, yet the diluted was significantly smaller due to experimental error.

Q4. For generating the basic hyperbolic Michaelis-Menton plot, a serial dilution of the substrate was used in order to always add a constant volume of substrate. Why was a constant volume needed? **(2 pts)**

A constant volume of substrate was needed in order to obtain data that determines how substrate concentration affects reaction rate and not how enzyme concentration affects it. We kept enzyme concentration the same for every solution because if we didn't, then we wouldn't know if it was the substrate

concentration that affected the reaction rate or the enzyme. In order to measure how the substrate concentration affected the reaction rate, we also had to keep the total volume the same by putting 50 microliters per tube via the serial dilution, varying the concentration of the substrate but not the volume to avoid other factors that impact rate changing the data.

Q5. Although the Lineweaver-Burk plot is most often presented in textbooks and the literature, it is in fact the least acceptable method for Km and Vmax estimates. Why is it considered a poor method? **(3 pts)**

The Lineweaver Burk plot is considered a poor method for Km and Vmax estimates due to the linear representation and the process of taking reciprocals, such as the negative reciprocal of the x-intercept to obtain the Km. The process of taking reciprocals can be inaccurate and amplify data errors while the line of best fit can make lower substrate values appear too big.