PUPIL RESEARCHER INITIATIVE

Seeing the Light

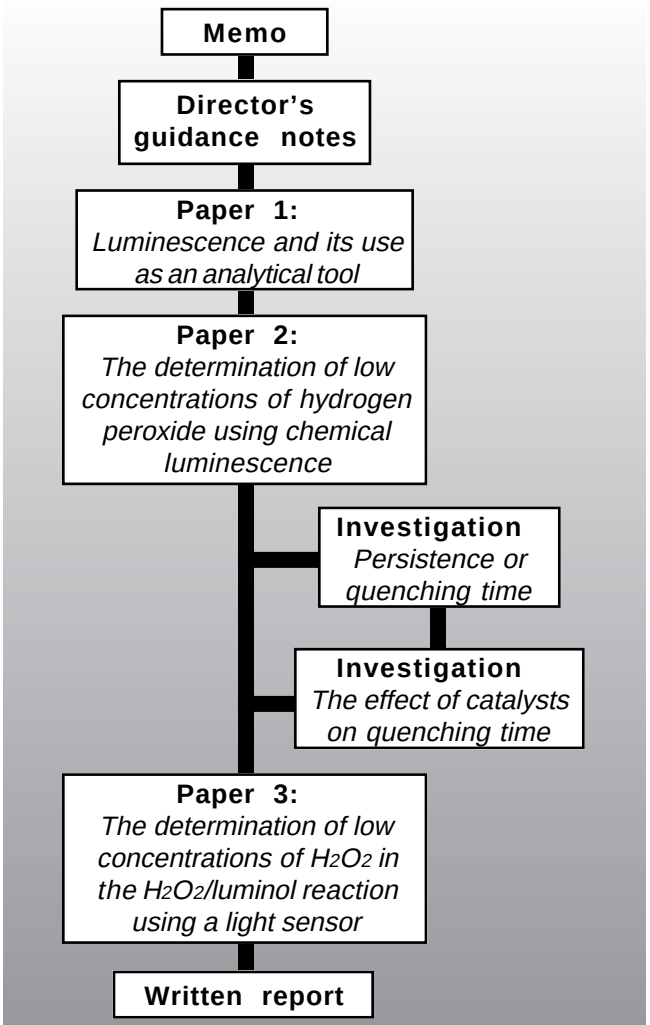
Pupil Research Brief

Teachers' Notes

Syllabus Coverage *Subject Knowledge and Understanding*

- ❑ the rate of a chemical reaction increases if the concentration of dissolved reactants increases
- ❑ the rate of a chemical reaction increases if a catalyst is used
- ❑ during a chemical reaction energy is released when bonds are formed

Route through the Brief



Introduction

This Brief is intended to be a demanding exercise for higher achieving pupils. It is also intended to provide an opportunity for assessing high level Sc1 skills in the context of a whole investigation.

Luminescence provides scientists with a tool to investigate a whole range of chemical and biological phenomena. Researchers have also been able to use it as an analytical tool to explore the processes underlying disease and its diagnosis.

Luminescence can be used to measure low levels of adenosine triphosphate (ATP) and therefore to detect the presence of bacteria at very low levels. This means that the levels of bacteria can be monitored easily and quickly in the food, drink and dairy industries. Traditional methods of monitoring depend on taking samples and culturing the bacteria which is time-consuming. The luminescence technique is also used in the testing of cosmetics, other personal care products and water quality.

Light-emitting proteins from jelly fish have enabled researchers to measure free calcium in living cells leading to discoveries about the role of calcium in cell behaviour and cell pathology. This has applications in research into multiple sclerosis - a disease caused by problems with the immune system.

Researchers at Glasgow Caledonian University have been using the chemiluminescence emitted by the luminol/hydrogen peroxide reaction to determine trace levels of H_20_2 in natural waterways. The principal gases contributing to acid rain are oxides of

sulphur and nitrogen and as these gases pass through the environment they are involved in a reaction with H_20_2. Thus, depletion of trace levels of H_20_2 is one approach to monitoring the condition of rivers and lochs, and can be used as an indicator of pollution levels. There are various methods for monitoring low levels of H_20_2 but some are time-consuming and do not give very good results. The Glasgow Caledonian researchers have been developing the luminescence method as a quick and reliable method for detection of trace levels of H_20_2.

