Identifying the type of inhibitors from experimental measurements

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I. Introduction

Enzymes are essential for many chemical reactions within an organism. They act as catalysts where they increase the rate of producing products from substrates. This is achieved through the binding of enzymes with substrates that form an enzyme-substrates. The enzyme-substrate then reacts to a product and the enzyme again. After the reaction, the enzyme is free to be used again.

Inhibitors control these processes. They are able to regulate the enzymes and enzyme-substrates such that the production of products is reduced. There are several types of inhibitors. Competitive inhibitors bind with the enzyme to stop the enzyme from acting as a catalyst. Uncompetitive inhibitors bind with the enzyme-substrate to stop it from producing its products. And lastly, non-competitive inhibitors bind with both the free enzyme and the enzyme-substrate.

This report will aim to identify the type of inhibitor from experimental data. It does this by first describing the enzyme kinetics without inhibitors and derive the Michaelis-Menten equation, which is an expression for the production rate for this particular system. It will then describe the Lineweaver-Burk method to create a linear form of the Michaelis-Menten equation. At last, the updated Michaelis-Menten equations are derived for the three types of inhibitors. These updated Michaelis-Menten equations are then used to identify the type of inhibitor from experimental data.

II. Theory

THE enzyme kinetics are described for a system without inhibitors. From this system, the Michaelis-Menten equation is derived. Lineweaver-Burk plots are discussed, which provide a way to obtain parameters of the Michaelis-Menten equation. Inhibitors are added to the enzyme kinetics. This is done for three types of inhibitors: competitive inhibitors, noncompetitive inhibitors, and noncompetitive inhibitors. For these systems, the production rate is also determined.

A. Enzyme kinetics

A catalyst increases the rate of the chemical reaction and is not used up during the reaction. Therefore, a catalyst remains in the system. Enzymes (E) acts as a catalyst during chemical reactions in the body. The enzyme and substrate (S) undergo a reversible reaction to form an enzyme-substrate. The enzyme-substrate then reacts to form a product (P) and the original enzyme in an irreversible reaction.

$$\mathrm{E} + \mathrm{S} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_{cat}} \mathrm{P} + \mathrm{E},$$

where the k symbols refer to the rate at which the reaction takes place.

Typically, k_+ and k_- are much larger than k_{cat}, and the concentration of the substrates is also much larger than the concentration of the enzymes. Therefore the concentration of ES remains constant. As such, the production rate of P is given by

$$v = k_{cat}[\mathrm{ES}]. \tag{1}$$

The production rate is therefore maximal when [ES] is as large as possible. [ES] can be at most $[\mathrm{E}_{\mathrm{total}}]$, where $[\mathrm{E}_{\mathrm{total}}]$ is the number of enzyme molecules. Thus the production rate is maximal for

$$V_{max} = k_{cat}[\mathrm{E}_{\mathrm{total}}]. \tag{2}$$

Thus when twice as much enzyme is added, V_{max} would also be twice as large.

This assumption does not hold when $[\mathrm{S}] \gg [\mathrm{E}]$, since then the limiting factor in the reaction is [S]. The production rate would then be maximal when [ES] is equal to $[\mathrm{S}_{\mathrm{total}}]$. Thus in the case of $[\mathrm{S}] \gg [\mathrm{E}]$ the maximum production rate would be

$$V_{max} = k_{cat}[\mathrm{S}_{\mathrm{total}}] \tag{3}$$

B. Michaelis-Menten Equation

The Michaelis-Menten Equation gives the rate of the production P as a function of [S]. The rate is given by Equation 1. [ES] is determined by looking at the steady-state solution. In the steady-state solution, [ES] remains constant, which is written as

$$(k_- + k_{cat})[\mathrm{ES}] = k_+[\mathrm{E}][\mathrm{S}]. \tag{4}$$

The Michaelis constant K_m is given by

$$K_m = \frac{k_- + k_{cat}}{k_+}. \tag{5}$$

Since the Michaelis constant does not depend on the concentration of the enzyme. Therefore, adding twice as much enzyme would not have any effect on K_m.

Equation 4 can be then be written as

$$K_m[\mathrm{ES}] = [\mathrm{E}][\mathrm{S}] \tag{6}$$

where K_m is the Michaelis constant.

Since $[\mathrm{E_{total}}] = [\mathrm{E}] + [\mathrm{ES}]$ this can be rewritten to obtain

$$[\mathrm{ES}] = \frac{[\mathrm{E_{total}}] - [\mathrm{ES}]}{K_m}[\mathrm{S}]. \tag{7}$$

Collecting the [ES] terms gives

$$[\mathrm{ES}] = [\mathrm{E_{total}}]\frac{[\mathrm{S}]}{K_m + [\mathrm{S}]}. \tag{8}$$

Substituting this into Equation 1 gives the Michaelis-Menten equation

$$v = k_{cat}[\mathrm{E_{total}}]\frac{[\mathrm{S}]}{K_m + [\mathrm{S}]} = V_{max}\frac{[\mathrm{S}]}{K_m + [\mathrm{S}]}. \tag{9}$$

From the Michaelis-Menten equation it can be seen that K_m controls the rate of production. Furthermore, when [S] is equal to K_m then v is exactly half of V_{max}.

