

**King's College London (KQC) University of London**

**SCIENCE SIMULATIONS LABORATORY**

**ENZYME KINETICS**

**STUDENTS' MANUALS (Version 1.02.2003)**

**Authors M.T. Heydeman and S. McCormick**

**Programmer: R. Lewis and R. Millwood (1982 GW Basic Version)**

**D. Terry (2003 Visual Basic Version)**

**Editors: S. McCormick, (1982 Version), C. Michelsen M.A. Ph.D. (2003 Version)**

**License granted to Michelsen Consulting by agreement with**

**King's College London May 10<sup>th</sup> 1988**

## STUDENTS' MANUAL A - ENZYMES AS CATALYST

Chemical reactions will only occur when the reacting molecules collide during random molecular movement. The speed of the reaction depends on the number of collisions. This in turn depends on the amount of random molecular movement and the concentrations of the molecules. The conditions within living organisms do not always favour a speed of reaction fast enough for the body's requirements. It is necessary for the body to produce and use substances called enzymes. Enzymes are biological catalysts which alter the rate of chemical reactions without affecting the products formed. An enzyme works by bringing together the reacting substances, the substrates, so that they are more likely to react and form the product. An enzyme does not actually take part, but acts as a surface on which the reaction can occur. This is illustrated in Figure A1.

A1 What factors affect the random movement of molecules?

A2 How can the concentration of reacting molecules alter the speed of a reaction?

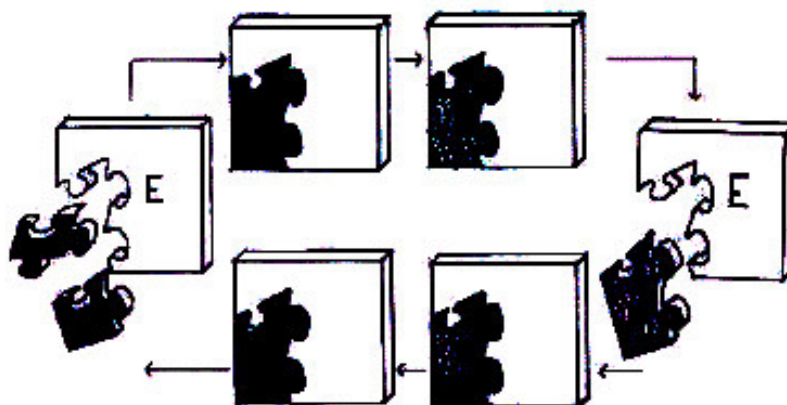


Figure A1 Diagram to show a simple enzyme-catalysed reaction. The enzyme E, links the substrates to its active centre. Then the substrate is converted to the product P while linked to the enzyme. Finally the product is released freeing the enzyme to be used again. Each stage in this process is reversible.

Enzymes are proteins composed of long chains of amino acids. Each has a precisely defined composition, with a complicated structure. The nature of the active site on any particular type of enzyme varies according to specific protein structure. Any one enzyme can only absorb certain substrates and so can only catalyse certain reactions. For this reason thousands of different enzymes are needed within living organisms. If any changes occur to the structure of an enzyme which affects the active site, the enzyme may be no longer able to function as a catalyst.

Although hundreds of different enzymes are known, the same general approach may be used for the study of any of them. The speed of any enzyme-controlled reaction can be measured either by the disappearance of the substrate or by the appearance of the product. The amount measured is plotted against time as shown in Figure A2.

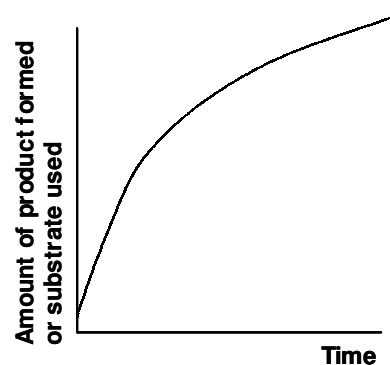


Figure A2 Course of an enzyme-catalysed reaction.

A3 Describe the course of an enzyme-catalysed reaction as shown by the shape of the curve in Figure A2.

A4 What conditions would change the shape of the curve?

The graph shown in figure A2 is known as a progress curve or an enzyme-controlled reaction. The speed or velocity of the reaction at any moment is the slope of the curve at that time, i.e. the rate at which the substrate is disappearing or the product is being formed. Experimenters try to measure the initial velocity of reaction, which is found by drawing a tangent to the progress curve at the start of the reaction as shown in Figure A3.

