International Baccalaureate
Diploma Programme

EXTENDED ESSAY
Chemistry HL

Topic:

Investigation into the

CHEMILUMINESCENCE

of

LUMINOL

Research Question:

How do variations in the concentration of Hydrogen Peroxide affect the intensity and the longevity of the light emitted by the chemiluminescent oxidation of Luminol (5-Amino-2,3-dihydro-1,4-phthalazinedione)?

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1. Introduction

Chemiluminescence is the phenomenon where light is emitted during a chemical reaction. The oxidation of Luminol (5-Amino-2,3-dihydro-1,4-phthalazinedione) with hydrogen peroxide and sodium hypochlorite classifies as a chemiluminescent reaction.[1] My interest was piqued in the subject of chemiluminescence in a curious experience that I had at a party I was attending. The entry permit for the party was a glowing wristband. When I first saw it, I assumed a light tube or LED lights were inside the band's casing. However, I cut it open later in the party when I was fiddling with the band. I was perplexed when I saw glowing liquid dripping from the wristband. This incident led to my discovery of chemiluminescence. Furthermore, I had also been to Mattu beach, , a month before the party, and there I had witnessed the shores glow at night and was utterly captivated by how beautiful the beach was at night. At the time, I discovered that it was due to a quality that the algae near the shores possessed: Bioluminescence. While researching chemiluminescence, I realized that bioluminescence was a type of chemiluminescence that occurs inside a living organism. After these two experiences, I wanted to further explore this peculiar phenomenon that I had stumbled across, which is why I was ecstatic when I realized that I could further investigate this phenomenon as my extended essay for the IBDP.

The uses of chemiluminescence are not limited to just wristbands; there are a plethora of varied practical applications. In forensics, Luminol is a significant chemical used to detect and date any blood present on crime scenes.[2] Also, Luminol-based chemicals are used as biological trackers by biologists. I chose to investigate chemiluminescence specifically as it marked the commencement of my intrigue in luminescence and also because I found it most appropriate to investigate in a laboratory environment. As it is a chemical reaction, it can be further investigated by changing various variables that are involved. Finally, I chose to determine the effect of changing the concentration of hydrogen peroxide, the oxidizing agent, on the intensity and longevity of the light produced. I aimed to find an optimum concentration for maximum intensity and longevity.

[1] (Britannica)
[2] (Hesskew)

2. Background Information

In my research, I found that the most suitable chemical reaction I should investigate would be the oxidation of Luminol. In this reaction, Luminol (5-Amino-2,3-dihydro-1,4-phthalazinedione) is initially dissolved in an alkaline solution and reacted with hydrogen peroxide in the presence of a catalyst. I chose to use Copper (II) sulphate as a catalyst, chiefly because it is a common chemical readily available in my school laboratory. I also was satisfied with the choice of hydrogen peroxide as the oxidizing agent because the O-O bond present is relatively weak but releases rather high amounts of energy when broken, which is energetically favourable. The reaction for the oxidation of Luminol is:

Luminol + H_2O_2 → 3-APA* + N_2 + Light[3]

In the above reaction, 3-APA represents the 3-aminopthaltate in its ground state, and 3-APA* is the 3-aminopthaltate in an electronically excited state. The process of this reaction has various stages. The reaction mechanism for the reaction mentioned above is given in Figure 1.