C. Lineweaver-Burk

When measured data is available, the measures reaction rates v can be plotted against [S]. A fit can be made through the data points using Equation 9 to calculate the Michaelis-Menten parameters for which the theoretical equation closest resembles the measurements. However, another way to obtain these parameters is by making a Lineweaver-Burk plot. This plot uses the inverses of the measured quantities to plot $\frac{1}{v}$ against $\frac{1}{[S]}$. Inverting the Michaelis-Menten equation yields

$$\begin{aligned}\frac{1}{v} &= \frac{1}{V_{max}} * \frac{K_m + [\mathrm{S}]}{[\mathrm{S}]} \\ &= \frac{1}{V_{max}}\left(1 + \frac{K_m}{[\mathrm{S}]}\right) \\ &= \frac{1}{V_{max}} + \frac{K_m}{V_{max}} * \frac{1}{[\mathrm{S}]},\end{aligned} \tag{10}$$

which is a linear relation between $\frac{1}{v}$ and $\frac{1}{[S]}$ with a slope $\frac{K_m}{V_{max}}$. This line intersects the vertical axis when $\frac{1}{[S]} = 0$, which gives $\frac{1}{v} = \frac{1}{V_{max}}$. The intersection with the horizontal axis when $\frac{1}{v} = 0$. Setting Equation 10 to zero results in

$$\begin{aligned}\frac{1}{V_{max}} + \frac{K_m}{V_{max}} * \frac{1}{[\mathrm{S}]} &= 0 \\ K_m * \frac{1}{[\mathrm{S}]} &= -1 \\ \frac{1}{[\mathrm{S}]} &= -\frac{1}{K_m},\end{aligned} \tag{11}$$

which means that the intersection points with the vertical and horizontal axes are located at $\{0, \frac{1}{V_{max}}\}$ and $\{-\frac{1}{K_m}, 0\}$, respectively.

Plotting measurements using the Lineweaver-Burk plot should be close to a linear dependence, so the line can be extrapolated to the axis intersections to obtain values for the parameters. This is much simpler than fitting the Michaelis-Menten equation to find optimal values since the parameters can be obtained by reading the intersection values. However, the values obtained in this way are not necessarily the same as the values calculated by fitting the Michaelis-Menten equation. If all measurements of [S] have the same absolute error, the smaller values will have the biggest relative error. When the inverse $\frac{1}{[S]}$ is then calculated, this value will have a much larger absolute error for smaller values of [S] than for larger values. The determined slope of the line then runs the risk of being inaccurate, since it will be disproportionally affected by the errors in these measurements.

D. Inhibitors

Enzyme inhibitors are molecules that regulate enzymes. They bond with the enzymes and are thus able to block the enzyme from acting as a catalyst on the substrates. There are three types of inhibitors: competitive inhibitors, uncompetitive inhibitors, and noncompetitive inhibitors. This section will explain the chemical reaction for all three inhibitors and see how they impact the Michaelis-Menten equation.

1) Competitive inhibitors: The competitive inhibitors are only able to react with free enzymes. Therefore they act as a direct competitor to the substrate since they are both reacting with the free enzymes.

$$\mathrm{E + I} \rightleftharpoons \mathrm{EI}$$

with Inhibition Constant

$$K_i = \frac{[\mathrm{I}][\mathrm{E}]}{[\mathrm{EI}]} \tag{12}$$

This reaction adds an extra component to $[\mathrm{E_{total}}]$, such that $[\mathrm{E_{total}}]$ becomes

$$[\mathrm{E_{total}}] = [\mathrm{E}] + [\mathrm{ES}] + [\mathrm{EI}]. \tag{13}$$

Rewriting Equation 12 for [EI] gives

$$[\mathrm{EI}] = \frac{[\mathrm{I}][\mathrm{E}]}{K_i}. \tag{14}$$

Substituting [EI] back into 13 finally gives the updated value for E_{total}

$$\begin{aligned}[\mathrm{E_{total}}] &= [\mathrm{E}] + [\mathrm{ES}] + \frac{[\mathrm{I}][\mathrm{E}]}{K_i} \\ &= [\mathrm{ES}] + \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{E}]\end{aligned} \tag{15}$$

The production rate v, given by Equation 1, is obtained by finding an expression for [ES]. This is obtained by Equation 6 and writing [E] as a function of $[\mathrm{E_{total}}]$.

