

14. Experiments with enzymes – planning an enzyme concentration vs. rate experiment

Student Sheet

In this experiment you will use your knowledge of previous rate experiments, and the information given below, to plan an investigation of the effect changing the concentration of an enzyme has on the rate of the reaction it catalyses. The reaction involved is the hydrolysis of urea, using urease as the enzyme.

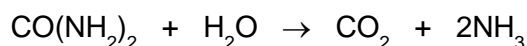
Intended lesson outcomes

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

- Produce a detailed plan of how the experiments will be performed
- Deduce the quantities of solutions to be used
- Identify the apparatus needed
- Decide on how the results will be analysed and evaluated
- Assess the risks involved and suggest appropriate safety precautions

Background information

Urease is an enzyme that breaks down urea, $\text{CO}(\text{NH}_2)_2$, into carbon dioxide and ammonia. The production of ammonia raises the pH of the solution and can be detected by using a suitable acid-base indicator.



Urease is active over a wide range of pH so any number of indicators can be used provided that the original solution is buffered at the right level to start with. You have the choice of two indicators, **bromothymol blue** and **phenolphthalein**. Don't forget that enzymes are temperature sensitive.

Bromothymol blue ($pK_{\text{in}} = 7.0$) has a colour change, from yellow in acid to blue in alkali, which occurs around pH 6 to 8. The mid-point colour, turquoise, occurs at about pH 7.5 and the colour transition from yellow, through turquoise to blue is quite sharp. A control tube containing pH 7.5 buffer and indicator may be used to help identify the end-point.

Phenolphthalein ($pK_{\text{in}} = 9.3$) has a colour change, from colourless in acid to red in alkali, which changes from colourless to pink at a higher pH than the change with bromothymol blue, and the change from colourless to pink occurs only very slowly. The colour becomes a more intense pink with time, so the end-point is the **first** faint pink coloration.

About 4 drops of either indicator would normally be used.

The method to be used in this investigation is based on a 'clock reaction' type of experiment for studying reaction rates – remember the 'iodine clock' experiment. The time taken for sufficient ammonia to be produced to overcome the buffer present is being measured. This is determined by measuring the time taken for sufficient ammonia to be produced to trigger the end-point of the indicator.

The pH of the buffer used **must not** be too close to the pH at the end-point of the indicator; otherwise the end-point will be reached too quickly.

Experimental details

In a similar experiment, a mixture containing 3.00 cm^3 of urea solution, 1.00 cm^3 of buffer solution, 1.50 cm^3 of urease solution, 0.50 cm^3 of water and 4 drops of indicator solution took 32 seconds to reach the end-point of the indicator.

Plan

Your plan should include the following:

- A description of **how** you would mix and **measure** the reagents. You may assume you are supplied with aqueous solutions of urea, urease, buffer (pH 5 or pH 7 as you choose) and your chosen indicator. You also have a supply of distilled water and whatever equipment you wish.
- Details of how you would ensure that your experiments provide a *fair test*.
- A description of how you would measure the time taken for the reaction to reach your selected end-point.
- Details of the number and range of experiments you would perform to enable you to determine the effect of enzyme concentration on the rate of the reaction.
- A results table showing how your results would be recorded and how these data would be manipulated so that you could analyse your results graphically.
- An explanation of how you would use graphical methods to determine the effect of enzyme concentration on the reaction rate. You should not invent results but you may sketch one or more graphs to help you in your explanation.
- A brief statement of how you analyse your graphical results and what further experiments you may need to perform as a result of your analysis.
- A brief outline of any safety factors involved and the measures you would take to reduce or eliminate them.

Safety

A full risk assessment with reference to materials safety data sheets should be carried out for your plan.

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Teachers' Notes

Planning exercises work best when students have sufficient knowledge of the topic not to need the support of books or notes. As planning may be a new skill to many students it is recommended that at the beginning of the course, group work is used so students can pool ideas and learn from each other. As students become more confident, they can progress to individual work, and finally to practising planning exercises under exam conditions.

From the student's point of view, often the most difficult part of a planning exercise is getting started. Be prepared to give help early on in the learning process, but encourage students to be more independent as the course progresses.

The main questions you might wish to consider when assessing the plan are:

- is the method used clearly described?
- have appropriate techniques, reagents and pieces of apparatus been selected?
- is there an appreciation of scale and precision (including relevant calculations)?
- is the suggested analysis/evaluation of the data obtained complete and workable?
- have all the appropriate safety factors been properly considered?

The following list of points will help you work through the students' plans methodically and give appropriate feedback or marks.

