General Certificate of Education
Advanced Level Examination
June 2011

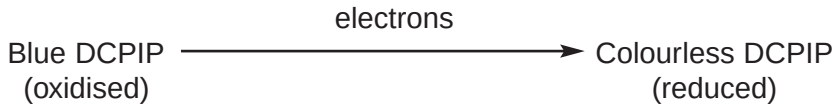
Biology BIO6T/P11/task

Unit 6T A2 Investigative Skills Assignment
Task Sheet

The effect of ammonium hydroxide on the time taken for chloroplasts to decolourise DCPIP

Introduction

In this investigation you will use a chloroplast suspension to investigate the role of chloroplasts in the light-dependent reaction of photosynthesis. Chlorophyll molecules release electrons in the presence of light. A blue dye, DCPIP, accepts these electrons and becomes colourless. This is shown in the diagram.



You will investigate whether the time taken for DCPIP to become colourless is affected by ammonium hydroxide. Ammonium hydroxide is a substance that affects the light-dependent reaction in a similar way to many weed killers.

You will collect data when ammonium hydroxide is **not** present. Then you will carry out **one** experiment to show the effect of ammonium hydroxide. For Stage 2 you will be supplied with more data showing the effect of ammonium hydroxide.

Materials

You are provided with

- spinach leaves
- access to a blender
- measuring cylinder
- muslin (or material for filtering)
- filter funnel
- beaker to use as beaker **1**
- large beaker to use as beaker **2**
- small beaker to use as beaker **3**
- ice
- isolation medium (cold)
- DCPIP solution (cold)
- distilled water (cold)
- ammonium hydroxide solution (cold)
- test tubes
- test tube rack
- syringes (1 cm^3 and 5 cm^3)
- piece of aluminium foil
- lamp
- marker pen
- timer

You may ask for any other apparatus you require.

Outline method

Read these instructions carefully before you start your investigation.

1. Put about 50 cm^3 of isolation medium into a beaker (beaker **1**).
2. Tear all 8 spinach leaves into small pieces and put the pieces into the isolation medium in the beaker. Do **not** put pieces of the midrib (the thickened central region of the leaf) or the leaf stalk into beaker **1**.
3. Half fill the large beaker (beaker **2**) with ice and place the small beaker (beaker **3**) on top of the ice.
4. Put three layers of muslin over the top of the filter funnel and wet it with isolation medium. Rest the filter funnel in beaker **3**.
5. Pour the contents of beaker **1** into the blender and blend for about 15 seconds. Pour the blended mixture back into beaker **1**.

You can carry out step 7 and part of step 10 while you are waiting to use the blender.

6. Pour a little of your blended mixture from beaker **1** through the muslin into beaker **3**. Carefully fold and squeeze the muslin to assist the filtering process. Repeat until most of the blended mixture has been filtered. Label this filtrate, which is now in beaker **3**, as your **chloroplast suspension**.
7. Label five test tubes **A**, **B**, **C**, **X** and **Y**. Stand these five tubes in the ice in beaker **2**. Position the lamp about 10 cm from the beaker so that all tubes are illuminated. Turn on the lamp.

8. Set up tubes **A** and **B** as follows.

   Tube **A**

   Put 5 cm^3 DCPIP solution + 1 cm^3 water + 1 cm^3 chloroplast suspension in the tube. **Immediately** wrap the tube completely in aluminium foil.

   Tube **B**

   Put 5 cm^3 DCPIP solution + 1 cm^3 water + 1 cm^3 isolation medium in the tube.

   Tubes **A** and **B** are control experiments. Leave both tubes until the end of your investigation.

9. Set up tube **C** as follows.

   Put 6 cm^3 water + 1 cm^3 chloroplast suspension in the tube.

   Tube **C** is for you to use as a standard to help you to determine when any colour change is complete.

10. Set up tube **X** as follows.

    Put 5 cm^3 DCPIP solution + 1 cm^3 water in the tube.