First the expression for [E] is obtained from Equation 15. Rewriting for [E] gives

$$[\mathrm{E}] = \frac{[\mathrm{E_{total}}] - [\mathrm{ES}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)} \tag{16}$$

Substituting [E] back into Equation 6 gives

$$K_m[\mathrm{ES}] = \frac{[\mathrm{E_{total}}] - [\mathrm{ES}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)}[\mathrm{S}], \tag{17}$$

which is then solved for [ES]

$$K_m[\mathrm{ES}] + \frac{[\mathrm{ES}][\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)} = \frac{[\mathrm{E_{total}}][\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)} \tag{18}$$

$$[\mathrm{ES}]\left(K_m + \frac{[\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)}\right) = \frac{[\mathrm{E_{total}}][\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)} \tag{19}$$

$$\begin{aligned}[\mathrm{ES}] &= \frac{[\mathrm{E_{total}}][\mathrm{S}]}{\left(K_m + \frac{[\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)}\right)\left(1 + \frac{[\mathrm{I}]}{K_i}\right)} \\ &= \frac{[\mathrm{E_{total}}][\mathrm{S}]}{K_m\left(1 + \frac{[\mathrm{I}]}{K_i}\right) + [\mathrm{S}]}\end{aligned} \tag{20}$$

The concentration [ES] is then substituted in Equation 1 to finally find

$$v = k_{cat}[\mathrm{E_{total}}]\frac{[\mathrm{S}]}{K_m\left(1 + \frac{[\mathrm{I}]}{K_i}\right) + [\mathrm{S}]} \tag{21}$$

$$= V_{max}\frac{[\mathrm{S}]}{K_m\left(1 + \frac{[\mathrm{I}]}{K_i}\right) + [\mathrm{S}]}. \tag{22}$$

This rate is maximal when $[\mathrm{S}] \to \infty$, where the term dependent on [I] and K_i becomes negligible and the rate goes to V_{max}. However, for v to remain the same for all values [S], K_m must compensate this term by a factor $K_m = \frac{1}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)}$.

When a Lineweaver-Burk plot is used to plot measurements with a competitive inhibitor, Equation 22 is then inverted to obtain the expected relation between $\frac{1}{v}$ and $\frac{1}{[\mathrm{S}]}$, which becomes

$$\begin{aligned}\frac{1}{v} &= \frac{1}{V_{max}} * \frac{K_m\left(1 + \frac{[\mathrm{I}]}{K_i}\right) + [\mathrm{S}]}{[\mathrm{S}]} \\ &= \frac{1}{V_{max}} + \frac{K_m\left(1 + \frac{[\mathrm{I}]}{K_i}\right)}{V_{max}} * \frac{1}{[\mathrm{S}]}.\end{aligned} \tag{23}$$

2) Uncompetitive inhibitors: The uncompetitive inhibitors do not directly act on the free enzymes, they, however, act on the enzyme-substrate, such that

$$\mathrm{ES} + \mathrm{I} \rightleftharpoons \mathrm{ESI}$$

with Inhibition Constant

$$K_i = \frac{[\mathrm{I}][\mathrm{ES}]}{[\mathrm{ESI}]} \tag{24}$$

This reaction adds an extra component to $[\mathrm{E_{total}}]$, such that $[\mathrm{E_{total}}]$ becomes

$$[\mathrm{E_{total}}] = [\mathrm{E}] + [\mathrm{ES}] + [\mathrm{ESI}]. \tag{25}$$

Rewriting Equation 24 for [ESI] gives

$$[\mathrm{ESI}] = \frac{[\mathrm{I}][\mathrm{ES}]}{K_i}. \tag{26}$$

Substituting [ESI] back into 25 finally gives the updated value for E_{total}

$$\begin{aligned}[\mathrm{E_{total}}] &= [\mathrm{E}] + [\mathrm{ES}] + \frac{[\mathrm{I}][\mathrm{ES}]}{K_i} \\ &= \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{ES}] + [\mathrm{E}]\end{aligned} \tag{27}$$

The production rate v, given by Equation 1, is obtained by finding an expression for [ES]. This is obtained by Equation 6 and writing [E] as a function of $[\mathrm{E_{total}}]$.

First the expression for [E] is obtained from Equation 27. Rewriting for [E] gives

$$[\mathrm{E}] = [\mathrm{E_{total}}] - \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{ES}] \tag{28}$$

Substituting [E] back into Equation 6 gives

$$K_m[\mathrm{ES}] = \left([\mathrm{E_{total}}] - \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{ES}]\right)[\mathrm{S}], \tag{29}$$

which is then solved for [ES]

$$K_m[\mathrm{ES}] + \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{ES}][\mathrm{S}] = [\mathrm{E_{total}}][\mathrm{S}] \tag{30}$$

$$[\mathrm{ES}]\left(K_m + \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{S}]\right) = [\mathrm{E_{total}}][\mathrm{S}] \tag{31}$$

$$[\mathrm{ES}] = \frac{[\mathrm{E_{total}}][\mathrm{S}]}{K_m + \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{S}]} \tag{32}$$