Hydrogen peroxide is also used widely as a bleach and disinfectant in manufacturing industry, and for commercial and domestic purposes. For example, it is used as a bleach in the pulp and paper industry; for the treatment of wastes; and as a disinfectant in fish farming, in the food industry and in health-care products, e.g. for cleaning and disinfecting dentures and contact lenses. It is clear there are many applications for H_20_2 and therefore improved methods for measuring low concentrations are frequently being sought.

Chemiluminescence is a fascinating process which seems to capture pupils' attention and imagination. However, pupils rarely have an opportunity to see a reaction in school, let alone carry out one for themselves. One such reaction is the luminol/H_20_2 reaction which lends itself quite well to use in the school laboratory.

In this Brief pupils 'work' as members of the Test Development Group in the Analytical Services Division of a university science faculty who are asked to investigate a test for determining low concentrations of H_20_2. The test is based on the luminol/H_20_2 reaction in the presence of a cobalt chloride catalyst. This is a chemiluminescence reaction which emits a low intensity blue luminescence. The intensity of luminescence or the amount of luminescence emitted can be measured for different concentrations of H_20_2 and a standard calibration method developed, from which 'unknown' concentrations of H_20_2 can subsequently be determined.

Experimental and investigative skills

- planning experimental procedures
- obtaining evidence
- analysing evidence and drawing conclusions
- evaluating evidence

Prior knowledge

Pupils should have some understanding of solution concentration and what the term *ppm* means. They should have met the terms catalyst, enzyme, and pH. It would be helpful if they were aware of adenosine triphosphate (ATP) and its role in cells. A basic knowledge of graphs and plotting 'lines of best fit' would be useful.

Running the Brief

Pupil grouping

Pupils could work in a number of groupings during this Brief. Suggestions are:

Initial briefing	-	whole class; teacher-led introduction
Introductory memo	-	'Test Development Teams' - groups of 4 (Note: 4 pupils are needed to perform the 'light pulse method' later)
Analysis of background papers and practical work	-	paper 1; tackled by all pupils in their teams of 4
	-	paper 2; teams can do 'light pulse' method and/or 'light persistence' investigation. Depending on time available some teams could do method 1, others method 2 . Teams could then report back their investigation to whole class. An optional investigation into the effect of a catalyst on the reaction could also be carried out
Analysis of results	-	individual, pairs or teams
Communication	-	individual, if written reports are to be assessed, otherwise pairs or teams
Optional extension investigation	-	paper 3; small groups 2-4

Timing

The Brief should take between 3-4 hours of classroom time. Extra time may be needed to write up individual investigation reports if these are to be used for examination assessment purposes. The optional investigation may add two hours or more, particularly if used for project work.

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Activities

Pupils should be issued with the **Study Guide**, which provides them with a summary of what they should produce as they work through the Brief. It can also be used as a checklist so that they can monitor their own progress.

It is worth spending a little time at the start to set the scene (see the introduction to these notes). Then, acting as the Director of Analytical Services give out the **memo** including the **Guidelines notes** and **Papers 1** and **2.** Section C of the Guidelines refers to optional **Paper 3**. Pupils are referred to you if they have some time, so you can decide whether they should do this section. Alternatively, you can do cutting and pasting and remove this section from the Guidelines and hold back Paper 3.

Pupils are guided through the Brief by the Guidance notes (G.N.) which are fairly prescriptive so that they can work systematically and pay attention to detail. All pupils should be able to do the tasks relating to **Paper 1** (Section A of G.N.) and either or both of the practical methods relating to **Paper 2** (section B of G.N.). Note, the 'persistence of luminescence' method is suggested as an idea for an investigation in a handwritten note from the Director at the end of **Paper 2**. However, some groups could do one method and others the other method and then report back to the whole class. You may want all pupils to do the 'persistence of luminescence' method since this is an investigation which they can plan for themselves. It is suggested that **Paper 3** tasks (Section C of G.N.) are suitable for higher achieving pupils and particularly useful for optional extension/project work and using IT. All pupils should produce a report of their findings to the Director (section D of G.N.).

Note: the article by Campbell on Luminescence in the first issue of *PRISM* (PRI pupil journal) is good stimulus and background reading material for this Brief. Other reading is referenced in Paper 1.