    Add 1 cm^3 chloroplast suspension to tube **X**, quickly mix the contents and start the timer. Record in seconds how long it takes for the contents of tube **X** to change colour from blue-green to green. This is when all signs of blue have disappeared. Use tube **C** to help you determine when the colour change is complete.

11. Repeat step 10 **four** more times. You may assume that this will give you sufficient data for statistical analysis in this investigation.

12. Set up tube **Y** as follows.

    Put 5 cm^3 DCPIP solution + 1 cm^3 ammonium hydroxide solution in the tube.

    Add 1 cm^3 chloroplast suspension to tube **Y**, quickly mix the contents and start the timer. Record in seconds how long it takes for the contents of tube **Y** to change colour from blue-green to green. Use tube **C** to help you determine when the colour change is complete. However, if this has not taken place within 300 seconds (5 minutes), record the colour at this point. Record your results in **Table 1** on page 4.

    You are **not** required to repeat step 12. Data will be supplied in Stage 2 for your statistical analysis.

Table 1

Tube	Contents	Time taken for solution to change from blue-green to green / seconds	Colour of mixture after 300 seconds
Y	DCPIP solution, ammonium hydroxide and chloroplast suspension		

13. At the end of your investigation, record the colour of the mixtures in tubes **A** and **B** in **Table 2** below.

Table 2

Tube	Contents	Colour of mixture
A	DCPIP solution, water and chloroplast suspension	
B	DCPIP solution, water and isolation medium	

You will need to decide for yourself

- when the colour change is complete.

Do not write outside the box

ISA BIO6T/P11 Candidate Results Sheet: Stage 1

The effect of ammonium hydroxide on the time taken for chloroplasts to decolourise DCPIP

Centre Number | | | | | | Candidate number | | | | |

Candidate Name .....

Record your data for tube **X** in a table in the space below.

Hand in this sheet at the end of each practical session.

There are no marks awarded for Stage 1.

Do not write outside the box

ISA BIO6T/P11 Candidate Results Sheet: Stage 2

The effect of ammonium hydroxide on the time taken for chloroplasts to decolourise DCPIP

Centre Number | | | | | | Candidate number | | | | |

Candidate Name .....

Use your data for tube **X** and the data in **Table 3** for tube **Y** in your statistical analysis.
The data for tube **Y** were obtained using the same method as in your investigation.

Table 3

Contents of tube Y	Trial number	Time taken for solution to change from blue-green to green / seconds
5 cm^3 DCPIP solution + 1 cm^3 ammonium hydroxide solution + 1 cm^3 chloroplast suspension	1	225
	2	198
	3	149
	4	215
	5	186

Analyse your data and the data supplied with a suitable statistical test. You may use a calculator and the AQA Students' Statistical Sheet that has been provided.

A sheet of graph paper is supplied. You may use this if you wish.

1 State your null hypothesis.

.....

.....

.....

.....

(1 mark)

2 (a) Give your choice of statistical test.

.....

(1 mark)

Do not write outside the box

2 (b) Give a reason for your choice of statistical test.

.....

.....

.....

.....

(1 mark)

3 Carry out the test and calculate the test statistic. Show your working.

(1 mark)

4 Interpret the test statistic in relation to your null hypothesis. Use the words *probability* and *chance* in your answer.

.....

.....

.....

.....

.....

