PUPIL
RESEARCHER
INITIATIVE

Seeing the light

Setting the Scene

You will be working as a member of the Test Development Group in a university science faculty. You are asked to investigate a test for determining low concentrations of hydrogen peroxide by measuring luminescence. You will be asked to try one or more methods and evaluate their usefulness.

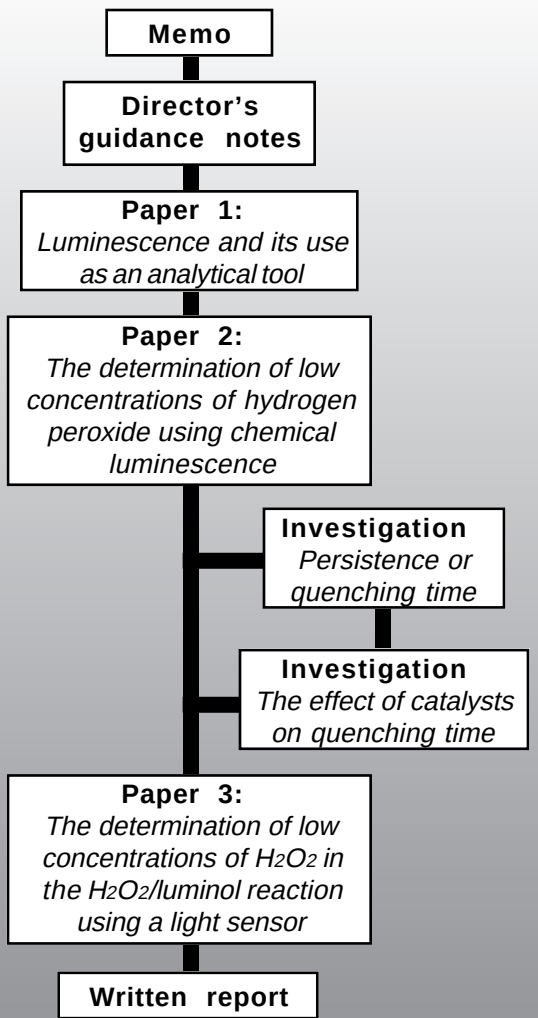
Pupil Research Brief

Study Guide

Syllabus Targets *Science you will learn about in this Brief*

- ❑ 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



Outcome Checklist

You will produce an evaluation report outlining your recommendations for an improved testing procedure for determining low concentrations of hydrogen peroxide. You should make sure you produce the following items as you work through the Brief.

Guidance notes and papers

- ❑ notes on luminescence and its uses
- ❑ investigation reports on methods undertaken
- ❑ notes on various tasks set in director's guidance notes
- ❑ written report for director

Faculty of Science

Memo

From Director, Analytical Services Division

To Test Development Group

Date

Re: Improved testing procedure for determining low concentrations of hydrogen peroxide, H_2O_2

I have been giving some thought to the possibility of us developing an improved test procedure for measuring low concentrations of H_2O_2 in the range 20 - 120 ppm. In view of the many uses of H_2O_2, I feel that there could be a market for such a test, particularly if it is quick, easy to use and reliable. After studying the research literature I've found a test that appears to work specifically for low concentrations of H_2O_2.

The test is based on the hydrogen peroxide/luminol reaction that emits low intensity blue light or luminescence. By measuring the intensity of the emitted light, the concentration of the H_2O_2 can be determined.

I've attached some research papers for you to consider which I hope will be useful. The first gives some helpful background information on luminescence and its uses as an important analytical tool.

The second paper describes a fairly quick and straightforward test for measuring the luminescence emitted in the luminol/H_2O_2 reaction which can be used to determine H_2O_2 concentration. I've suggested an idea for another method, which I've scribbled on the paper, perhaps you could also investigate this.

If you have time to look at Paper 3 come and see me. This paper was presented at a conference I attended recently in Budapest and describes a method for measuring luminescence using a light meter. Although the paper claims the test is reliable and gives good results, I have tried the test myself and cannot seem to get results which are as good as the paper claims. (I've attached my results for you to consider, in the Guidance notes).

The Guidance notes outline what I'd like you to investigate, and how I'd like you to report your findings.

I look forward to receiving your report as soon as possible.

Guidance notes for tackling luminol/H_2O_2 investigations, and writing each section of your report.

Section A

Read through Paper 1 by Mary Lynn Gray.

If you are interested in further reading try some of the references given by Gray at the end of her paper. I suggest you make notes on the following which will be useful as a brief introduction for your report.