Investigation details

The brief suggests three methods for measuring the light emitted during the reaction, two of which are described in **Papers 2** and **3**. The methods have been developed for use by pupils.

1. *Light pulse method* - intensity or brightness of the light is measured by the naked eye using a simple brightness scale e.g. 1,2,3,4 to place the different concentrations in order. Most of the early work in this field was done using the naked eye. The method can give appropriate results but has obvious limitations. Pupils will need to rehearse the procedure, be well organised as a group to work it satisfactorily, and follow procedures carefully.

2. *Persistence of luminescence method* - the time taken from appearance to disappearance (quenching time) of luminescence is used to compare the amount of light emitted for each H_20_2 concentration. This method is only suggested as an idea to the pupils so that they can devise their own investigation. This method works well. Thorough mixing of solutions is important. Our results give a graph which is a curve. It is suggested that this is set as an investigation so that pupils can plan and design their own experiment themselves. It is set up by a note from the Director written on Paper 2. Details of the method are set out in the next section*.

3. *Light sensor method* - a light sensor linked to a PC with datalogging facility is used to measure the initial intensity of the light pulse for each H_20_2 concentration. Pupils could experiment with this method and suggest their own ideas - a good project which could form the basis for a CREST Award.

A key aim of this activity is also to get pupils to consider the validity and reliability of results.

Basic information on the calculation of errors and the use of error bars in drawing graphs is provided. Able pupils should be able to cope with this and apply it to their own results.

Our results give a straight line for the light sensor method described in Paper 3.

Technical details

All of the information required, such as apparatus, solutions, experimental data, etc. is given in Papers 2 and 3.

Solution concentrations are as follows.

Luminol solution:	1000 ppm - 0.1g/100 cm^3 of pH11 buffer solution. (The buffer keeps the pH at a constant level during the reaction.)
pH11 buffer solution:	solution A - 2.12g of sodium carbonate made up to 100cm^3 with water solution B - 1.68g of sodium hydrogen carbonate made up to 100cm^3 with water.

Teachers' Notes continued

	Adding 85 cm^3 of A to 15 cm^3 of B gives 100cm^3 of pH 11 buffer solution.
Hydrogen peroxide solution :	120 ppm (1 cm^3 of 20 vol (6% weight/volume) of H_20_2 soln. made up to 500cm^3 with water). From this stock solution a series of standard solutions of 20 ppm, 40 ppm, 60 ppm and 80 ppm are made. An additional 100cm^3 of say 40 ppm and/or 80 ppm H_20_2 solution can be used for the 'unknown' concentration.
Cobalt chloride solution:	Dissolve 0.24g of cobalt chloride in distilled water and make up to 1 litre.

Solutions should be prepared and used within 24 hours.

Pupils should allow about 5 minutes for their eyes to adapt to the dark before making observations.

Luminol can be obtained from *Sigma Chemicals:* Catalogue number A8511. Price (July 1996) £5.80 - 1g (excluding VAT). 1g/l gives 1000ppm solution: 0.1g/100cm^3 should be enough for a class practical.

* **Measuring persistence of luminescence to determine low concentrations of H_20_2 in the H_20_2 /luminol reaction.**

In this method the time taken from appearance to disappearance (quenching time) of luminescence is used to compare the amount of light emitted for each H_20_2 concentration.

The concentrations of the luminol and cobalt chloride catalyst solutions are the same as for the light pulse method. H_20_2 standards of 20, 40, 60, 80, 100 and 120 ppm are used for this experiment. An additional 100 cm^3 of say 40 ppm or 80 ppm H_20_2 solution is made up for the unknown. The apparatus is also the same except for the addition of a stop-clock. The same safety procedures are also followed and are given on the safety information sheet in Paper 2.

Method

Work area conditions are the same as for the light pulse method. Wearing plastic gloves, 1cm^3 of the luminol solution (1000 ppm) is added to the sample tube. 1cm^3 of the 20 ppm H_20_2 solution is then added.

In the dark, 1cm^3 of the cobalt chloride catalyst solution (syringe or pipette) is quickly added to the sample tube, and the stop clock started at the same time. The sample tube is continually shaken gently to ensure that the solutions are mixed.

