Practical Booklet 10
Measuring the effect of gibbererllin on the amylase activity of germinating maize

Cambridge International AS & A Level Biology 9700

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Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations

2. collect, record and present observations, measurements and estimates

3. analyse and interpret data to reach conclusions

4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To expose germinating maize grains to different concentrations of applied gibberellin (gibberellic acid) to stimulate amylase activity. To then assay the activity of the enzyme using cut halves of the maize grains and starch agar and measuring the area of starch digestion.

Outcomes

Syllabus sections 15.2 (c), 16.3 (d) and 17.1 (c)

Skills included in the practical

A Level skills	How learners develop the skills
Planning	Decide how to dilute a stock solution

Analysis	Calculate area of a circle
	Calculate rate of reaction
	Draw a graph and add standard error bars
	Calculate standard deviation (s) and carry out a t-test, including:
	  • stating a null hypothesis
	  • calculating t
	  • calculating degrees of freedom
	  • use a probability table
	  • decide if results are significant
	Produce a calibration curve to find the actual concentration of amylase – extension work, optional

Evaluation	Evaluate the method used and suggest sources of error and how these might be improved
Conclusions	Describe the effect of the concentration of gibberellin on the activity of starch amylase in the maize halves with the embryo and those without the embryo Explain their results using appropriate theory

This practical provides an opportunity to build on essential skills introduced at AS Level.

| AS Level skills	How learners develop the skills
MMO collection	Record quantitative results, measuring diameter and using a grid
PDO recording	Record quantitative results in appropriate tables

Guidance for teachers, *continued*

Method

- Cereal grains, such as barley and maize, contain an embryo and endosperm, which is a storage tissue. During germination, amylase enzymes are produced in the aleurone layer around the endosperm. These enzymes diffuse into the endosperm and catalyse the breakdown of starch reserves to maltose.

- The production of amylases in the aleurone layer is triggered by the release of gibberellin from the embryo in response to water. Gibberellin is also known as gibberellic acid and as GA3.

- Amylase activity is measured by the breakdown of starch to give the reducing sugar maltose. It is possible to measure amylase activity by placing grains that are cut in half onto starch-agar in Petri dishes and measuring the area of starch digested. Learners should be reminded about AS knowledge of enzymes and how to test for the presence of starch and reducing sugars.

- The natural concentration of gibberellin in plant tissue is very low, approximately 346×10^{-6} g dm^{-3} which is equivalent to 1.0 μmol dm^{-3}. Gibberellins can be supplied to seeds to promote germination and in the brewing industry are sprayed onto germinating barley to increase maltose production from starch.

- This investigation has three main stages and two periods of time where the investigation has to be left for at least 24 hours. This means planning lesson time to take this into account.

Stage	Activity	Approximate time to complete / minutes
1	Making solutions and soaking grains. These need to be left for **24 hours**. Optional: learners make their own starch agar plates.	40 40–60
2	Cutting maize grains and placing then onto starch-agar plates. These need to be left for **24 hours**.	30
3	Measuring areas on agar plates.	40

- Learners should be provided with dry maize grains and a 3 mmol dm^{-3} stock solution of gibberellin and sodium hypochlorite solution. The learners are asked to decide how to dilute the stock solution to give a suitable range of concentrations of gibberellin to test on the maize. This provides an opportunity to review AS knowledge of serial dilution and simple dilution and to discuss which would be most appropriate for this investigation. Learners should also discuss how many different dilutions should be used in terms of the range, intervals between concentration as well as the time and equipment available to carry out the investigation.

- Learners should then prepare the dilutions they have decided. They will surface sterilise maize grains and place 6 grains in each dilution. Barley can be used for this investigation, but the grains are much smaller and difficult to handle. These will need to be left for 24 hours to soak. If learners are to make their own starch-agar plates this should also be prepared and left for 24 hours. If resources are limited then this can be carried out by pairs of learners.
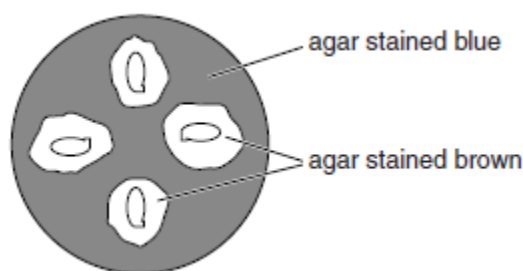
Guidance for teachers, *continued*