Equipment

- Use of syringe/pipettes/burettes to measure volumes
- Suitable container of specified capacity
- If appropriate, a suitable device for holding the containers – e.g. a test tube rack
- Timer reading to a specified degree of precision

Procedure

1. Chooses bromothymol blue as indicator. Explains choice in terms of sharp end-point and rapid transit through colour change.
2. If using bromothymol blue, sets up a control test-tube by adding sensible volume, e.g. 5 cm³, of pH 7.5 buffer and adding 4 drops of the indicator.
3. If using phenolphthalein, reference to the first sign of pink colour.
4. Specifies an appropriate buffer solution; pH 5 for bromothymol blue or pH 7 for phenolphthalein
5. Specifies a suitable **number** and **range** of experiments to be done.
6. Keeps volume constant by adjusting the volume of water added.
7. Keeps all solution volumes, apart from the enzyme solution, constant.
8. Adds 4 drops of chosen indicator.
9. Sensible mixing procedure, adding the enzyme last.
10. Adds final reagent and starts timer.
11. Stops timer at defined/described end-point.
12. Records data in results table to a degree of precision appropriate to the apparatus used.
13. Repeats with different mixtures.

Appendix 2

14. Suggests the use of an effective form of temperature control.

Analysis

1. Calculates $1/\text{time}$ to represent rate. (May suggest logarithmic graphs to determine rate order.)
2. Sketches expected graphs of reaction time (t) against volume of urease, and rate ($1/t$) against volume of urease.

Evaluation

Suggests how the analysis might be evaluated in terms of:

1. Identifying anomalous results
2. Comparing the graphical results with known outcomes.
3. Deducing that the graph of rate vs. volume graph should show a straight line, starting from origin.

Safety

Identifies **two** appropriate risks and suggests appropriate targeted precautions.

Technical information

- Access to hazard cards or equivalent safety data
- When candidates have some experience in planning, this exercise could be carried out in exam-style conditions with a time limit of around 1 hour

Extension work

You may wish to perform this exercise as a class practical, either by using the students' own plans, or by using the method described below. Students should prepare their own results table and plot the appropriate graphs. They should then analyse and evaluate their results.

The effect of enzyme concentration on reaction time

The background information for this exercise is as for the planning exercise.

Procedure

1. Decide which indicator you are going to use (either phenolphthalein or bromothymol blue).
2. If using bromothymol blue, set up a control test-tube by adding 5 cm^3 of pH 7.5 buffer and adding 4 drops of the indicator. This shows the turquoise mid-point colour change that can be used to determine a 'standard' end point for the reaction.
3. Label 8 test tubes 1 to 8.
4. Add the required buffer solution (pH 5 for bromothymol blue and pH 7 for phenolphthalein), water and urea solution to each of the tubes as shown in **the table below**.
5. Add 4 drops of the indicator to each tube.
6. Add the required amount of urease (from **the table below**) and start timing.
WARNING: Tubes 7 and 8 can be very quick!
7. Shake the tubes to ensure the contents are mixed thoroughly. It may be necessary to shake the tubes during the experiment to ensure the colour is the same throughout the solution
8. Record the time when each tube shows the correct end-point colour. This is either turquoise for bromothymol blue or the faintest hint of pink if using phenolphthalein.

Appendix 2

9. Plot the reaction time against the volume of urease used.
10. The rate of reaction is fastest in the mixture that gives the shortest reaction time; so the rate of the reaction can be approximated to $1/\text{reaction time}$. Plot a graph of rate of reaction against the volume of urease used.

Test tube	Urea / cm ³	Water / cm ³	buffer* / cm ³	Urease / cm ³
1	3.00	1.90	1.00	0.10
2	3.00	1.80	1.00	0.20
3	3.00	1.70	1.00	0.30
4	3.00	1.60	1.00	0.40
5	3.00	1.40	1.00	0.60
6	3.00	1.20	1.00	0.80
7	3.00	1.00	1.00	1.00
8	3.00	0.50	1.00	1.50

- Use pH 5 buffer if using bromothymol blue.
- Use pH 7 buffer if using phenolphthalein.

Technical information

Requirements per student/group

Apparatus

- nine test tubes (eight of them labelled 1 to 8) and rack
- access to distilled water
- three 1 cm³ syringes and one 5 cm³ syringe, or equivalent pipettes / graduated pipette
- stopclock/timer

Materials

- access to bromothymol blue solution
- access to phenolphthalein solution
- access to 3% **urease** solution: add 3.0 g of urease active meal to 100 cm³ of distilled water. Stir/shake the solution for at least 5 minutes to extract the urease. Filter off the undissolved material (this can be quite slow). This solution should be freshly prepared.
- access to 2% **urea** solution: dissolve 20 g urea in 1 dm³ of distilled/deionised water.
- access to pH 5 **buffer** solution: add 2.4 g of buffer powder pH 5 to 200 cm³ of distilled water and stir until dissolved.
- access to pH 7 **buffer** solution: add 4.8 g of buffer powder pH 7 to 200 cm³ of distilled water and stir until dissolved.
- access to pH 7.5 **buffer** solution: add 0.80 g of buffer powder pH 7.5 to 100 cm³ of distilled water and stir until dissolved.

Safety

MSDS sheets should be consulted for any materials used.