

Give all answers to at least **4 significant figures**.

1. Characterizing the properties of enzymes is a very important endeavor in the biological sciences. Many enzymes follow Michaelis-Menten kinetics and have the following form for their reaction rates:

$$R([S]) = \frac{V_{max}[S]}{K_m + [S]}, \quad (1)$$

where $[S]$ is the concentration of the substrate that the enzyme is catalyzing, $R([S])$ is the rate of production of the product, V_{max} is the maximum rate of the reaction (and depends on the enzyme concentration used), and K_m is the Michaelis-Menten constant, which characterizes a particular enzyme.

Below are data from Schmider, et. al. [1] for cytochrome P450 mediated demethylation of amitriptyline (AMI) to nortriptyline (N) by human liver microsomes.

[AMI] (μM)	N formation nmol/min/mg
5	0.20
10	0.45
15	0.60
25	0.80
50	1.35
100	2.17
200	2.68
260	2.89
330	3.05
500	3.12

From this table, we let $[S] = [\text{AMI}]$ and $R([S]) = \text{N formation}$. We want to find the best values of V_{max} and K_m to characterize the enzyme cytochrome P450.

a. One of the easiest methods for finding the constants V_{max} and K_m is using the Lineweaver-Burk plot as discussed in lecture. To do this, you take the data and create a new table with the values $x = 1/[S]$ and $y = 1/R([S])$. Create this new table in Excel, then plot the data y vs x . Use Trendline to find the best fit to this straight line. The y -intercept has the value $1/V_{max}$ and the slope has the value K_m/V_{max} . Use this information to find V_{max} and K_m .

b. With this information, plot the original data with the Michaelis-Menten reaction formula for $[S] \in [0, 500]$. With the values of V_{max} and K_m computed in Part a, compute the sum of square errors between the nonlinear model and the data. Again using the nonlinear Michaelis-Menten reaction formula, predict the production of nortriptyline (N) when [AMI] is $[S] = 15, 50, 100, 200,$ and 500 and calculate the percent error between the model and the actual data (with the assumption that the data values are the best values). Find all intercepts and any asymptotes for this function for $[S] \geq 0$.

c. Use Excel's Solver to find the nonlinear least squares best fit of the parameters V_{max} and K_m to the nonlinear function $R([S])$ in Eqn. (1). Write the sum of square errors and compare it to

the one calculated in Part b. Assuming that these nonlinear fit constants are the better constants, compute the percent error between the values of V_{max} and K_m calculated in this part and Part a.

d. With the information in Part c, plot the original data with this Michaelis-Menten reaction formula for $[S] \in [0, 500]$. Find the prediction from this Michaelis-Menten reaction formula for the production of nortriptyline (N) when [AMI] is $[S] = 15, 50, 100, 200,$ and 500 . Calculate the percent error between the model and the actual data (with the data again consider to be the best value). Find all intercepts and any asymptotes for this function for $[S] \geq 0$. Which model would you say is better and why?

2. This problem is probably best done using Maple. Consider the quadratic function

$$f(x) = x^2 - 3x - 5$$

and the rational function

$$g(x) = \frac{20x}{1.4 + x}.$$

(You will probably want to graph these functions on the interval $x \in [-10, 10]$ with the range restricted to $y \in [-50, 50]$.)

a. Find the x and y -intercepts for both of these functions. Find the vertex of the quadratic function, $f(x)$. Give both x and y values. List any asymptotes (vertical and horizontal) for the rational function, $g(x)$.

b. Find all points of intersection between the graphs of $f(x)$ and $g(x)$.

References

[1] Schmider J., Greenblatt D.J., Harmatz J.S., Shader R.I., (1996) Enzyme kinetic modelling as a tool to analyse the behaviour of cytochrome P450 catalysed reactions: application to amitriptyline N-demethylation, *Brit. J. Clin. Pharmacol.* **41**, 593-604.