The concentration [ES] is then substituted in Equation 1 to finally find

$$\begin{aligned} v &= k_{cat}[\mathrm{E_{total}}] \frac{[\mathrm{S}]}{K_m + \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{S}]} \\ &= V_{max} \frac{[\mathrm{S}]}{K_m + \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{S}]} \end{aligned} \tag{33}$$

This rate is maximal when $[\mathrm{S}] \to \infty$, where K_m becomes negligible and the rate goes to $v_{max} = \frac{V_{max}}{1+\frac{[\mathrm{I}]}{K_i}}$. Thus, for the maximum v to remain the same, V_{max} must compensate this change to become $V_{max} = v_{max} * \left(1 + \frac{[\mathrm{I}]}{K_i}\right)$, where v_{max} would be the maximum reaction rate if there was no inhibitor. After substituting this, the equation becomes

$$\begin{aligned} v &= \frac{v_{max} * \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{S}]}{K_m + \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{S}]} \\ &= v_{max} \frac{[\mathrm{S}]}{\frac{K_m}{\left(1+\frac{[\mathrm{I}]}{K_i}\right)} + [\mathrm{S}]} \end{aligned} \tag{34}$$

which means that K_m must also increase by the same factor for the rate v to always be unchanged by the inhibitor.

When a Lineweaver-Burk plot is used to plot measurements with an uncompetitive inhibitor, Equation 33 is then inverted to obtain the expected relation between $\frac{1}{v}$ and $\frac{1}{[\mathrm{S}]}$, which becomes

$$\begin{aligned} \frac{1}{v} &= \frac{1}{V_{max}} * \frac{K_m + \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{S}]}{[\mathrm{S}]} \\ &= \frac{1 + \frac{[\mathrm{I}]}{K_i}}{V_{max}} + \frac{K_m}{V_{max}} * \frac{1}{[\mathrm{S}]}. \end{aligned} \tag{35}$$

3) Noncompetitive inhibitors: The noncompetitive inhibitors act both on the free enzymes and the enzyme-substrates. They block the forming of enzyme-substrates and block the production of a product from the enzyme-substrates. The two reactions are given by

$$\mathrm{E + I} \rightleftharpoons \mathrm{EI}$$

and

$$\mathrm{ES + I} \rightleftharpoons \mathrm{ESI}$$

with Inhibition Constant

$$K_i = \frac{[\mathrm{I}][\mathrm{E}]}{[\mathrm{EI}]} = \frac{[\mathrm{I}][\mathrm{ES}]}{[\mathrm{ESI}]} \tag{36}$$

This reaction adds two extra components to $[\mathrm{E_{total}}]$, such that $[\mathrm{E_{total}}]$ becomes

$$[\mathrm{E_{total}}] = [\mathrm{E}] + [\mathrm{EI}] + [\mathrm{ES}] + [\mathrm{ESI}]. \tag{37}$$

Rewriting Equation 36 for [EI] and [ESI] gives

$$[\mathrm{EI}] = \frac{[\mathrm{I}][\mathrm{E}]}{K_i} \tag{38}$$

and

$$[\mathrm{ESI}] = \frac{[\mathrm{I}][\mathrm{ES}]}{K_i}. \tag{39}$$

Substituting [EI] and [ESI] back into 37 finally gives the updated value for E_{total}

$$\begin{aligned} [\mathrm{E_{total}}] &= [\mathrm{E}] + [\mathrm{ES}] + \frac{[\mathrm{I}][\mathrm{E}]}{K_i} + \frac{[\mathrm{I}][\mathrm{ES}]}{K_i} \\ &= \left(1 + \frac{[\mathrm{I}]}{K_i}\right)([\mathrm{ES}] + [\mathrm{E}]) \end{aligned} \tag{40}$$

The production rate v, given by Equation 1, is obtained by finding an expression for [ES]. This is obtained by Equation 6 and writing [E] as a function of $[\mathrm{E_{total}}]$.

First the expression for [E] is obtained from Equation 40. Rewriting for [E] gives

$$[\mathrm{E}] = \frac{[\mathrm{E_{total}}] - \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{ES}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)} \tag{41}$$

Substituting [E] back into Equation 6 gives

$$K_m[\mathrm{ES}] = \frac{[\mathrm{E_{total}}] - \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{ES}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)}[\mathrm{S}], \tag{42}$$

which is then solved for [ES]

$$K_m[\mathrm{ES}] + [\mathrm{ES}][\mathrm{S}] = \frac{[\mathrm{E_{total}}][\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)} \tag{43}$$

$$[\mathrm{ES}]\left(K_m + [\mathrm{S}]\right) = \frac{[\mathrm{E_{total}}][\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)} \tag{44}$$

$$[\mathrm{ES}] = \frac{[\mathrm{E_{total}}][\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)\left(K_m + [\mathrm{S}]\right)} \tag{45}$$

The concentration [ES] is then substituted in Equation 1 to finally find

$$\begin{aligned} v &= k_{cat}[\mathrm{E_{total}}] \frac{[\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)\left(K_m + [\mathrm{S}]\right)} \\ &= V_{max} \frac{[\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)\left(K_m + [\mathrm{S}]\right)}. \end{aligned} \tag{46}$$