(i) Explain what is meant by luminescence, giving examples of chemiluminescence and bioluminescence.

(ii) Summarise the luminol/H_2O_2 reaction identifying the factors which affect the intensity of luminescence.

(iii) Give examples of the uses of H_2O_2 and suggest why it is important to be able to detect low concentrations of H_2O_2.

Section B

Read Paper 2 and familiarise yourselves with the method for testing for low concentrations of H_2O_2 using the luminol/ H_2O_2 reaction. Try the experiment making sure to follow the method as outlined in the paper. Make brief notes on the questions and activities in this section. If you try my suggestion for the 'persistence or quenching time' method, make notes on these activities as well.

(PLEASE KEEP TO THE SAFETY INSTRUCTIONS GIVEN IN THE 'SAFETY INFORMATION SHEET', which can be found at the end of Paper 2.)

For the 'light pulse method'

(i) Tabulate your results i.e. light intensity (according to your chosen scale) against concentration of H_2O_2 standard solutions.

(ii) Use your method to determine the concentration of a sample or samples of unknown H_2O_2 concentration. Comment on the effectiveness of this method for determining low concentrations of H_2O_2 i.e. in the range approximately 20 - 100 ppm of H_2O_2. How easy was it to judge the intensity of light emitted? Did you get your standard solutions in the right order? Check with me to see if your 'unknown solution' fits in the right place on your scale.

(iii) Discuss the practicality of the method as a 'quick and easy test' and comment on changes, if any, that might be made to improve it.

If you carry out the 'Persistence or quenching time' of luminescence as an investigation which you have planned:

(i) Tabulate your results and plot a graph which would allow you to determine unknown concentrations of H_20_2.

(ii) Use the graph to determine the concentration of a sample or samples of unknown H_2O_2 concentration. How effective or reliable is the test for determining low concentrations of H_20_2?

(iii) How do you feel that the quenching time and light pulse methods compare in their effectiveness as tests?

(iv) Carry out a further investigation into the effect, if any, of different concentrations of the cobalt chloride catalyst on quenching times.

(v) Discuss the practicality of the method as a 'quick and easy test' and comment on any changes, if any, that might be made to improve it.

(vi) Consider follow-up work, e.g. would it be possible to design a 'black box' method for carrying out the test in daylight or outside the lab?

Section C (before you begin this, discuss it with me)

Read Paper 3 and familiarise yourselves with the method described by Walton and Wilson. Make brief notes on the questions and activities in this section.

(i) Carefully examine Walton and Wilson's results (Table 1) and their calibration graph (Figure 2). Study how they have estimated the error for the average light intensity value for 20 ppm H_2O_2 concentration and work out the errors corresponding to each of the other H_2O_2 concentrations. Do they match the error bars on their graph?

(ii) I have repeated their experiment exactly and tabulated my results in Table A below.

Table A. Light intensity (lux)

	4 readings for each H_2O_2 concentration			
H_2O_2 Conc. (ppm)	**1**	**2**	**3**	**4**
20	8.2	9.1	9.5	10.1
40	9.7	28.2	26.1	33.9
60	39.5	20.9	47.9	26.7
80	31.7	70.1	39.2	70.1
100	73.4	105.9	46.4	61.1
120	104.5	87.8	55.6	54.2

(iii) Comment on the two sets of results (theirs and mine) and associated errors, saying which seem to be the most reliable and why.

(iv) Are there any of the four readings within a set of my results which you feel ought to be eliminated or at least re-checked before averaging?

(v) Considering the very low concentrations of H_2O_2 we are working with (e.g. 20 ppm = 0.02g of H_2O_2 in 1000cm^3 water) and the fairly basic light sensor being used, do you think we should expect any greater reliability in the results? Although we have assumed the H_20_2 solutions have been made up accurately, there will be some error in the measurements and procedures. What do you think could be the possible sources of error in making up the H_2O_2 solutions?

(vi) Can you suggest what the most likely causes of error might be that affect the accuracy and repeatability of the light intensity results?

(vii) If time allows try the experiment yourselves and follow exactly the method as described in paper 3. How well does the consistency of your results compare with Walton and Wilson's? Are they as good? Are the errors of a similar range or do they match more closely with mine?

(viii) In your opinion how useful and reliable is this method of detection?