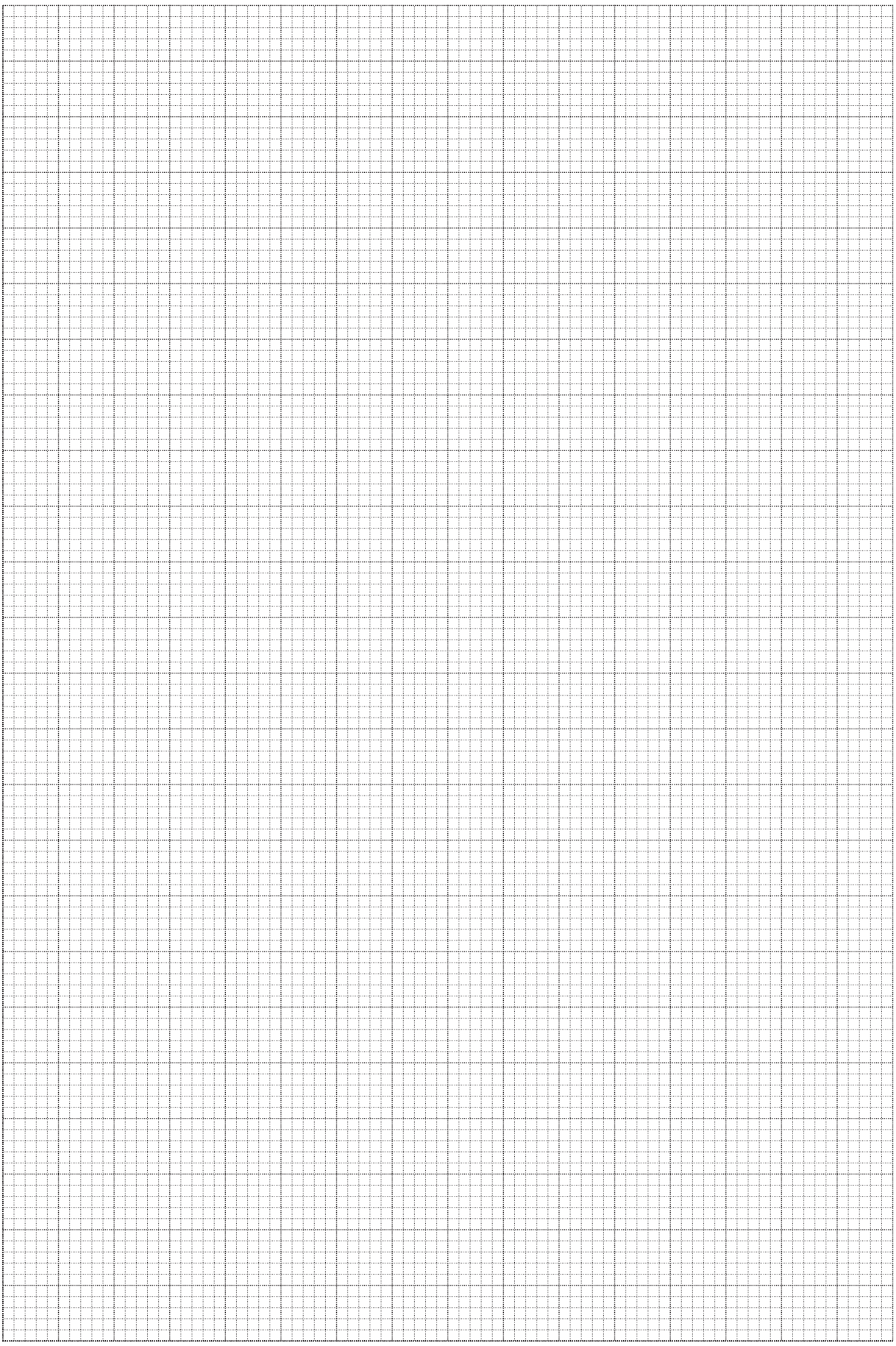
.....

(2 marks)