This rate is maximal when $[\mathrm{S}] \to \infty$, where K_m becomes negligible and the rate goes to $v_{max} = \frac{V_{max}}{1+\frac{[\mathrm{I}]}{K_i}}$. Thus, for the maximum v to remain the same, V_{max} must compensate this change to become $V_{max} = v_{max} * \left(1 + \frac{[\mathrm{I}]}{K_i}\right)$, where v_{max} would be the maximum reaction rate if there was no inhibitor. After substituting this, the equation becomes

$$\begin{aligned} v &= \frac{v_{max} * \left(1 + \frac{[\mathrm{I}]}{K_i}\right)[\mathrm{S}]}{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)(K_m + [\mathrm{S}])} \\ &= v_{max}\frac{[\mathrm{S}]}{K_m + [\mathrm{S}]} \end{aligned} \tag{47}$$

which means that K_m does not need to change for v to always be unchanged by the inhibitor.

When a Lineweaver-Burk plot is used to plot measurements with a noncompetitive inhibitor, Equation 46 is then inverted to obtain the expected relation between $\frac{1}{v}$ and $\frac{1}{[\mathrm{S}]}$, which becomes

$$\begin{aligned} \frac{1}{v} &= \frac{1}{V_{max}} * \frac{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)(K_m + [\mathrm{S}])}{[\mathrm{S}]} \\ &= \frac{1 + \frac{[\mathrm{I}]}{K_i}}{V_{max}} + \frac{\left(1 + \frac{[\mathrm{I}]}{K_i}\right)K_m}{V_{max}} * \frac{1}{[\mathrm{S}]}. \end{aligned} \tag{48}$$

III. Method

THE Michaelis-Menten equation was implemented in Python, and this section further describes the methods used to analyse the Lineweaver-Burk plots and the inhibitors.

A. Lineweaver-Burk

The Michaelis-Menten parameters are approximated from measurements of $[\mathrm{S}]$ and v by fitting the Michaelis-Menten equation to the data points using the `scipy.optimize.curve_fit` function. The parameters are also obtained from the Lineweaver-Burk plot by fitting a linear relation function to $\frac{1}{[\mathrm{S}]}$ and $\frac{1}{v}$, where the function is given by

$$\frac{1}{v} = a + b\frac{1}{[\mathrm{S}]}. \tag{49}$$

The curve fitting function then returns the fitting parameters a and b. Since the theoretical intersection points are known from section II-C, the parameter values can be deduced as follows. The linear relation function intersects the vertical axis when $\frac{1}{[\mathrm{S}]} = 0$, so then $\frac{1}{v} = a (= \frac{1}{V_{max}})$. The intersection with the horizontal axis happens when $\frac{1}{v} = 0$, which means $\frac{1}{[\mathrm{S}]} = -\frac{a}{b} (= -\frac{1}{K_m})$. Thus, once a and b are known, the parameter values can be calculated by $V_{max} = \frac{1}{a}$ and $K_m = \frac{b}{a}$.

B. Inhibitors

The Lineweaver-Burk plot can be used to determine the type of an inhibitor from measurements of $[\mathrm{S}]$ and v for different concentrations $[\mathrm{I}]$. Equation 23 shows that for a competitive inhibitor, only the slope of the line in the Lineweaver-Burk plot depends on the concentration $[\mathrm{I}]$ while the intersection of the line with the vertical axis stays unchanged. Equation 35 shows that only the intersection with the vertical axis depends on $[\mathrm{I}]$ for an uncompetitive inhibitor, while the slope remains constant. Finally, Equation 48 shows that both the slope and vertical intersection point depend on $[\mathrm{I}]$ for a noncompetitive inhibitor. Thus, if measurements of $[\mathrm{S}]$ and v are available for different concentrations of $[\mathrm{I}]$, the type of inhibitor can be determined by checking how the slopes and intersection points change.

IV. Results

THIS section will show the impact of V_{max} and K_m on the Michaelis-Menten equation. Furthermore, the Michaelis-Menten parameters will be obtained from experimental data. This is performed both with and without the Lineweaver-Burk method. Lastly, the type of inhibitor is found using the Lineweaver-Burk method from experimental data.

A. Michaelis-Menten Equation

The Michaelis-Menten equation without inhibitors, Equation 9, is tested for different values for K_m and V_{max}. The production rate is then plotted as a function of $[\mathrm{S}]$. This is shown in Figure 1. It shows that the maximum production rate converges towards V_{max} as $[\mathrm{S}]$ increases. The speed at which convergence happens is controlled by the K_m parameter. It converges faster for lower values of K_m.