(ix) I felt that improving the mixing of the solutions at the point where the catalyst is added to the sample tube could be a key factor in getting reproduceable results. Could you explore this and suggest any improvements? Perhaps some sort of injection technique? Also, is there an optimum concentration for the catalyst?

(x) Have you any other ideas for improving this method? If so, it could possibly lead to the development of a field test kit - what do you think? Any suggestions for the design of a test kit that could be used out of the lab in day light conditions?

Section D (your group report)

I need this report as soon as possible, so it may be that individual group members or pairs take responsibility for reporting back on different methods and sections. However, the final group report should be compiled and should cover the following areas.

(a) **Background**

Title: Evaluation of luminescence methods for determining low H_2O_2 concentrations.

Introduction: Brief coverage of luminescence and its use as an analytical tool (see section A of Guidance notes and Paper 1).

(b) **Test methods investigated**

Brief review of the method(s) you investigated. It should include a summary of your investigations, including brief methods and results. (See sections B and C of Guidance Notes and Papers 2 and 3)

(c) **Summary and recommendations**

This section should compare each of the methods you investigated and include, as appropriate, a summary of your answers to the questions I posed in the relevant sections of the Guidance notes. It should finally make recommendations as to which of the tests your group investigated should be seriously considered for further development as a possible commercial field test kit for determining H_2O_2 concentrations.

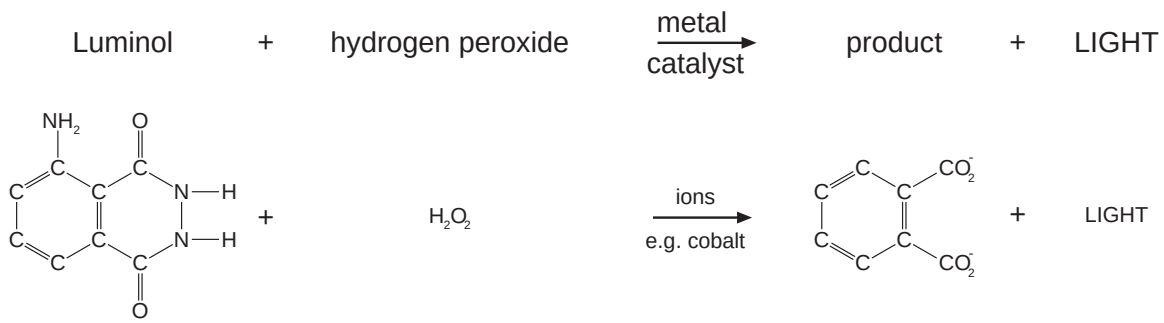
The luminol/H_2O_2 reaction is well known. Very low levels of hydrogen peroxide can be detected using simple analytical techniques involving this reaction.

***Mary Lynn Gray** outlines some of the possible applications of the luminol/H_2O_2 reaction in monitoring very low hydrogen peroxide levels in industrial wastes.*

Luminescence and its use as an analytical tool

Mary Lynn Gray
Dept. of Chemistry
Winston University
Grangetown, N.T. 0707

Chemiluminescence is observed when light is emitted from a chemical reaction. If the reaction occurs in a living system the process is called bioluminescence. A well known and very useful example of chemiluminescence is the reaction between:



The intensity of light increases at the start of the reaction and then decreases as the reactants are used up. The rate of the reaction determines the intensity of light emitted. So altering the rate, by changing the concentration of H_2O_2 for example, affects the intensity of light given out. The reaction can also be affected by other factors such as the pH, the type of solvent, the purity of reactants, presence of a catalyst and the temperature. Burgess and Hepher (1994) at Glasgow Caledonian University and other workers have used this important chemiluminescence reaction to determine H_2O_2 concentrations in a range of applications where its detection is of importance. For example, in determining the extent of acid rain pollution in lakes and rivers where changes in very low concentrations of H_2O_2 can be used as an indicator of pollution levels.

Hydrogen peroxide is also used widely as a bleach and disinfectant in manufacturing industries, and for commercial and domestic purposes. For example:

- as a bleach in the pulp and paper industry
- for the treatment of wastes
- 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. In the latter case, hydrogen peroxide is used as a key ingredient in the lens cleaning solution. It is essential that the lens is thoroughly rinsed to remove traces of hydrogen peroxide which can cause discomfort and possibly damage the cornea, even at very low concentrations.

continued

The detection of low concentrations of H_2O_2 could be required in a number of research applications, for example in research into the catalytic conversion of H_2O_2 to oxygen and water using manganese dioxide catalyst.