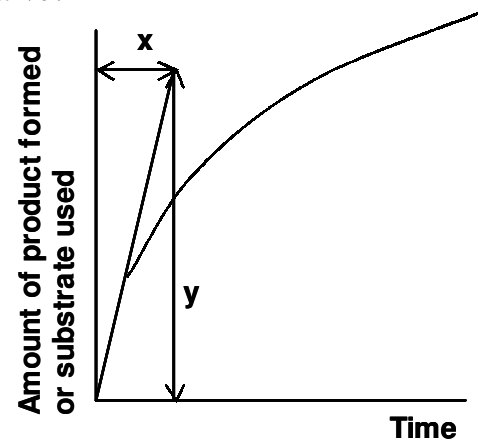


Figure A3 Measuring the initial velocity from the progress curve

It is only at the moment when the reaction starts that all the factors are controlled. From then on substrate concentration falls, back reaction or inhibition may begin and some enzyme may even be destroyed, as proteins are very sensitive to high temperature and extreme pH. However, in practice, measurement over a period of a few minutes will usually give a progress curve that is straight enough for its average slope to be a good approximation to the initial slope. Then in order to obtain the initial velocity of reaction divide the amount of substrate consumed or product formed by the time, i.e.  $y/x$ .

The initial velocities of reaction can be obtained under different conditions and compared to find out the characteristics of an enzyme. The pH, substrate concentration, enzyme concentration, incubation time and temperature can be varied. Usually only one parameter is varied at a time, the rest being kept constant at what are thought to be suitable values to obtain meaningful results. Because this kind of study is concerned with rates of reaction – and hence the idea of movement – it is termed enzyme 'kinetics' from the Greek word meaning 'move'.

## STUDENTS' MANUAL B - THE HYDROLYSIS OF STARCH

The enzyme amylase is found in the saliva in most mammals. It catalyses the breakdown of the large polysaccharide, starch, into the disaccharide, maltose. The reaction is one of hydrolysis, whereby water molecules react at certain parts of the starch molecule releasing links and so breaking the molecule into smaller units as shown in figure B1.

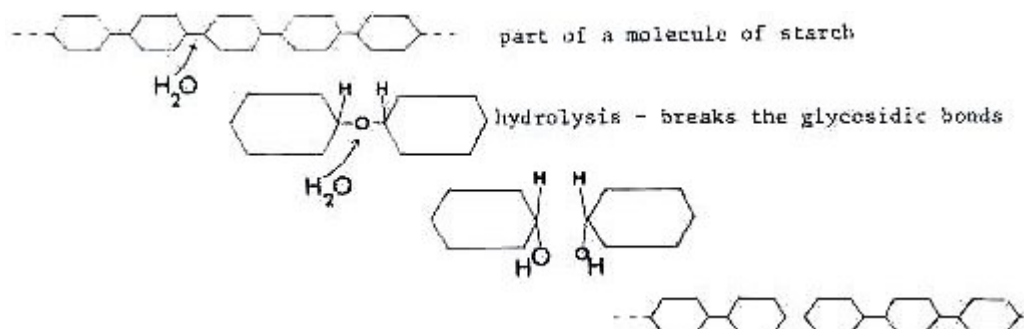


Figure B1 Diagram to illustrate the hydrolysis of starch.

This reaction can be easily demonstrated in the laboratory using human saliva and freshly prepared starch solution.

*B1 This reaction normally occurs in the mouth of a mammal or within the bolus of food as it is swallowed and passed along the oesophagus to the stomach. What conditions of temperature and pH do you consider most suitable for the enzyme reaction?*

The speed of this reaction can be shown either by the progressive disappearance of the substrate, starch, or by the gradual appearance of the product, maltose.

*B2 If a certain amount of saliva was added to a quantity of starch, what chemical tests could you perform to demonstrate:*

- a starch decreases in quantity.*
- b maltose increases in quantity. (Remember, although maltose is a disaccharide, it is a reducing sugar).*

*B3 At what point would you know when the reaction was complete?*

*B4 How could you accurately measure the time it takes for the reaction to be completed?*

Use your ideas from your answers to questions *B1*, *B2*, *B3* and *B4* to design an experiment to demonstrate whether the speed of hydrolysis of starch in the presence of amylase is greater when:

- a There are equal quantities of enzyme and substrate.*
- b There is more enzyme than substrate.*
- c There is more substrate than enzyme.*

When planning your experiments consider the following points:

- a The temperature and pH must be maintained at a constant level throughout the experiment.
- b The experiment should be repeatable so that all solutions used should be of known concentrations and in specific quantities.
- c The experiment should be controlled.

