

Practical 6 - The effect of inhibitors on enzyme activity




This practical focuses on making measurements and observations, recording and presenting data, analysis, drawing conclusions and evaluating methods. You will also develop other assessed skills throughout the practical.

Intended learning outcomes

By the end of this practical you should be able to:

- Experience relevant methods, analysis and conclusion.
- Describe and explain the effect of a non competitive inhibitor on enzyme activity.
- Evaluate procedures.

Safety Information

	You should wear eye protection throughout this practical.
	Amylase is harmful . Avoid contact with eyes or skin.
	Iodine solution is harmful . Avoid contact with eyes or skin. It will stain skin or clothes.

Background information

- A non competitive inhibitor binds to a part of the enzyme away from the active site
- The shape of the enzyme is changed, thus changing the shape of the active site
- No enzyme-substrate complexes can be formed, hence no product produced
- Increasing the amount of substrate does not overcome the effect of this type of inhibitor

You will investigate the effect of increasing the amount of Lead nitrate on the hydrolysis of starch by the enzyme amylase.

- Read the information above
- Identify and write down the dependent and independent variables
- Write down a hypothesis
- Draw a sketch graph to show what you think will happen
- Identify any variables that should be controlled and outline how this should be done

A colorimeter should be used to compare the colours of the solutions obtained after a given time.

Method

Preparations and making observations

1. Set up a thermostatically controlled water bath set at 40°C
2. Label six boiling tubes A – F and place in a test tube rack
3. Add 10cm³ starch solution to each tube
4. Add the following quantities of Lead nitrate to 5cm³ distilled water in six test tubes labelled 1 – 6 : 0g, 0.1g, 0.2g, 0.3g, 0.4g, 0.5g and shake to ensure it dissolves
5. Pour contents of tube 1 into boiling tube A, tube 2 into boiling tube B etc.
6. Add 5cm³ amylase solution to each of the boiling tubes, agitate well and start the timer after placing the tubes into the water bath
7. Allow the reaction to proceed for 20 minutes
8. During this time adjust the colorimeter using a solution of 1cm³ iodine solution in a boiling tube containing 10cm³ starch solution and 5cm³ distilled water. Set the colorimeter to 0% transmission with this solution
9. After 20 minutes add 1cm³ iodine solution to each tube
10. Test each of the tubes A – F in the colorimeter noting down the absorbance for each tube and record in a table. (Note for centres without access to a colorimeter the method could be adapted to using a spotting tile with one drop of iodine in each well. At intervals of one minute a drop of the reaction mixture is placed on the tile and the time taken for the black colour to disappear noted.)

Write-up

- Record your results in a clear table ensuring units are put in headers where possible.
- Plot a graph of transmission against mass of lead nitrate added. (If experiment done using spotting tile method a graph of rate of reaction against mass of lead nitrate added should be drawn)
- Explain your findings using your knowledge of enzymes and inhibitors.
- Assess the reliability of the results obtained and suggest any modifications you could make to improve the experiment.
- Why was it necessary to control the temperature of the reaction?
- What further experiments could be done to investigate non-competitive inhibitors?
- What are the advantages of repeating an experiment?
- Suggest why the iodine solution was not added at the same time as the other solutions

Practical 6 - Lesson Plan

The effect of inhibitors on enzyme activity

Context

A practical investigation set in the context of 9700 syllabus – Enzymes and enzyme inhibitors

Key aims of the lesson

This practical is designed to develop the skills of observation, analysis and evaluation.

Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain the effect of a non competitive inhibitor

Resources required

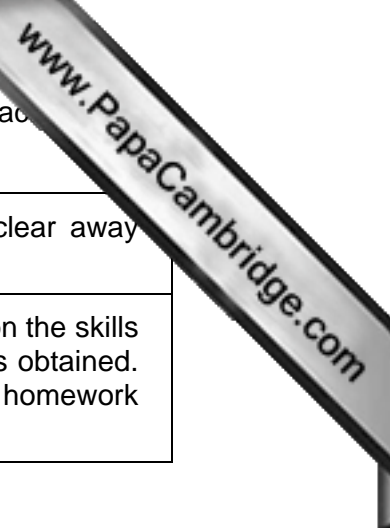
White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	Preparation – Student worksheet given out for students to read in preparation for the practical lesson. To consider identification of the variables, formulate a hypothesis and review previous learning on enzymes.
0 - 3	Introduction to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	Context – review of enzyme action,
5 - 8	Introduction to method – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns with the use of chemicals.
8 - 40	Carrying out the practical – students carry out the practical work. Whilst they are waiting for the 20 minute period they can write up the first part, identifying variables, hypothesis, results table. Teacher to demonstrate the use of colorimeter to those students unfamiliar with this piece of equipment.



40 - 50	Obtain results – Students enter results into table and clear away apparatus as soon as they have finished
50 - 60	Drawing together the threads – Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Practical write up to be completed in flowing lesson or as homework activity

Useful information

- For centres without access to a colorimeter the practical results will not be as accurate but will still be of an objective nature. The pupils could evaluate to suggest improvements to include a more objective measurement.
- If the centre does not have access to thermostatically controlled water baths, manually controlled ones could be substituted or left out altogether but the need for temperature control needs to be discussed in the evaluation.

Other Discussion / evaluation points should include:

- explanation of the shape of the graph
- the differences between competitive and non competitive inhibitors needs to be emphasised
- for students unable to obtain a full set of results the following could be used for analysis

Mass of Lead nitrate / g	Transmission / arbitrary units
0.0	85
0.1	52
0.2	37
0.3	27
0.4	23
0.5	21

Practical 6 - Technical information

The effect of inhibitors on enzyme activity

The apparatus and materials required for this practical are listed below.


The amount of apparatus listed is for one student or one group of students if they are to work in groups.


1. 6 boiling tubes.
2. 6 test tubes
3. test tube rack
4. labels/marker pen
5. 1% starch solution – allow 100cm³ per repeat
6. Amylase solution 1% - allow 50cm³ per repeat
7. Iodine solution – allow 10cm³ per repeat
8. Distilled water – 50cm³ per repeat
9. 10cm³ graduated pipette / measuring cylinder / syringe
10. Stopclock
11. colorimeter cuvettes

Additionally each student will require access to a colorimeter, thermostatically controlled water bath, sink & running water.

If using spotting tile method also add 1 x spotting tile and glass rods.

Safety Precautions/Risks.

Amylase = H 

Iodine solution = H 

A risk assessment should be carried out as a matter of course.