Get some extra practice…

…working with enzyme kinetics

1. The table below shows the result of an experiment designed to determine the apparent Michaelis constant (K_M) and the maximum velocity (V_{max}) for a particular enzyme. The kinetic data shows how the initial rate of reaction, measured in a spectrophotometer as the change in absorption per minute, varies as the substrate concentration is increased. Use this information to determine the value of K_M and V_{max} for the enzyme by plotting the data using a suitable graphical method.

[S]/mM	ΔA/min
2.50	0.26
5.00	0.37
10.00	0.53
15.00	0.60
20.00	0.63
30.00	0.67

2. The kinetic data showing substrate concentration and initial rate of reaction for an enzyme-catalysed reaction in the presence and absence of an inhibitor is given in the table below. Use this information to determine the apparent Michaelis constant (K_M) and the maximum velocity (V_{max}) for the enzyme by plotting the data using a suitable graphical method and thus determine the nature of the inhibition.

[S] / μmol L^{-1}	V_o / μM min^{-1}	V_o / μM min^{-1}
	No inhibitor	+ inhibitor
1.50	21.00	8.00
2.00	25.00	10.00
3.00	28.00	12.00

4.50	33.00	13.00
8.00	44.00	16.00
16.00	46.00	18.00

3. Describe how a non-competitive inhibitor affects the rate of an enzyme-catalysed reaction. Suggest how you might show experimentally that a particular inhibitor of an enzyme is indeed a non-competitive inhibitor. Sketch a Lineweaver-Burk double reciprocal plot of initial rate (V_o) against substrate concentration that would be expected for an enzyme showing Michaelis-Menten kinetics.

Scroll to the following pages to check your answers.

Answers

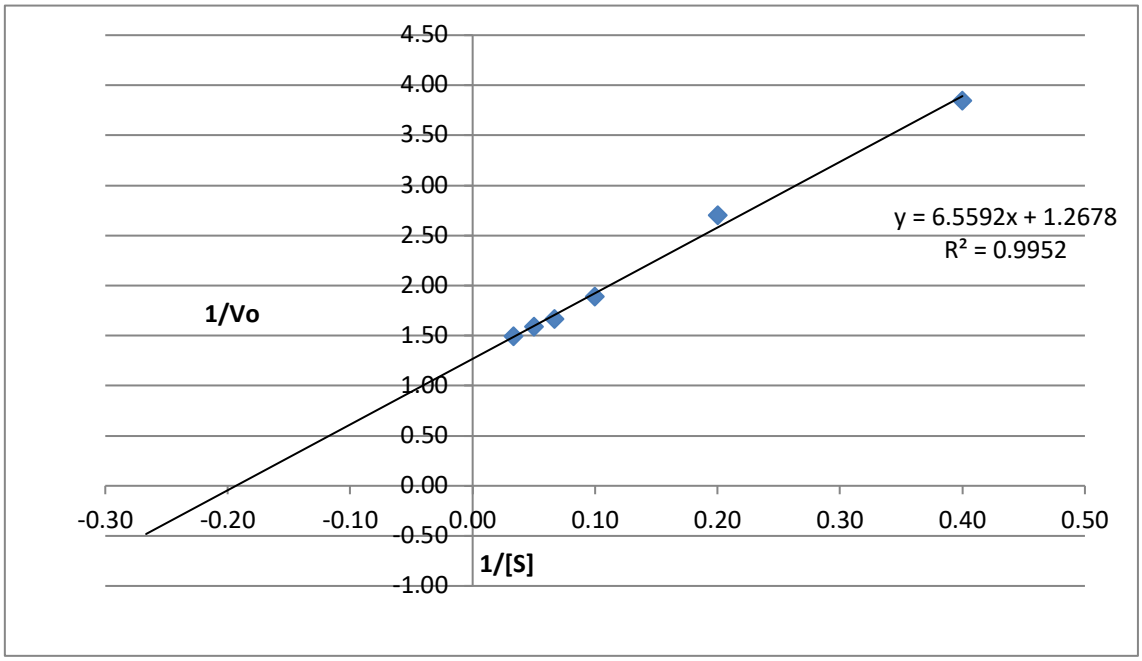
1. The table below shows the result of an experiment designed to determine the apparent Michaelis constant (K_M) and the maximum velocity (V_{max}) for a particular enzyme. The kinetic data shows how the initial rate of reaction, measured in a spectrophotometer as the change in absorption per minute, varies as the substrate concentration is increased. Use this information to determine the value of K_M and V_{max} for the enzyme by plotting the data using a suitable graphical method.