It is clear then that there are many applications for hydrogen peroxide and therefore a need for improved methods of measuring low concentrations.

Experimental details for a relatively quick and straightforward procedure to test for H_2O_2 using the luminol/H_2O_2 reaction are described in another article by the author (Gray, 1994).

Bioluminescence, a 'living' example of chemiluminescence, has a very important application in the food and health care industries. Because of its use in detecting the presence of microorganisms it is used to monitor levels of contamination in foods and dairy products, products in the pharmaceutical industry and in the water industry.

The mechanism by which bioluminescence occurs was identified by William McElroy in 1947 when he analysed how fireflies produce a flash of light.

The tail of the firefly contains the substances luciferin and the enzyme luciferase. Light is produced when the luciferin reacts with adenosine triphosphate (ATP) present in the living cells of the firefly. The reaction is catalysed by the enzyme luciferase. The amount of light emitted is directly proportional to the amount of ATP present. Since all life-forms contain ATP, applications of bioluminescence in microbiology are based on capturing the microorganisms, releasing the ATP from within the cell and measuring the amount of bioluminescence generated. A high reading of light units indicates a high number of microorganisms. Traditional methods of testing for microorganisms using agar plates, which may take several days to grow a culture, is slow. Results from a bioluminescent reaction can be obtained quickly, since light is produced in seconds and can be measured with a **luminometer** - a light measuring instrument.

There are therefore many examples of luminescence in chemistry and in nature and they have widespread applications as analytical tools in research, industry and medicine. Further information on luminescence and its uses can be found in other works, for example by Campbell (1988, 1996) and De Silva (1996).

References

1. Burgess, A. E. and Hepher, M. J., Enhanced Measurement of Hydrogen Peroxide by Stimulated Light Emission. *Proceedings, 2nd International Symposium on Environmental Contamination in Central and Eastern Europe*, Budapest, Hungary, 20 - 23 September, 1994, pp35 - 37
2. Campbell, A. K., *Chemiluminescence: Principles and Applications in Biology and Medicine*. Ellis Harwood, 1988. ISBN 0-89573-501-6.
3. Campbell, A.K., *PRISM,* Issue No. 1. 1996.
4. De Silva, A.P., Light Messages from Molecules. *Chemistry Review*, January 1996.
5. Gray, M.L., The determination of Low Concentrations of Hydrogen Peroxide using Chemical Luminescence. *IJAS*, 47, 11, 1994.

The determination of low concentrations of hydrogen peroxide using chemical luminescence

Mary Lynn Gray, Department of Chemistry, Winston University

Introduction

Methods are being developed and tested for detecting very low levels of hydrogen peroxide. For example in monitoring trace levels in waterways, special equipment with sensitive detection devices is required (Burgess and Hepher, 1994). The method is based on the H_2O_2 /luminol reaction (in the presence of a cobalt chloride catalyst) which emits a low intensity blue luminescence. This paper describes a relatively quick and straightforward procedure for detecting H_2O_2 concentrations in the range 20 - 80 ppm using the naked eye and readily available equipment.

Test development and method

The light pulse method to determine low concentrations of H_2O_2 in the H_2O_2 /luminol reaction

In this method, sight is used as a means to determine the intensity of light emitted. After a number of trials, the most favourable conditions and procedures were determined.

Equipment requirements:

- flashlight with red filter
- sample tubes (5 x 5cm^3 or 10cm3^3) - glass or perspex
- pipettes (1cm^3) + pipette fillers or other 1cm^3 measuring device, e.g. plastic syringe body (no needle)
- plastic gloves
- luminol solution (20cm3^3)
- hydrogen peroxide solutions 20, 40, 60, 80 ppm, and solutions of unknown concentration within the range 20 - 80 ppm (10cm^3 of each)
- cobalt chloride solution (20cm^3).