*B5 Explain why different members of the class are likely to obtain slightly different results even if they use exactly the same method with the same quantities and conditions.*

To produce a progress curve for any enzyme reaction (Students' Manual A - figure A2), where the amount of substrate or product is plotted against time, quantitative results are needed. This means that actual measurements of the quantity of substrate, such as starch remaining, or product, such as maltose accumulating, must be taken at regular time intervals. This involves using an instrument such as a colorimeter or chemical analysis procedures both of which are more complicated and time consuming, but necessary in the study of enzyme kinetics. Table B1 shows some typical results that can be obtained by measuring the amount of maltose present at various time intervals during the hydrolysis of starch. The temperature and pH were kept constant.

Time /min	Maltose /mg
0	0
2	1.1
4	1.9
6	2.6
8	3.1
10	3.5
12	3.8
14	3.85
16	3.95
18	4.0
20	4.0

Table B1 The amount of maltose measured at various time intervals during the hydrolysis of starch.

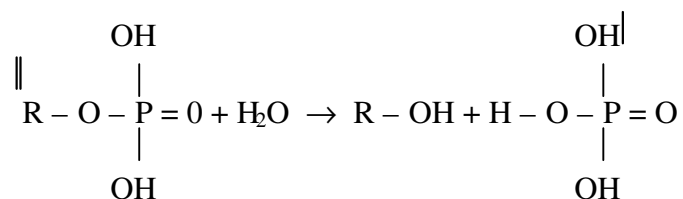
Use these results to draw a progress curve of this enzyme-catalysed reaction.

*B6 What factors do you think could affect the final amount of product formed?*

*B7 What factors could affect the speed of the reaction?*

**STUDENTS' MANUAL C – THE HYDROLYSIS OF NITROPHENYL PHOSPHATE**

The object of this experiment is to follow the course of an enzyme-catalysed reaction, plot the progress curve and estimate the initial velocity of reaction. The enzyme used, a phosphatase, is of widespread distribution. There are phosphates in all living cells and most of these phosphates are hydrolysed by phosphatases. There are several kinds of phosphatases and most are not highly specific, so in our experiments a wide range of organic phosphates can serve as substrates. The type of reaction phosphatases catalyse is shown by the following general equation, in which R stands for any of a wide range of organic chemical groups.



The pH at which the enzyme works may be high or low. You will be told whether 'acid phosphatase' or 'alkaliphosphatase' is provided for your use.

A buffer solution is used to stabilise the pH at a suitable value. You are also provided with a colourless substrate for the enzyme, 4-nitrophenyl phosphate,  $\text{NO}_2\text{C}_6\text{H}_4\text{H}_2\text{PO}_4$ . On hydrolysis this yields a yellow product, 4-nitrophenol,  $\text{NO}_2\text{C}_6\text{H}_4\text{OH}$ . The reaction is as shown in the equation, with R replaced by  $\text{NO}_2\text{C}_6\text{H}_4$ .

The intensity of the yellow colour, which results from absorption of light in the blue-violet region of the spectrum, can be used to measure the concentration of nitrophenol without having to separate it from the other components of the reaction mixture. The more nitrophenol there is in the light path, the more blue-violet light will be absorbed. If we make sure that light always goes through the same thickness of solution then the amount absorbed will be directly proportional to the concentration of nitrophenol in the solution. An instrument called a calorimeter is used to measure the absorption. It is most sensitive when light which is not absorbed by the test solution is excluded. In this experiment a violet or blue filter is used, so that only light of this colour can pass through the mixture. To find out the relationship between the colour measured by the calorimeter and the concentration of the nitrophenol, the colour of each of a series of standard solutions is measured. A graph of the results is plotted; this is called a standard curve. This graph is then used to convert calorimeter readings into concentrations of nitrophenol when unknown solutions are measured.

Sodium carbonate is added to each solution before it is measured to intensify the colour of the nitrophenol and stop any further enzymic action. The sodium carbonate also makes the measurements more sensitive and less urgent, as samples may be kept for an hour or so until it is convenient to measure them.

**Standard curve preparation**

- 1 Make a range of dilutions of nitrophenol in a series of labelled test tubes. First pipette the amounts of 2mM nitrophenol solutions shown in the following table and then use another

pipette to add the amounts of water shown to make a total volume of  $1.5 \text{ cm}^3$  in each test tube.