[S]/mM	ΔA/min
2.50	0.26
5.00	0.37
10.00	0.53
15.00	0.60
20.00	0.63
30.00	0.67

Plotting the data graphically in its present form produces a hyperbolic curve (Michaelis-Menten plot), which does not give very accurate values of the enzyme parameters required. A more common approach to obtain reasonably accurate values for K_M and V_{max} is to transform the data to produce a linear graph, which intercepts both the x-axis and y-axis. One such plot is the double reciprocal or Lineweaver-Burk plot.

[S] mM	ΔA/min (V_o)	1/[S]	$1/V_o$
2.50	0.26	0.40	3.85
5.00	0.39	0.20	2.70
10.00	0.58	0.10	1.89
15.00	0.63	0.07	1.67
20.00	0.66	0.05	1.59
30.00	0.78	0.03	1.49

You can solve this problem with the aid of the Excel spreadsheet supplied with this worksheet. A plot of 1/[S] on the x-axis against $1/V_o$ on the y-axis gives the following graph:



The kinetic parameters can be calculated from the properties of the linear graph as follows.

The x-intercept is equal to $-1/K_M$ while the y-intercept is $1/V_{max}$.

Since $-1/K_M = -0.192$, $K_M = 5.217$ mM (the reciprocal of $-1/K_M$ multiplied by –1).

And $1/V_{max} = 1.268$ so $V_{max} = 0.789$ mM min^{-1}.

Other types of linear plots, such as the Hanes-Woolf plot, will produce similar (but not identical) results. To see the differences for yourself, plot the values of [S] against [S]/V and compare the values of K_M and V_{max} obtained by the different approaches.

2. The kinetic data showing substrate concentration and initial rate of reaction for an enzyme-catalysed reaction in the presence and absence of an inhibitor is given in the table below. Use this information to determine the apparent Michaelis constant (K_M) and the maximum velocity (V_{max}) for the enzyme by plotting the data using a suitable graphical method and thus determine the nature of the inhibition.

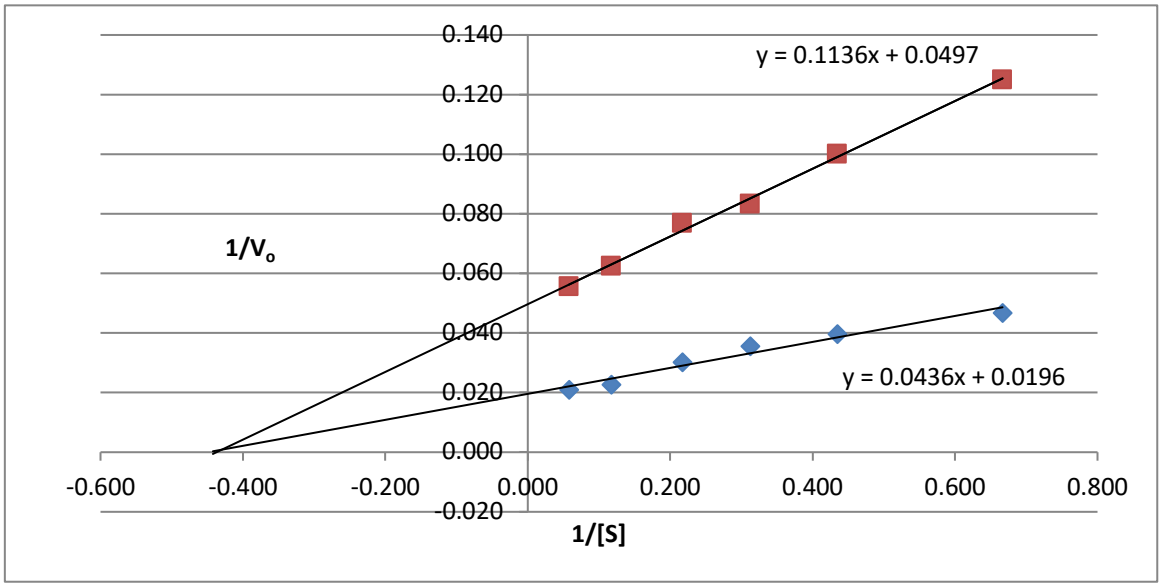
[S] / μmol L^{-1}	V_o / μM min^{-1}	V_o / μM min^{-1}
	No inhibitor	+ inhibitor
1.50	21.00	8.00
2.00	25.00	10.00
3.00	28.00	12.00
4.50	33.00	13.00
8.00	44.00	16.00
16.00	46.00	18.00