B. Obtaining Michaelis-Menten parameters

Figure 2 shows the measures data points and the corresponding curve fit. This fit yielded the parameter values $V_{max} = 9.9537$ and $K_m = 0.46236$, and matches well with the data points. Figure 3 shows these same data points in a Lineweaver-Burk plot, where they show a linear relation as expected. A linear fit is plotted according to equation 49. The first data point $(0, 0)$ was discarded for this plot, since the inverse of those values is infinity. This linear fit gives values $a = 0.10084$ and $b = 0.04404$, from which the Michaelis-Menten parameters were calculated to be $V_{max} = \frac{1}{a} = 9.9168$ and $K_m = \frac{b}{a} = 0.43673$. These values deviate somewhat from the values from the standard curve fit. Figure 3

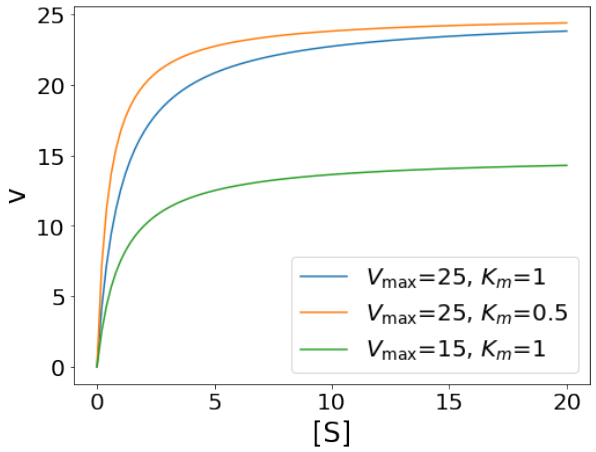


Fig. 1: The production rate as a function of [S]. This is shown for three different sets of parameter values.

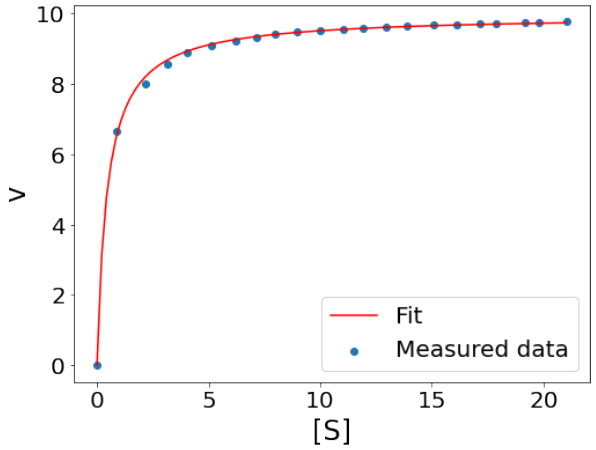


Fig. 2: A curve fit using the Michaelis-Menten equation through the measured data of [S] and v. The fit has parameters $V_{max} = 9.9537$ and $K_m = 0.46236$.

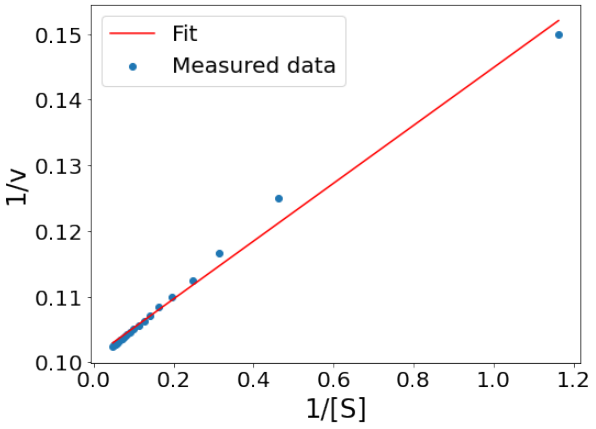


Fig. 3: Lineweaver-Burk plot of the measured data of [S] and v, along with a fit using Equation 49 that yields $a = 0.10084, b = 0.04404$. From these values, the corresponding Michaelis-Menten parameters were calculated to be $V_{max} = 9.9168$ and $K_m = 0.43673$.

shows that the highest point could be the cause of this discrepancy because the rest of the points indicate a steeper slope that is possibly decreased because of the error in that point. This is one of the previously described downsides of determining the parameters from the Lineweaver-Burk plot.

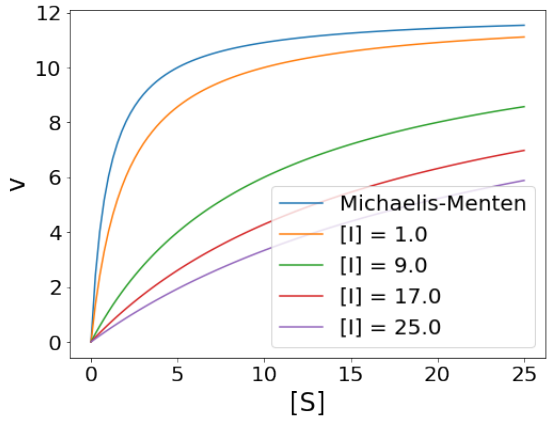
C. Inhibitors

Equations 22, 33 and 46 were implemented, which describe the dependence of the rate v on the concentration [S] for competitive, uncompetitive and noncompetitive inhibitors, respectively. The dependence of these functions on [I] and K_i was investigated for parameter values $V_{max} = 12$ and $K_m = 1$.