Volume of nitrophenol/ $\text{cm}^3$	0	0.05	0.1	0.2	0.4	0.7	1.0
Volume of water/ $\text{cm}^3$	1.5	1.45	1.4	1.3	1.1	0.8	0.5

( $1 \text{ cm}^3$  of a 2mM solution contains  $2 \mu\text{mol}$  of the solute)

- 2 Add  $1 \text{ cm}^3$  buffer solution and  $1 \text{ cm}^3$  sodium carbonate solution to each test tube, so that there is a total volume of  $3.5 \text{ cm}^3$ .
- 3 Using water as a blank, measure the light absorbed by each solution in a calorimeter fitted with a violet (or blue) filter.
- 4 Draw a graph of the results, with the quantity of nitrophenol in  $3.5 \text{ cm}^3$  on the x-axis and the calorimeter reading on the y-axis. This is your standard curve and will be used to convert readings into micromoles of nitrophenol in subsequent work. The x-axis is marked in these units because you will have  $3.5 \text{ cm}^3$  samples and will want to know how many micromoles of nitrophenol have been liberated by the enzyme. You can read it off the graph directly.

#### Measuring enzyme activity

- 1 Incubate in a water-bath at  $30^\circ\text{C}$  five test tubes labelled 30, 20, 10, 5 and 0. The number on each tube is the number of minutes for which each is to be incubated with enzyme and substrate.
- 2 In each test tube place  $0.5 \text{ cm}^3$  4-nitrophenyl phosphate solution (substrate),  $1.0 \text{ cm}^3$  buffer solution and  $0.5 \text{ cm}^3$  water.
- 3 To start the reaction, add  $0.5 \text{ cm}^3$  enzyme solution to tube 30, noting the time. Mix the contents of the tube well and replace it in the  $30^\circ\text{C}$  water-bath to be incubated. You will be stopping the reaction in this tube just 30 minutes after the observed starting time.
- 4 Similarly start the reaction in the other tubes, except for the tube labelled 0, at intervals of about 1 minute, noting the time exactly for each. To tube 0 add  $1.0 \text{ cm}^3$  sodium carbonate solution before you add the enzyme.
- 5 Stop the reaction in each tube after the appropriate number of minutes, by adding  $1.0 \text{ cm}^3$  sodium carbonate solution and mixing well. Measure the colour in each mixture as before, using a blank of water.

Use your standard curve to convert calorimeter readings to  $\mu\text{mol}$  nitrophenol in  $3.5 \text{ cm}^3$ . These amounts are those actually released by the enzyme, as the total volume measured was  $3.5 \text{ cm}^3$ . Plot the results, with amount of nitrophenol in  $3.5 \text{ cm}^3$  on the y-axis and incubation time on the x-axis.

*C1 Is the curve you obtain similar to the progress curve shown on Students' Manual A, Figure A2?*

Draw a tangent to the curve at the start of the reaction and measure its slope.

*C2 Over what period of time after starting was the reaction rate constant?*

*C3 What is the initial velocity of reaction (in  $\mu\text{mol}$  nitrophenol per  $\text{cm}^3$  of enzyme per hour)?*

*C4 What are the sources of error in measuring enzyme activity in the Laboratory?*

**STUDENTS' MANUAL D - SIMULATED ENZYME-CATALYSED REACTIONS**

A thorough investigation in the laboratory of an enzyme catalysed reaction is time consuming. However, so that you can have experience in planning investigations, drawing conclusions from the results and using your conclusions as the basis of new investigations, the computer program *Enzyme Kinetics* simulates a series of enzyme-catalysed reactions and gives the results as soon as you have put in the conditions of the reaction.

The simulation allows you to choose, for any of the six enzymes, one varying factor. You state the range over which it varies and set values for the other factors. For example you may want to set values for pH, temperature and starting volumes of substrate and enzyme and consider the product formed over time intervals varying between 0 and 60 minutes. Similarly you may prefer to maintain a constant incubation time, but vary another factor such as temperature over a certain range. In this way the program *Enzyme Kinetics* allows you to perform quickly and easily a range of investigations on the six enzymes.

The minimum and maximum possible values for the range of any of the factors and the minimum possible interval between them are shown in Table D1.

Factor	Minimum value	Maximum value	Minimum interval
pH	0	14	1
Substrate volume/cm <sup>3</sup>	0	9	1
Enzyme volume/cm <sup>3</sup>	0	9	1
Incubation time/min	0	60	10
Temperature/°C		100	10

Table D1 Table showing the acceptable range of values for any of the factors in *Enzyme Kinetics*.

In the simulation the test tube always has at least 1 cm<sup>3</sup> buffer solution in it. The total volume in which the reaction takes place is 10 cm<sup>3</sup> the capacity of the test tube. If the volumes you provide exceed 9 cm<sup>3</sup> you will be asked to restate new volumes. If the total volumes are less than 9 cm<sup>3</sup>, extra water is added to the test tube.