Reasonably accurate values for K_M and V_{max} may be obtained by transforming the data in order to plot a linear graph using either a Lineweaver-Burk or Hanes-Woolf method.

[S] μmol L^{-1}	V_o μM min^{-1} no inhibitor	V_o μM min^{-1} inhibitor	1/S	$1/V_o$ no inhibitor	$1/V_o$ inhibitor
1.50	21.40	8.00	0.667	0,047	0.125
2.30	25.20	10.00	0.435	0.040	0.100
3.20	28.00	12.00	0.313	0.036	0.083
4.60	33.00	13.00	0.217	0.030	0.077
8.50	44.00	16.00	0.188	0.023	0.063
17.30	47.50	18.00	0.058	0.021	0.056

It is most efficient to use an Excel spreadsheet to plot the values of 1/[S] on the x-axis against $1/V_o$ on the y-axis. This transforms the hyperbolic Michaelis-Menten curve into a straight line, both in the absence and presence of the inhibitor. The

properties of the straight line enable us to calculate the kinetic properties of K_M and V_{max}.



Investigate the Excel spreadsheet supplied and convince yourself that by knowing the x-intercept, the y-intercept and the slope of the line, the values of K_M and V_{max} for the enzyme can be calculated as per the following table.

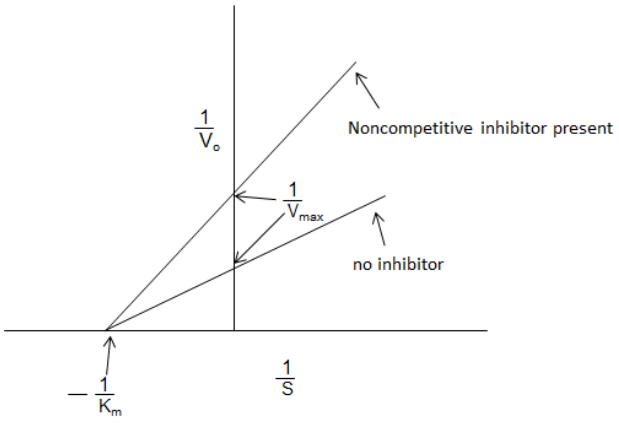
	Enzyme in the absence of inhibitor	**Enzyme in the presence of inhibitor**
slope	0.044	0.114
x-intercept	-0.438	-0.422
y-intercept	0.020	0.048
V_{max}	51.08 μM min^{-1}	2.37 μM min^{-1}
K_M	20.13 μmol L^{-1}	2.30 μmol L^{-1}

3. Describe how a non-competitive inhibitor affects the rate of an enzyme-catalysed reaction. Suggest how you might show experimentally that a particular inhibitor of an enzyme is indeed a non-competitive inhibitor.

Sketch a Lineweaver-Burk double reciprocal plot of initial rate (V_o) against substrate concentration that would be expected for an enzyme showing Michaelis-Menten kinetics.

Non-competitive inhibitors are reversible inhibitors, which bind to an enzyme at a site other than the active site. The binding alters the three-dimensional structure of the enzyme such that the substrate, although still able to bind at the active site, forms the transition state with less efficiency such that the enzyme activity is reduced.

An experiment to determine the Michaelis constant and the V_{max} for the enzyme in the presence and absence of a non-competitive inhibitor gives a characteristic graphical analysis:



The sketch shows that the K_M of the enzyme (the concentration of substrate when the enzyme is functioning at half its V_{max}) remains unchanged in the presence of a non-competitive inhibitor, while V_{max} is reduced.