At the moment the cobalt chloride catalyst was added to the sample tube a pulse of luminous blue light was given out. This light slowly dims in the darkness of the room. At the moment when the light became no longer visible, the clock was stopped and the time noted for the luminescence to 'quench'.

This procedure is repeated for the other H_20_2 solutions and the 'unknown', writing down the concentration of the H_20_2 and the quenching time in each case. The samples are treated in order of increasing H_20_2 concentration. Measurements are made at least twice in order to check the reliability of the readings. The two sets of results are recorded in a table and an average value calculated and recorded.

A graph of average quenching times plotted against corresponding H_20_2 concentrations (ppm) gives a calibration or standardisation curve that can be used to deduce the concentration of an unknown H_20_2 sample.

Technical details

The results for the light sensor detection method in Paper 3 were obtained using data supplied by the Data Harvest - Sense and Control - Easy Log (Interface) and Light Level Sensor (cat. no. 6120)

Alternative systems that might be used to obtain similar data include:

1. Philip Harris - Light Sensor Meter (cat. no. E30280/1) with either the DL Plus Interface (cat. no. E40000/8). or Universal Interface (cat. no. E11500/4)

2. Fisher Scientific Log IT Portable Data Logger System (cat. no. CRD-200-Y) + Light Level Sensor (cat. no. CRD-220-500U.)

Safety issues

A safety information sheet for pupil use is provided as an appendix to Paper 2 (page SL.10).

Although safety data is given for 20 vol H_2O_2 pupils will only be working with solutions of concentration 20-120ppm. They will also only be working with 1000ppm luminol solution.

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PLEASE NOTE: It is also important that you prepare your own risk assessments for the practical work in this Brief in the usual way. It is advisable for pupils to wear goggles and plastic gloves when handling chemicals.

Luminol (3-Aminophthalhydrazide): minimal hazard

Hydrogen Peroxide 20vol: irritant - dangerous if swallowed

If concentration greater than 5.9M (71 vol): corrosive. Dangerous with organic compounds.

at 20-120 ppm: minimal hazard.

If swallowed: wash mouth and give water to drink. Seek medical attention as soon as possible.
If in eyes: flood eye with flowing tap water for at least 10 minutes. Seek medical attention.
If on skin: flood area with water. Remove contaminated clothing. If skin blistered or large area affected - seek medical attention.

Wear eye protection.

Cobalt Chloride: minimal hazard.
If swallowed: wash mouth and give water to drink. Seek medical attention.
If in eyes: flood eye with flowing tap water for at least 10 minutes. Seek medical attention.
If on skin: wash area with water. Remove contaminated clothing.

Wear eye protection.

Assessment issues for *Experimental and Investigative Science* (National Curriculum for England and Wales, Northern Ireland Curriculum)

P	Planning	O	Obtaining evidence
A	Analysing evidence	E	Evaluating evidence

This Brief provides opportunities for pupils to gain high marks in all four **Skill Areas.** The amount of guidance given to planning (mainly in Papers 2 and 3) will have to be considered when assessing **Skill Area P**, although there is enough scope for the full range of marks, particularly if pupils follow up the suggestion to vary the amount of catalyst (see Guidance notes, section B, part (iv). The activities in section 3 of the Guidance notes offer opportunities to develop high marks in **Skill Areas A and E**.

Scottish syllabus coverage

Standard Grade Biology - *Biosphere*

Further pupil research opportunities

The activities relating to Paper 3 offer plenty of scope for an extended investigation.
See section C of Director's Guidance notes. Other useful ideas can be found in the references below.

Useful references

1. Chemiluminescence, Principles and Applications in *Biology and Medicine*; A.K. Campbell, Publisher Ellis Horwood, 1988, ISBN 0-89573-501-6.

2. Chemiluminescence Analysis; M.L Grayeski, *Analytical Chemistry*, Vol 59, No 21, November 1987, p.1243-1256.

3. Bioluminescence and Chemiluminescence: fundamentals and applied aspects, *Proceedings of the 8th International Symposium*, Cambridge, September 1994. Ed. A.K Campbell, L. J. Kricha, P.E. Stanley. Part 5: Luminescence in Education.

Note : references in Papers 1 and 3 of the pupil Brief are real papers except for Gray, M.L. (1994) which is a simulated paper.