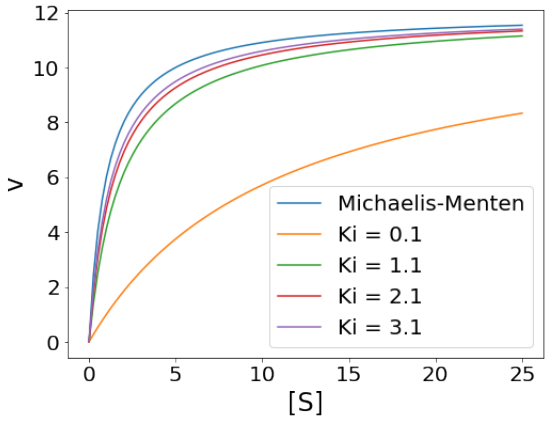
In Figure 4, the function for the competitive inhibitor is plotted for different values of [I] and K_i. While v still converges to V_{max} in all cases, the function converges more slowly for higher values of $\frac{[I]}{K_i}$, while it is equal to the Michealis-Menten equation when that fraction goes to zero. The competitive inhibitor I is a direct competitor for S, as it binds to the free enzyme. When the concentration of S is low compared to that of I, the inhibitor will be able to bind more often with the enzyme than S which suppresses the reaction rate v. Similarly, Equation 14 shows that a lower K_i will increase the concentration of EI, which leaves less free enzyme for S to bind to and also decreases v. However, when the concentration of S goes to infinity, the concentration of I will be so comparatively low that almost all enzymes can still bind to the substrate, so the maximum reaction rate stays the same.

In Figure 5, the function for the uncompetitive inhibitor is plotted for different values of [I] and K_i. Here, v converges to a lower maximum value for higher values of $\frac{[I]}{K_i}$, but it converges to that maximum more quickly than for lower values. Here, I binds to the enzyme-substrate complex ES. Even when [S] becomes very large, the production rate v will still be lower when I is present, because I can bind to ES anyway to form ESI, which is not a reaction that S competes with. Equation 26 shows that a lower K_i will increase the concentration of ESI. Because ESI can not react to product P, the production rate v is thus lowered when [I] increases or K_i decreases. In the opposite cases, the concentration of ESI decreases, allowing more P to be produced, increasing v.

Finally, Figure 6 plots the function for the noncompetitive inhibitor for different values of [I] and K_i. Now, v also converges to a lower maximum value for higher values of $\frac{[I]}{K_i}$, but does so just as slowly - the halfway point to the maximum v is the same. Again, the concentration of ESI increases for higher values of [I] and lower

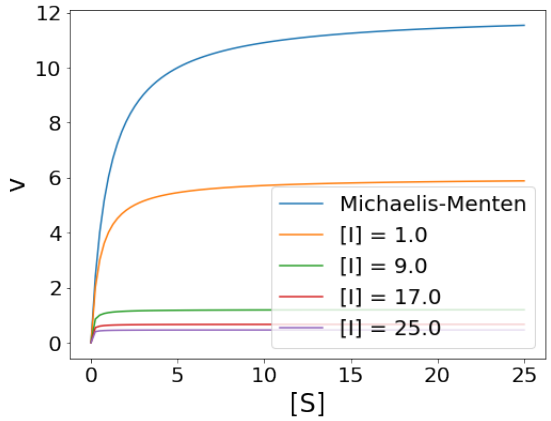


(a) Different values of [I].

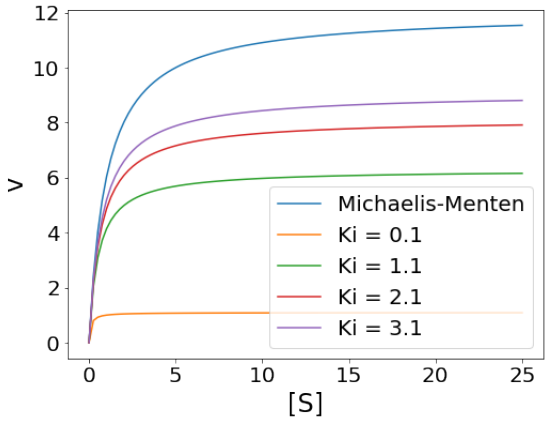


(b) Different values of K_i.

Fig. 4: The Michaelis-Menten equation plotted together with the function for v against [S] of a competitive inhibitor for different values of [I] and K_i. All functions used parameters $V_{max} = 12$ and $K_m = 1$.