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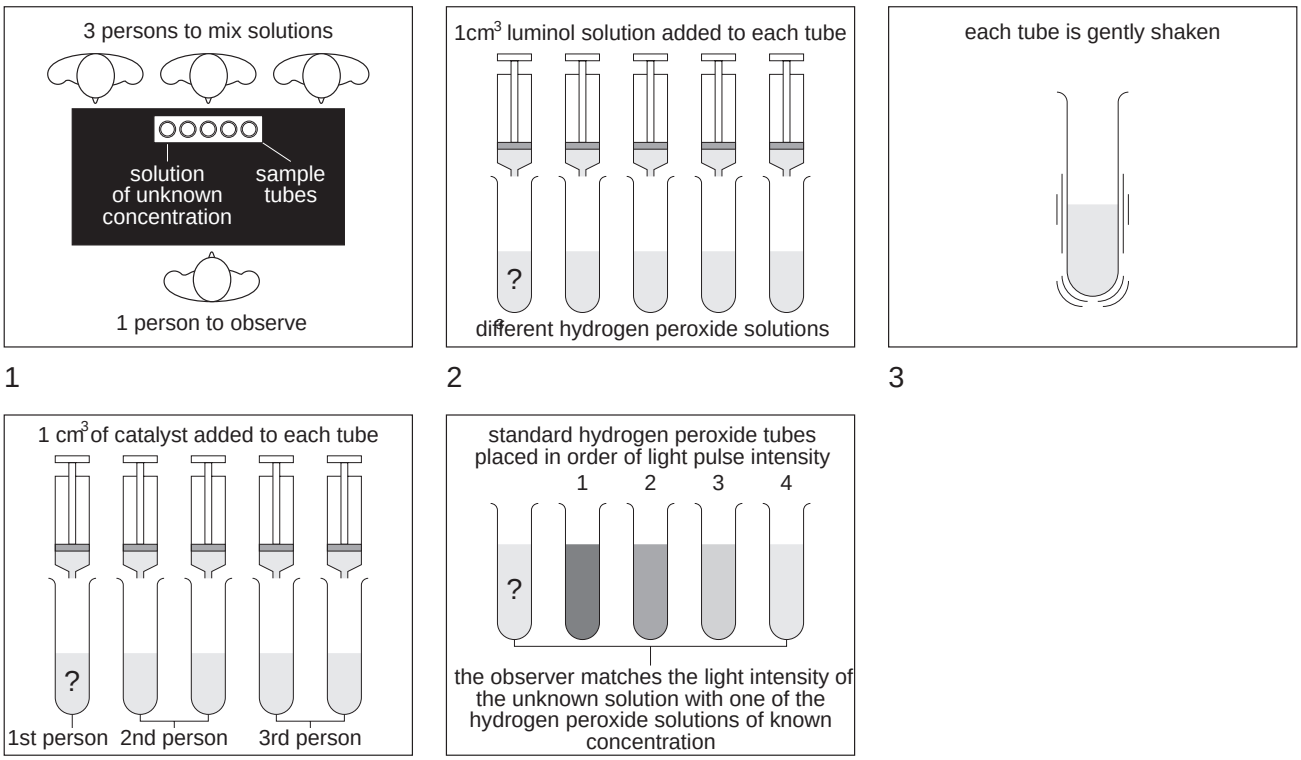
Figure 1. The test method



Method

Safety procedures are given on the *Safety information sheet* (appendix to this paper).

A minimum of four people are needed to perform the experiment, three to mix the solutions and at least one person to act as observer. The investigation is carried out in a darkened room. A blackout or darkroom gives best results. A flashlight with a red filter is used to set up the experiment, but all observations are made in the dark. At least five minutes must be allowed for the eyes to adapt to the dark before making any observations.

It helps if the surface is covered with black sugar paper, although it is not essential. The five sample tubes are arranged in a line at one side of the table. The observer should be seated on the opposite side of the table facing the sample tubes. Make sure that the observer knows the tubes that will contain each of the standard hydrogen peroxide solutions and the tube that will hold the solution of 'unknown' concentration.

Wearing plastic gloves, one person introduces 1cm^3 of the luminol solution into each tube (using a pipette or syringe). 1cm^3 of each standard hydrogen peroxide solution and the 'unknown' are added to their own tube. Each tube is gently shaken to mix the two solutions. Two people then add 1cm^3 of cobalt chloride catalyst into the tubes containing the standard solutions (two tubes each) and one person adds 1cm^3 of catalyst to the tube containing the 'unknown'. **The addition of the catalyst solution must be made quickly** (using syringes or pipettes) **and simultaneously by all three investigators (some practice may be needed).**

continued

The observer should attempt to place the tubes in order according to the intensity of the pulse of light that is given out by each solution at the moment that the catalyst is added to the sample tubes. A crude scale may be used to place the tubes in order, e.g. 1,2,3,4. The observer then attempts to match the intensity of light observed in the 'unknown' solution with the intensity of light produced by one of the standard solutions, i.e. if the intensity of light given out by the 'unknown' most closely matches that produced by standard solution 4 (80ppm), then it could be assumed that the 'unknown' is of similar concentration, i.e. 80 ppm.