- Learners should use two starch-agar plates for each dilution of gibberellin. They are instructed to label the Petri dishes on the underside with their name and the dilution of gibberellin that grains have been soaked in. Learners should then select four maize grains from the highest concentration of gibberellin and cut them vertically into two halves. The halves from two of the grains should then be placed cut surface downwards on one of the labelled starch-agar plate, as shown below. The embryo should be removed from the remaining two grains and then placed cut surface downwards on the second starch agar plate. This should then be repeated for each concentration of gibberellin. Learners should be instructed to take care to keep the lids on the Petri dishes containing starch-agar and to only lift them when placing the maize grins onto the starch-agar.

halves of maize grains with cut surface on agar containing starch
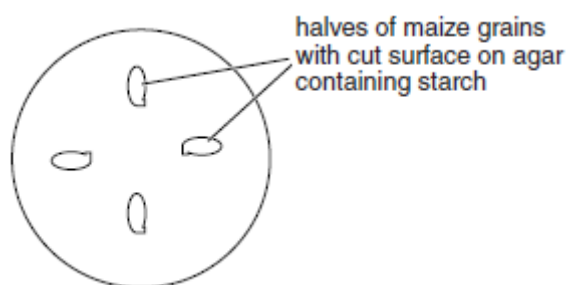
- Once all the starch-agar plates have been completed the learners should stack the Petri dishes on top of each other and leave them for 24 hours in a dark place. If the results cannot be obtained after 24 hours, the plates can be left for 48 hours at room temperatures of 20 °C – 25 °C. At higher temperatures they should be placed in a refrigerator after 36 hours to prevent the digested areas from overlapping.

- To obtain results, learners are instructed to add 10 drops of iodine solution and swirl the plate to spread the iodine over the plate.

  The expected appearance is shown in the diagram.

agar stained blue

agar stained brown

Results

1. Learners are instructed to measure the area of the starch-agar plate that has been digested. This provides an opportunity to discuss how this might be achieved. Learners should be able to suggest measuring the diameter of the brown zone and using the formula πr^2. Some may also suggest tracing onto graph paper and counting squares.

   Learners should be directed to observe the shape of the areas stained brown and led towards the idea of measuring several diameters to obtain a mean to use in the calculation of area. Learners should also be introduced to transparent grids as an alternative to graph paper.

Guidance for teachers, *continued*

2. Learners should construct a table to record their results for each dilution of gibberellin for both the halves with the embryo and those without the embryo. They may need to be reminded that since there are 4 halves on each starch–agar plate, a mean can be calculated. A graph showing the concentration of gibberellin and the area of starch digestion should be plotted. If the exact time is known then a rate can be worked out by dividing the area by the time. Learners should be reminded about the rules for orientating both tables and the graphs and that units are need.

3. Learners should then describe the effect of concentration of gibberellin on the activity of amylase and also comment on any differences between the maize halves with the embryo and those without the embryo.

4. Learners should then be directed to think about the reasons for their observed results and draw conclusions about the effect of gibberellin.

5. To carry out a *t*-test, class results need to be pooled to give sufficient data. 10 sets of data should be sufficient. Individual values should be used, so 3 students will have a total of 12 values for each of the maize halves with the embryo and those without the embryo.

   Learners should:

   - explain why the *t*-test can be used for the results of this investigation

   - state a null hypothesis

   - use the formula to find the standard deviation (*s*) for maize halves with the embryo and for those without the embryo and the *t*-test to obtain a value of **t**.

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}} \quad \text{and} \quad t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

   - work out the number of degrees of freedom (*v)*

   - use a probability table to find the critical value at 0.05 probability

   - decide whether the difference in amylase activity of the maize halves with the embryo and those without the embryo is significant.

   Learners often find statistical analysis confusing. It is important that learners understand that a null hypothesis always makes an assumption that there is no significant difference between the two populations being tested, in this case, amylase activity of the maize halves with the embryo and those without the embryo. A null hypothesis needs to state clearly what is being compared.