Values set are displayed in the table at the top of the screen. When all values have been entered click on the *Process* button and the computer program will calculate the amount of product at eleven, about equally-spaced time intervals within your chosen range. The results are displayed in a table and plotted on a graph as shown in Figure D2.

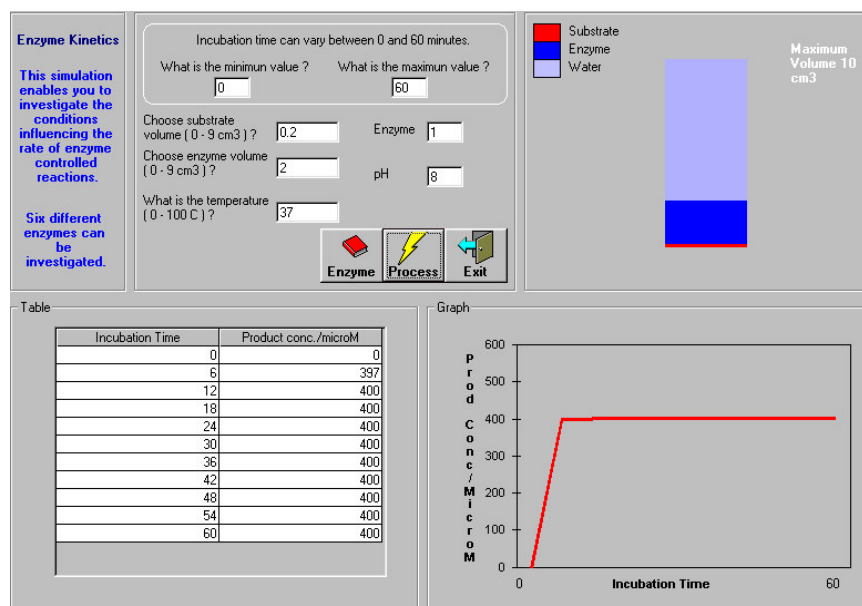


Figure D2 Screen printout from *Enzyme Kinetics* showing typical results.

The substrate solution has a concentration of 20 mM and so when the substrate volume is set the initial concentration of substrate in the reaction mixture can be calculated in the following way:

$$\begin{aligned}
 \text{Volume 20 mM substrate used} &= 0.2 \text{ cm}^3 \\
 \text{Total volume of reaction mixture} &= 10 \text{ cm}^3 \\
 \text{Initial concentration of substrate} &= 20 \times \frac{0.2}{10} \text{ mM} \\
 &= 0.4 \text{ mM} \\
 &= 400 \text{ } \mu\text{M}
 \end{aligned}$$

After the results have been plotted, you can change any of the conditions. When you have entered the new value click on the *Process* button to obtain the new results.

To become familiar with using the program, try the following investigation. Using enzyme 1 at pH 8 and temperature 22°C produce a progress curve by varying the incubation time between 0 and 50 minutes. Select an enzyme volume of 2 cm<sup>3</sup> and a substrate volume 0.2 cm<sup>3</sup>. Record your results and repeat the experiment using a substrate volume of 5 cm<sup>3</sup>.

*D1* How do your computer outputs compare with the progress curve in *Students' Manual A, Figure A2*?

*D2* Why is the product concentration unchanged in the last 10 minutes of the first reaction?

*D3* What differences in the results are there between the first and second reactions?

**STUDENTS' MANUAL E - FINDING THE OPTIMUM pH**

Enzymes are sensitive to pH. For every enzyme there is an optimum pH at which it functions most effectively and for most there is only a restricted range of pH in which they will work at all. For this reason the pH must be maintained at certain levels within the body for the correct functioning of particular enzymes.

*E1 What mechanisms exist within a mammal to maintain the correct pH within:*

- a the stomach?*
- b the duodenum?*
- c the blood stream?*

The existence of an optimum pH is a consequence of the acidic and basic groups present in enzymes and other proteins. These groups change their state of charge as pH varies as shown in Figure E1. In a low pH, acid conditions, the additional hydrogen ions ( $H^+$ ) tend to ionise the basic  $-NH_2$  groups so that they become positively charged ( $-NH_3^+$ ). In a high pH, hydrogen ions tend to be removed from acidic groups ( $-COOH$ ) leaving them negatively charged ( $COO^-$ ). If a basic or acidic group should be located in or near an enzyme's active site its state of charge will affect enzyme activity. If there are two such groups, one may permit activity only below a particular pH and the other only above some other pH. The enzyme will therefore be active only over a narrow range of pH. This situation is the rule, although there are exceptions. The substrate may also vary in charge according to the pH. An enzyme is so specific that it normally accepts its substrate in only one of the substrate's states of charge.