Conclusions

The test described in this paper provides a quick, straightforward and satisfactory method of determining low-level concentrations of H_2O_2 and has a number of useful applications as an analytical tool.

Appendix - Safety information sheet

Wear goggles and plastic gloves when handling chemicals.

LUMINOL (3-Aminophthalhydrazide) (1000 ppm) - The *Material Safety Data Sheet* (Fisons) classifies luminol as presenting 'no significant hazard' and lists no Risk or Safety phrases.

HYDROGEN PEROXIDE (20-120ppm) - No significant hazard.

COBALT CHLORIDE (solid) - No significant hazard.

In each case, follow the general first aid instructions provided below. Clean up all spillages immediately.

First aid measures

Skin Contact	Flood the splashed area with large quantities of running water.
Eye Contact	Wash the eyes out with water or saline solution for at least 5 minutes.
Ingestion	If the chemical has been confined to the mouth give large quantities of water as a mouth wash. Ensure the mouth wash is not swallowed. If the chemical has been swallowed, give about 250cm of water to dilute it in the stomach. In severe cases obtain medical attention.

I wonder if it might be possible to use the time it takes from the appearance to the disappearance of luminescence (quenching time or persistence of luminescence) to compare the amount of light emitted for each H_2O_2 concentration?
You could investigate this and design a suitable method. Try it out and see if your results produce a suitable graph or 'standardisation curve' that can be used to determine the concentration of an unknown H_2O_2 concentration.

Proceedings of the 3rd International Symposium on
Environmental Contamination in Central Europe.

Academy of Science, Budapest.
20-21 September 1995

The determination of low concentrations of H_2O_2 in the H_2O_2/luminol reaction using a light sensor

Peter Walton and Richard Wilson

Abstract

The paper describes a quick and straightforward method of determining low H_2O_2 concentrations using a readily available light detection device. A graph of light intensity versus H_2O_2 concentration gives a satisfactory straight line of best fit.

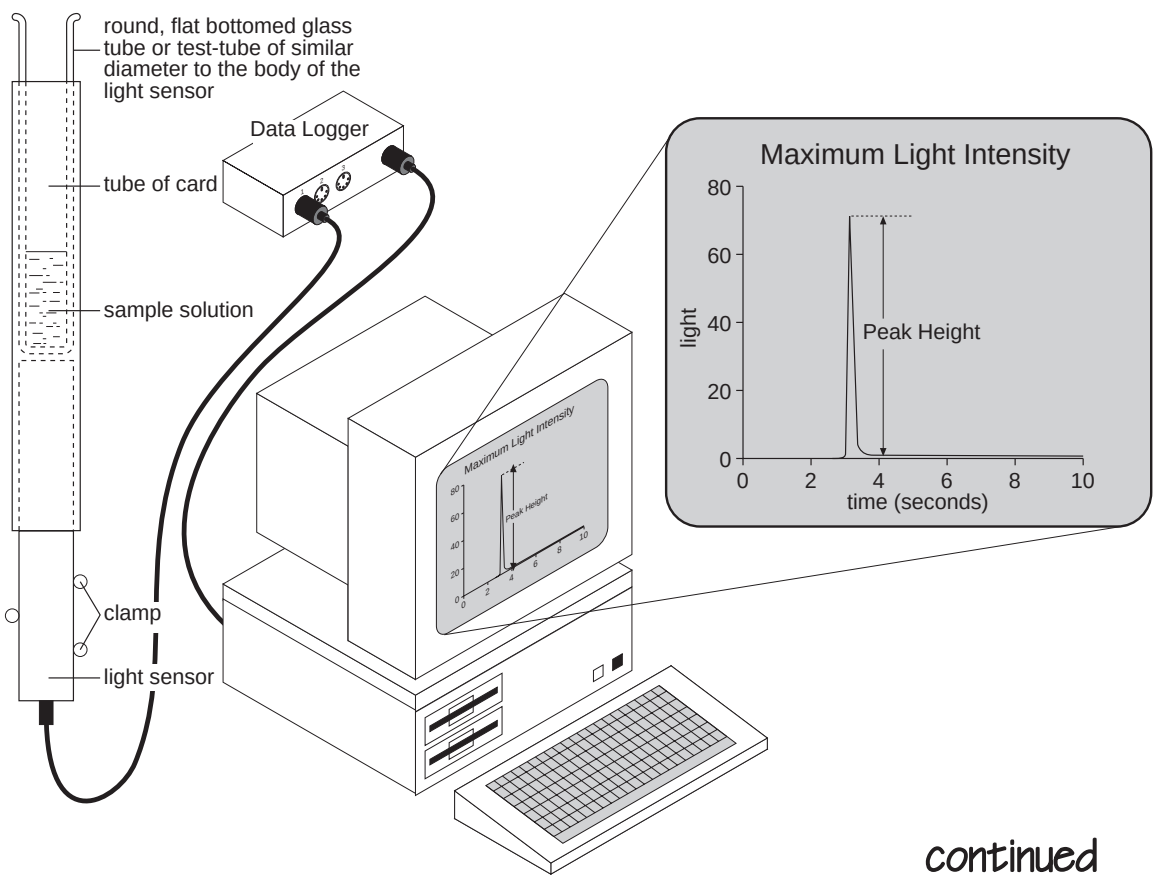
Considering the basic instrumentation and the low concentrations of H_2O_2 being measured the method gives acceptable results. The results are encouraging and further work is in progress on the design of a portable test kit for routine use in the field in daylight conditions.