   Learners also need to understand that degrees of freedom are worked out from the number of samples. Assuming that 12 measurements are recorded for maize halves with the embryo and those without the embryo, then n = 12 and:

   - degrees of freedom for maize halves with the embryo is n – 1, i.e. 12 – 1 = 11

   - degrees of freedom for maize halves without the embryo is also 12 – 1 = 11

   - the total degrees of freedom is 22, which is the row of the probability table that the learners need to use to find the critical value with which to compare the calculated value of **t.**

Guidance for teachers, *continued*

Interpretation and evaluation

Learners should evaluate the reliability of the results. This could be done by discussing the methods used for obtaining the results, listing all the possible sources of error and deciding if there is any way the reliability could be improved.

- Some problems identified may include measuring the area of an irregular shape, observing the 'edge' where the colour changes and being unable to calculate the rate of starch breakdown accurately. They should also suggest alternative ways of estimating the amylase activity, such as making an extract from the grain and testing it with a starch solution of known concentration.

- Learners should also be directed to think about a control and whether this could be used to improve the reliability of the results.

- The way in which variables that have been standardised should also be considered to decide if all the variables that might influence the results have been taken into account and whether the method of standardising could be improved.

- Other issues such as uncontrolled or non-standardised variables could also be considered, such as rate of diffusion of enzyme from the grain and the pH of the starch-agar. This provides an opportunity to discuss the idea that there may be variables that cannot be standardised.

Extension

1. A calibration curve could be made by using a known concentration of starch and measuring the rate of disappearance of starch with different known concentrations of amylase.

   - Learners should be provided with 2% amylase solution to make a simple dilution series of: 1.5%, 1%, 0.5%, and 0.25% amylase solution.

   - A 1 cm^3 sample of each amylase solution is added to 1 cm^3 of a 1% starch solution and tested at 30 second intervals using a spotting tile.

   - Learners place drops of iodine solution in rows on a tile and remove a sample from each dilution of amylase at 30 second intervals until the iodine remains brown. The rate of starch hydrolysis can be calculated in mg s^{-1}.

   - Learners can calculate the number of mg of starch in 1 cm^3 of a 1% starch solution and divide by time in seconds.

   - The calibration curve is plotted with concentration of amylase as the *x*-axis and rate of starch hydrolysis as the *y*-axis.

2. To find the amylase concentration in a maize grain, the two halves of a grain should be crushed in water and filtered. 1 cm^3 of the filtrate is added to 1 cm^3 of a 1% starch solution and tested in the same way as the known amylase concentrations. The rate of reaction is then found on the *y*-axis of the calibration curve and the amylase concentration found on the *x*-axis.

Information for technicians

Each learner will require:

Stage 1

- 1% sodium hypochlorite (sodium chlorate) solution for surface sterilising the grains **[H]**
- 1 g dm-3 solution of gibberellin (gibberellic acid)
  1 g dm-3 solution is approximately 3 × 10-3 mol dm-3 (3 mmol dm-3) solution of gibberellin
- 5 × containers for making dilutions, e.g. 100 cm^3 beakers
- supply of distilled or deionised water
- 36 maize grains
- 4 × 2 cm^3 syringes or pipettes

Stage 2

- 20 starch-agar plates
- 1 × sharp scalpel or single edge razor blade
- 1 × forceps
- 1 × tile or cutting board

Stage 3

- Iodine in potassium iodide solution
- 1 × transparent 2 mm grid
- 1 × 15 cm or 30 cm ruler with mm divisions

Additional instructions

- Barley can be used for this investigation, but the grains are much smaller and difficult to handle.

- To make gibberellin solution, 2 cm^3 of 95% ethanol per dm^3 should be added to dissolve the gibberellin before adding distilled water. Learners will use this to make their own dilutions so sufficient quantity should be prepared for the number of learners in a class.

- Starch-agar plates may be prepared as follows using sterile apparatus:

  1. Take 100 cm^3 of water.

  2. Use a small volume of this water to make a paste with 1 g soluble starch.

  3. Boil the rest of the water and add to the starch paste. Stir until dissolved.

  4. Add 2 g of agar powder until the agar is dissolved.

  5. Boil the starch-agar solution for several minutes.

  6. Allow the starch-agar to cool to about 60 °C and pour into sterile Petri dishes to a depth of approximately 3 mm.

  7. Leave the agar to set.

  8. Store the starch-agar plates in a refrigerator until required.

Information for technicians, *continued*

Transparent grids can be made by photocopying 2 mm graph paper onto heat resistant transparencies or printing using a laser printer from an internet source onto heat resistant transparencies. Ink jet printers are not suitable.