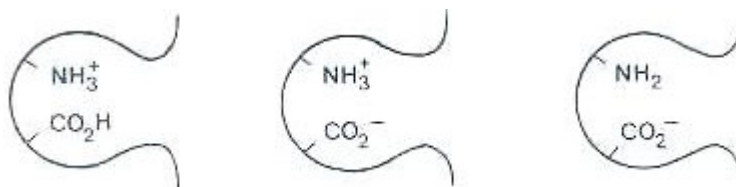


Figure E1 The effect of pH on the basic ( $-NH_2$ ) and acidic ( $-CO_2H$ ) groups.

Use the program to find the optimum pH of the enzyme-catalysed reaction, that is the pH giving the most product. You may need to run the program more than once, perhaps with a wide range and then with a narrower range of pH values. Use  $1\text{ cm}^3$  of enzyme solution and a large substrate volume, and allow the reaction to run for 10 minutes. When you want to rerun the program with different ranges of pH select vary pH (*What is to be varied?*), enter the new maximum and minimum values of the range and click on the *Process* button to obtain the results.

*E2 What is the optimum pH of the enzyme investigated?*

The enzyme activity at the optimum pH can be expressed in micromoles of product formed per minute per  $\text{cm}^3$  of enzyme solution. For example a final micromolar product concentration of 500 obtained after 10 minutes using  $1\text{ cm}^3$  of enzyme solution has an enzyme activity of:

$$500 \times \frac{10}{1000} \times \frac{1}{10} = 0.5 \text{ micromoles per minute per } \text{cm}^3 \text{ of enzyme}$$

*E3 What is the enzyme activity at the optimum pH?*

### STUDENTS' MANUAL F – VARYING THE TEMPERATURE AND INCUBATION TIME

When you know the optimum pH for any enzyme you can investigate the relationship between product concentration and time by running the program at different temperatures.

Enzymes like other proteins are affected by extreme temperature changes. The protein structure on which the activity of the enzyme depends becomes irreversibly changed at high temperatures and the protein is said to be denatured. At low temperatures the structure may become temporarily distorted by the formation of ice crystals in certain parts of the molecule. In either case if the structure of the active site is altered the enzyme will no longer be able to adsorb substrates and catalyse reactions.

Try using the program to investigate

- a a temperature you consider very suitable for life,
- b a temperature 15-20°C lower,
- c a temperature 15-20°C higher.

Each run should be at the optimum pH for the enzyme and use 1 cm<sup>3</sup> of enzyme solution, a large substrate volume and vary time between 0 and 60 minutes. Record the results you obtain from each run and plot a set of three progress curves on the same axes.

*F1 What is the relationship between the product concentration and the time at the three temperatures?*

*F2 Suggest two possible reasons why the progress curves are not straight lines?*

*F3 Why do the progress curves have different initial slopes?*

*F4 Why do the progress curves have different degrees of curvature?*

If time permits you can try other temperatures and other conditions to support your answers to these questions. Try varying the temperature with two or three different fixed times to answer this question.

*F5 Is there a fixed 'optimum temperatures' for the enzyme-catalysed reaction, which does not depend upon time?*

**STUDENTS' MANUAL G – VARYING ENZYME AND SUBSTRATE CONCENTRATIONS**

In the investigations described in the previous Manuals both the enzyme and substrate concentrations were kept constant. However, they are factors that may vary in nature and the concentrations available for the enzyme catalysed reaction may affect the course of the reaction.

*G1 What factors could vary the concentrations of substrates in nature?*

*G2 Why are there variations in the amounts of enzyme available for reactions?*

Use the computer program to investigate the effect of varying substrate concentrations. Choose a small enzyme volume and select an appropriate pH and temperature.

*G3 At what initial substrate concentrations is the most product formed?*

*G4 Is more product formed when the initial concentration of substrate is increased?*

*G5 Is the optimum substrate concentration the same for all six enzymes?*

Now try varying the enzyme concentration. Choose a small substrate volume and select a suitable pH and temperature for the enzyme.