Test development and methodology

Gray (1994) describes a method of measuring the light emitted during the luminol/H_2O_2 reaction using the naked eye which can be used to determine H_2O_2 concentrations. However, an alternative method is to use a light sensor linked to a PC with datalogging facility to measure the initial intensity of the light pulse. Figure 1 shows the arrangement of apparatus and equipment.

The solutions used for this investigation were the same as those used in the light pulse method described by Gray (1994), but in addition, H_2O_2 concentrations of 100 and 120ppm were used.

Figure 1. Experimental set-up for light sensor detection method



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1 Preparation

The light sensor was clamped in a vertical position with the working end pointing upward. A sample tube holder was made with white card, wrapped around the body of the light sensor and protruded above the working end, such that approximately three quarters of the sample tube was resting inside when measurements were taken. The tube of card was kept in place with adhesive tape (see Figure 1).

The experiment should be carried out in a darkened room. Total darkness is not necessary. The sensor is surrounded by the tube of card which only allows light to enter from above.

The datalogger was set up to measure light intensity (in lux units) against time (in seconds).

It was calibrated to measure light intensity over the range 0 - 80 lux over a time period of 10 seconds. The upper limit of 80 lux was determined by taking initial readings using the most concentrated standard hydrogen peroxide solution.

The light pulse given out by the reaction mixture was almost instantaneous. Therefore the time scale on the datalogger plot was of little importance to the investigation. It was possible to make use of the 'overlap' facility on the datalogger to run each set of four samples on the same charge by staggering the injection time of the cobalt chloride catalyst.

2 Method

Safety procedures are given on the Safety information sheet given in the paper by Gray (1994).

1cm^3 of luminol solution (1000ppm) was added to the sample tube, followed by 1cm^3 of the 20ppm standard hydrogen peroxide solution and the tube carefully shaken to mix the contents.

The sample tube was placed in the card sample tube holder fitted to the light sensor.

1cm^3 of cobalt chloride catalyst solution was introduced to the mixture in the sample tube using a plastic syringe, the datalogger being started at the same time. A peak was recorded by the datalogger almost immediately.

The sample tube was washed out with distilled water between each reading and dried with a tissue.

The same procedure was repeated until four recordings of peak heights had been taken for each of the standard hydrogen peroxide solutions, four for the 'unknown' solution and four for a 'blank' solution in which the hydrogen peroxide was replaced with distilled water.

3 Results and conclusions

Results are shown in Table 1.

Average values were calculated for each of the sets of four peak height readings for each of the H_2O_2 standard solutions. Also, errors associated with each of the average values were estimated and included as error bars on the graph to show the spread of readings above and below the average value.

continued

Proceedings of the 3rd International Symposium on Environmental Contamination in Central Europe.

Academy of Science, Budapest. 20-21 September 1995

A graph of average light intensity values was plotted against the corresponding H_2O_2 concentrations. Figure 2 shows the 'line of best fit' passing within the range of the error bars.

Estimation of errors

The vertical error bars extend above and below the average value of the four readings of light intensity for each H_2O_2 concentration. I have assumed that the errors in the concentration of H_2O_2 are very small and are included within the diameter of the circles. Hence it is unnecessary in this particular case to use horizontal error bars on the graph.