Hazard symbols

C = corrosive substance

H = harmful or irritating substance

N = harmful to the environment

F = highly flammable substance

O = oxidising substance

T = toxic substance

Worksheet

Aim

To expose germinating maize grains to different concentrations of applied gibberellin (gibberellic acid) to stimulate amylase activity. To then assay the activity of the enzyme using cut halves of the maize grains and starch agar and measuring the area of starch digestion.

Method

You are provided with a 1 g dm^{-3} solution of gibberellin. You must decide how to dilute this stock solution to give a suitable range of concentrations of gibberellin to test on the maize.

Stage 1

1. Prepare the dilutions of gibberellin solution that you have decided to use.

2. Surface sterilise the maize grains using the sodium hypochlorite solution.

3. Put 6 maize grains in each dilution and leave to soak for 24 hours.

Stage 2

4. Label 2 starch- agar plates with your name and the highest concentration of gibberellin solution you have made.

5. Remove 2 maize grains from the solution containing the highest concentration of gibberellin and cut them vertically into two halves.

6. Lift the lid of one Petri dish and place these halves face downward onto the starch-agar as shown in the diagram. Replace the lid as quickly as possible.
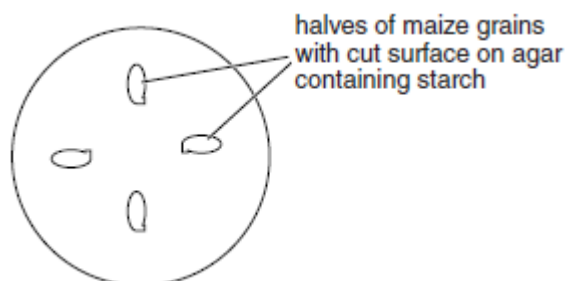
7. Remove another 2 maize grains from the solution containing the highest concentration of gibberellin and cut them vertically into two halves.

8. Remove the embryos from the bottom of the grains and then place the grain halves face down onto the starch-agar in the second Petri dish.

9. Repeat steps **3** to **6** for each of the concentrations of gibberellin solution you have made.

10. Leave the Petri dishes containing starch agar to incubate for 24 hours.

Stage 3

11. Add 6 drops of iodine solution to the surface of the starch agar and rotate the petri dish to spread the iodine solution evenly.

Worksheet, *continued*

Results

1. Prepare a table to record the results for each concentration of gibberellin solution. This should have sufficient cells to record, separately, each of the 4 maize halves with the embryo and each of the four halves without the embryo and to calculate the mean for each concentration.

2. Measure the area of the starch-agar stained brown. Use two different methods:

   - use a ruler to measure the diameter of the brown area and use the formula for the area of a circle to calculate the area

   - turn the starch-agar plate upside down and place the transparent 2 mm grid over the brown area. Trace the shape of the area onto the grid and then use systematic counting to find the area.

3. Plot a graph of the mean area of starch-agar stained brown against the concentration of gibberellin solution. If you know the exact time for which the starch-agar plates were left, then calculate the rate as $mm^2\ h^{-1}$.

4. Describe the effect of the concentration of gibberellin on the activity of starch amylase in the maize halves with the embryo and those without the embryo.

5. Use your knowledge of the role of gibberellin to give reasons for your results and explain any difference between the maize halves with the embryo and those without the embryo.

6. Choose a concentration of gibberellin that appears to have a clear difference between the maize halves with the embryo and those without the embryo. Use the pooled class results to collect at least 6 other results for the maize halves with the embryo and those without the embryo from the same concentration of gibberellin solution.

7. Use the data you have collected in step **6** to find out if the difference between the maize halves with the embryo and those without the embryo is significant, using the formulae for standard deviation (*s*) and the *t*-test.

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}} \quad \text{and} \quad t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

Interpretation and evaluation

Evaluate the reliability of the results by discussing the methods used for obtaining the results, listing all the possible sources of error and describing any ways the reliability could be improved. You should consider:

- problems such as measuring the area of an irregular shape

- the role of a control

- whether all the variables that might affect the results have been taken into account

- whether there are any variables that cannot be controlled.