*G6 How does the enzyme concentration affect the amount of product and the speed at which it is formed?*

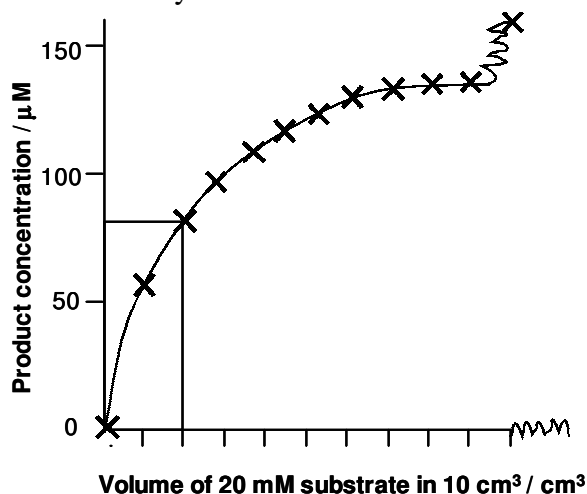
*G7 Is this the same for all six enzymes?*

The initial substrate concentration will determine the amount of product that can be formed, but the rate at which this occurs will depend on the amount of substrate compared to enzyme since the enzyme molecules provide the 'active sites' for the reaction to occur. The more enzyme molecules present the more substrate molecules can react at any one time. In addition enzymes have different speeds of action. This is known as the turnover number, that is the number of substrate molecules that one molecule of enzyme turns into product per minute. This varies from a few to several million, depending on the enzyme. The enzyme catalase found in the liver has one of the highest turnover numbers known. It can 'process' almost six million substrate molecules in one minute. Its action can be demonstrated by dropping a small amount of fresh liver into hydrogen peroxide solution. The formation of one of the products, oxygen, can be seen by the fizzing of the solution.

## STUDENTS' MANUAL H – THE MICHAELIS CONSTANT

Two important quantities can be derived from your results on the effect of varying substrate concentration – the maximum velocity of reaction of which the enzyme is capable,  $V$ , and the substrate concentration corresponding to half that velocity of reaction, known as the Michaelis constant,  $K_m$ . These two quantities can be estimated by finding the value towards which the product concentration tends as the enzyme becomes saturated by an infinitely large substrate concentration. This is proportional to  $V$  and the substrate concentration which gives half this product concentration will be  $K_m$ . The two values must be in the correct units. The Michaelis constant,  $K_m$ , should be in milli or micromolar final substrate concentration.  $V$  should be in units of micromoles product formed per minute per  $\text{cm}^3$  of enzyme solution.

Figure H1 Results derived from a computer simulation of the effect of initial substrate concentration on the velocity of the reaction. The mixture was incubated for 2 minutes at pH 8.1 and a temperature of  $30^\circ\text{C}$  with  $1 \text{ cm}^3$  of enzyme.



The following worked example uses the data in Figure H1. From the graph it looks as though the product concentration will never go higher than  $155 \mu\text{M}$ , but does eventually reach this value. The substrate concentration giving half this much product,  $77.5 \mu\text{M}$ , is  $0.203 \text{ cm}^3$  of 20 mM solution in  $10 \text{ cm}^3$  final volume.

The concentration of  $0.203 \text{ cm}^3$  of a 20 mM solution in a final volume of  $10 \text{ cm}^3$

$$= 20 \times \frac{0.203}{10} \text{ mM}$$

$$= 0.406 \text{ mM}$$

The Michaelis constant is therefore 0.406 mM

The amount of product at a concentration of 155 mM in  $10 \text{ cm}^3$

$$= 155 \times \frac{10}{1000} \mu\text{mol}$$

$$= 1.55 \mu\text{mol}$$

If  $1 \text{ cm}^3$  enzyme produces  $1.55 \mu\text{mol}$  in 2 min, it produces  $\frac{1.55}{2} = 0.775 \mu\text{mol}$  in 1 min.

Therefore in our example,  $V$  is  $0.775 \mu\text{mol min}^{-1} \text{ cm}^{-3}$ .

H1 What are the values of  $V$  and  $K_m$  for your enzyme, estimated by this method?

## STUDENTS' MANUAL I – THE MICHAELIS-MENTEN EQUATION

The velocity of reaction is related to the substrate concentration by an equation involving  $V$  and  $K_m$ , known as the Michaelis-Menten equation:

$$v = \frac{V S}{S + K_m} \quad \text{where } v = \text{velocity of reaction} \\ \text{and } S = \text{substrate concentration}$$

This gives a curve such as that shown in Figure H1 on Manual H. However, if the equation is inverted, it becomes a straight line:

$$\frac{1}{v} = \frac{S + K_m}{VS} \\ = \frac{S}{VS} + \frac{K_m}{VS} \\ = \frac{1}{v} + \frac{K_m}{V} \times \frac{1}{S}$$