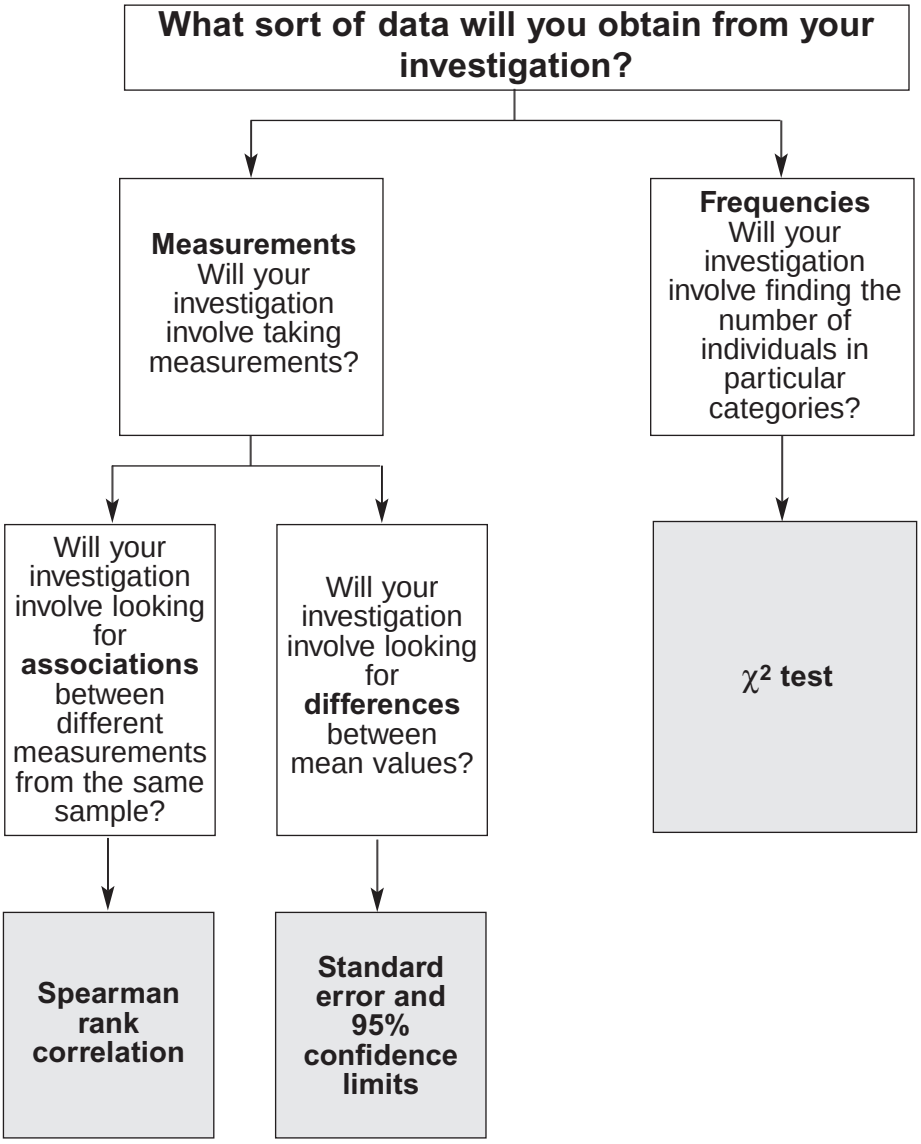
END OF QUESTIONS

6

Do not write outside the box



AQA Students' Statistics Sheet Version 3



Standard error and 95% confidence limits

Calculate standard error, SE, for each sample from the following formula

$$SE = \frac{SD}{\sqrt{n}}$$

where SD = standard deviation
and n = sample size

95% confidence limits = $2 \times SE$ above and below the mean

For use in the ISA and EMPA assessment

The χ^2 test

The chi-square (χ^2) test is based on calculating the value of χ^2 from the equation

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

where O represents the results you observe in the investigation
and E represents the results you expect.

Table showing the critical values of χ^2 at P = 0.05 for different degrees of freedom

Degrees of Freedom	Critical value
1	3.84
2	5.99
3	7.82
4	9.49
5	11.07
6	12.59
7	14.07
8	15.51
9	16.92
10	18.31

Spearman rank correlation test

Calculate the value of the Spearman rank correlation, r_s, from the equation

$$r_s = 1 - \left[\frac{6 \times \Sigma D^2}{n^3 - n}\right]$$

where n is the number of pairs of items in the sample and D is the difference between each ranked pair of measurements.

Table showing the critical values of r_s at P = 0.05 for different numbers of paired values

Number of pairs of measurements	Critical value
5	1.00
6	0.89
7	0.79
8	0.74
9	0.68
10	0.65
12	0.59
14	0.54
16	0.51
18	0.48

For use in the ISA and EMPA assessment