The method used for calculating the errors associated with the average values is that recommended by Pentz and Shott (1988) and is shown below for only one of the average light intensity values i.e. that for H_2O_2 concentration of 20 ppm. The others are calculated in exactly the same way.

Step 1

Calculate the average light intensity value (12.8) for the four readings taken for 20 ppm H_2O_2. The readings are spread out between 9.5 and 16.3.

Step 2

Calculate the negative and positive deviations from the average value using the lowest and highest readings, e.g.

9.5 ◄——— 12.8 ———► 16.3
(-3.3) (+3.5)

Step 3

Average the negative and positive deviations showing a spread of + or -3.4.

H_2O_2 conc (ppm)	Light intensity (Lux) 4 readings for each H_2O_2 conc			
	1	2	3	4
100	10.1	9.5	16.3	15.3
120	28.2	26.1	33.9	30.7
60	39.5	47.9	38.9	46.2
80	48.7	51.2	60.1	58.7
100	72.1	70.1	63.5	65.0
120	87.8	77.4	90.1	75.9

Table 1

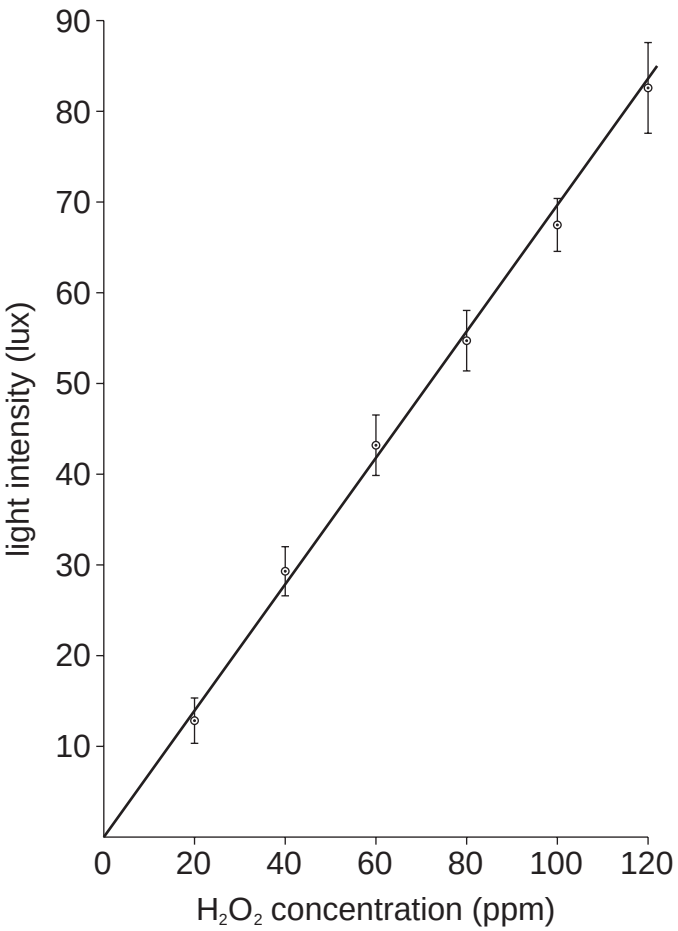

Figure 2. Calibration graph with line of best fit and showing error bars

continued

Hence, the error associated with the average value of light intensity in this case is 12.8 + or - 3.4. But this is the maximum error. We normally assume that experimental error is never always at the maximum, but would accept, on average, an error at two thirds of the maximum. Therefore, the error bar for the point on the graph representing light intensity at 20 ppm H_2O_2 would be + or -2.3.

12.8 + or - 2/3 of 3.4 (= 2.3) or 12.8 + or - 2.3

The error bars for the other values have been calculated and are shown on the graph. The graph should be a straight line for light intensity versus H_2O_2 concentration and should ideally pass through the error bars of each point on the graph (Fig. 2.).

In conclusion, the usefulness of the graph in Figure 2 as a standard calibration graph was confirmed by making up accurate, known concentrations of H_2O_2, reacting them with luminol (as described in the method) and checking that the light intensity values matched well with the calibration graph. Future, unknown concentrations of samples could thus be determined using the standard calibration graph.

References

Gray, M. L., The determination of low concentrations of hydrogen peroxide using chemiluminescence. *International Journal of Analytical Science*, vol.47, No ll, 1994.

Pentz, M. and Shott, M., *Handling Experimental Data*. Open University Press, 1988. ISBN 0-335-15897-8.