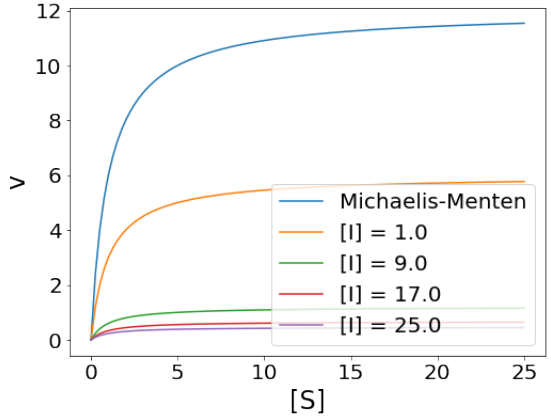


(a) Different values of [I].

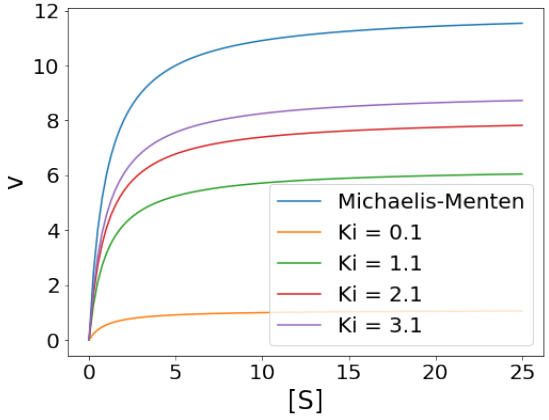


(b) Different values of K_i.

Fig. 5: The Michaelis-Menten equation plotted together with the function for v against [S] of an uncompetitive inhibitor for different values of [I] and K_i. All functions used parameters $V_{max} = 12$ and $K_m = 1$.



(a) Different values of [I].



(b) Different values of K_i.

Fig. 6: The Michaelis-Menten equation plotted together with the function for v against [S] of a noncompetitive inhibitor for different values of [I] and K_i. All functions used parameters $V_{max} = 12$ and $K_m = 1$.

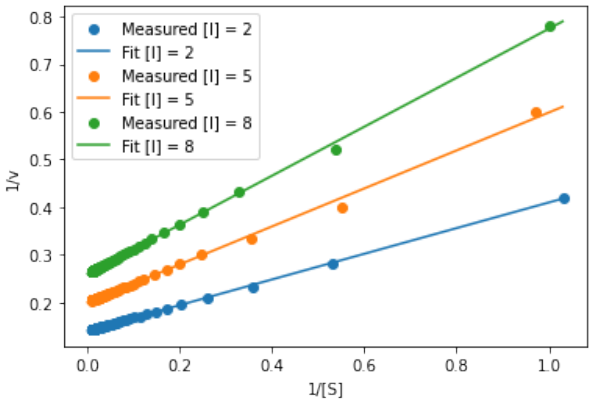


Fig. 7: Lineweaver-Burk plot of measurements of [S] and v for different concentrations [I], together with the linear fits of these measurements using Equation 49.

TABLE I: The obtained fitting parameters a and b for measurements of different concentrations [I].

[I]	**a**	**b**
2	0.140257	0.269287
5	0.199909	0.398514
8	0.260184	0.513969

values of K_i, which prevents P from being produced. Equation 38 and 39 show that the concentrations of EI and ESI are then increased by the same factor, meaning concentrations of E and ES are also decreased by that same factor. This means the production of P will behave the same as in a system with no inhibitor, with a lower concentration $[\mathrm{E}_{\mathrm{total}}]$. From Equation 2, this system would have a lower V_{max}, while K_m stays the same. This is different from the uncompetitive inhibitor which does not decrease [E] and so allows more P to be produced at smaller concentrations [S].

These behaviours correspond to the predictions made in section II-D, only because V_{max} and K_m are fixed in this analysis, their apparent values change in the opposite direction.

Figure 7 shows the Lineweaver-Burk plot for measurements of [S] and v with an unknown inhibitor for different concentrations [I]. The linear relationship from Equation 49 is plotted for the different measurements. Table I shows the obtained fitting parameters a, the intersect with the vertical axis, and b, the slope. It is visible that both the slope and intercept depend on the concentration [I]. This behaviour corresponds to a noncompetitive inhibitor.

V. Discussion & Conclusion

This report was able to derive the Michaelis-Menten equation and show how V_{max} and K_m influence the production rate v. It was shown that this equation can be rewritten to show a linear dependence using the Lineweaver-Burk plot, which allows for an easy way to approximate the Michaelis-Menten parameters from measurements. A drawback of the method is that linear fits can be very heavily influenced by errors in small values from measurements. Future research could investigate if it is possible to make a fit that gives less weight to these points with a high relative error, and whether this could improve the results.

Furthermore, this report was able to derive the updated Michaelis-Menten equations for three types of inhibitors: competitive inhibitors, uncompetitive inhibitors, and noncompetitive inhibitors. The influence of these inhibitors on the reaction rate was analysed. The report was able to identify the type of inhibitor from experimental data, using these equations and the Lineweaver-Burk plot.