If  $\frac{1}{v}$  is plotted against  $\frac{1}{S}$  this will give a straight line of slope  $K_m/V$  cutting the  $1/v$  axis at  $1/V$  (figure I1)

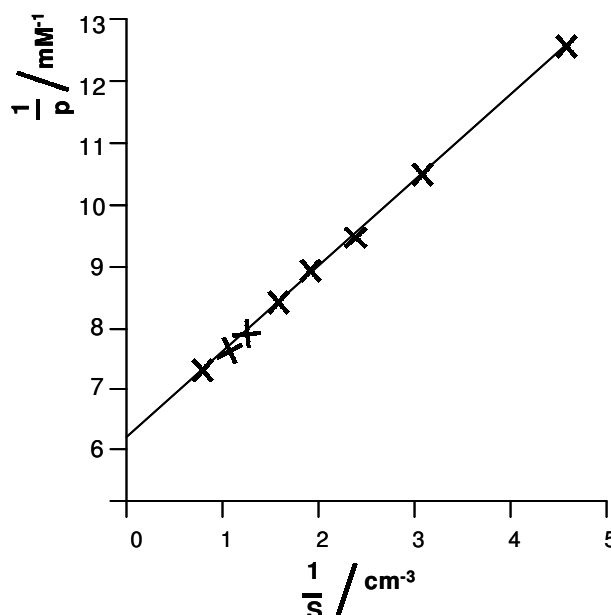


Figure I1 Double-reciprocal (Lineweaver-Burk) plot of the data in Figure H1.

This is known as the Lineweaver-Burk plot, after two biochemists who devised it in the 1930s. It is also called the 'double-reciprocal' plot. As the final product concentration,  $p$ , is proportional to  $v$  and the substrate volume,  $s$ , is proportional to the substrate concentration,  $S$ ,  $1/p$  and  $1/s$  may be plotted instead of  $1/v$  and  $1/S$ . Figure I1 shows the data of Figure H1 replotted as a double-reciprocal plot using the following values:

Substrate volume, $s/\text{cm}^3$	$\frac{1}{S} / \text{cm}^{-3}$	Product concentration, $p/\mu\text{M}$	$\frac{1}{p} / \text{mM}^{-1}$
0.0	–	0	–
0.1	10.00	51	19.61
0.2	5.00	77	12.99
0.3	3.33	94	10.64
0.4	2.50	105	9.52
0.5	2.00	113	8.85
0.6	1.67	119	8.40
0.7	1.43	123	8.13
0.8	1.25	127	7.87
0.9	1.11	130	7.69
1.0	1.00	132	7.58

You can calculate the values of  $V$  and  $K_m$  from the data in Figure I1 in the following way.  $1/V$  is the value of  $1/v$  when  $1/S$  is zero. When  $1/s$  is zero  $1/p$  is 6.21 mM.

$$\begin{aligned} p &= \frac{1}{6.21} \text{ mM} \\ &= 0.161 \text{ mM} \\ &= 161 \text{ } \mu\text{M} \end{aligned}$$

The amount of product at a concentration of 161  $\mu\text{M}$  in 10  $\text{cm}^3$

$$\begin{aligned} &= 161 \times \frac{10}{1000} \text{ } \mu\text{mol} \\ &= 1.61 \text{ } \mu\text{mol} \end{aligned}$$

This amount of product is formed in 2 minutes using 1  $\text{cm}^3$  enzyme.

$$\begin{aligned} V &= \frac{1.61 \text{ } \mu\text{mol min}^{-1} \text{ cm}^{-3}}{2} \\ &= 0.805 \text{ } \mu\text{mol min}^{-1} \text{ cm}^{-3} \end{aligned}$$

The slope in the line in Figure I1 is proportional to  $K_m / V$ , so that multiplying by the product concentration gives the substrate concentration corresponding to  $K_m$ . From Figure I1 the slope is 1.35.

Substrate volume corresponding to  $K_m$

$$\begin{aligned} &= 1.35 \times 0.161 \text{ cm}^3 \\ &= 0.217 \text{ cm}^3 \end{aligned}$$

0.217  $\text{cm}^3$  of a 20 mM solution in 10  $\text{cm}^3$  is a concentration of  $20 \times \frac{0.217}{10}$  mM

or 0.434 mM

Therefore  $K_m$  is 0.434 mM

This ' double-reciprocal' plot will only give a good estimate of  $K_m$  and  $V$  if the experimental errors are minute or absent as in this simulated reaction. Real data usually have enough variation to make this method inadequate, although it is still used by biochemists who should know better!