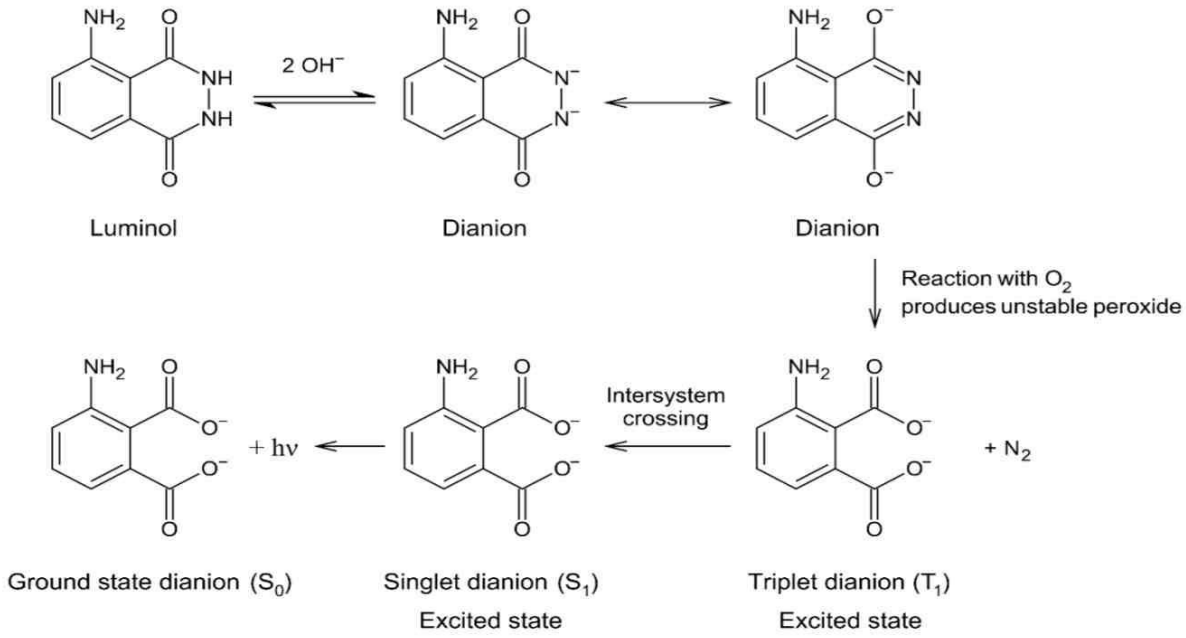

Figure 1[4]

[3] (Wikipedia)
[4] (Wikimedia)

It begins with the base converting the luminol to a resonance-stabilized dianion by removing the protons from the nitrogen atoms. Then the oxidizing agent produces oxygen which then performs cyclic addition to form a cyclic peroxide. This cyclic peroxide is then converted to 3-APA*, the 3-aminopthaltate in its excited state, a dicarboxylate anion, by removing the two nitrogen atoms as nitrogen gas. Later, as this compound descends to its ground state, the emission of visible light occurs.

When combined, the Luminol solution and hydrogen peroxide produce a bright blue glow, eventually forming a brown-black colour. The blue glow is actually made up of two distinct colours with two distinct wavelengths. When 3-APA* ions are hydrogen bonded or fully protonated, they emit light at a wavelength of 424 nm, whereas when they are not, they emit light at 485 nm.[5]

3. Hypothesis

I hypothesize that the intensity of light emitted will prove to be directly proportional to the concentration of hydrogen peroxide and thus will be greatest when the concentration is the highest and least when the concentration is minimum. Conversely, the longevity of the light emitted may decrease with an increase in the concentration of hydrogen peroxide, as I predict that a stronger and more rigorous reaction with a higher concentration will result in a higher reaction rate.

4. Methodology

4.1. Synthesis of Luminol

My quest for the most appropriate recipe for the oxidation of Luminol was one of the most arduous tasks in my research for this project. However, it was also a profusely informative task. I found that there are many procedures with slight variations in reactant or catalyst choice. Finally, after a lot of deliberating, I arrived at two possible reactions. The first reaction was the oxidation of Luminol with hydrogen peroxide as the oxidizing agent and Copper (II)Sulphate as the catalyst; this was also the one I decided to implement in my experiment. Another possible

[5] (Fleming)

reaction employed Sodium Hypochlorite as the oxidizing agent with Copper (II)Sulphate. I concluded that using Sodium Hypochlorite would not be a prudent choice as it is a chemical known to drastically affect any water-based life forms; hence, if I disposed of it in a way that would result in its presence in water bodies, it would harm the environment and the ecosystem.[6] Also, it is known to develop into a highly poisonous chemical if it interacts with ammonium salts. Since ammonium carbonate [(NH4)2CO3] is an active reactant in the recipe that I am using for Luminol, Sodium Hypochlorite is not the appropriate choice.[7] Finally, the Luminol solution that was used in my experiment was created as follows:

- Add 0.4g Sodium Carbonate, 0.2g Luminol, 24g Sodium Hydrogen Carbonate, 0.5g Ammonium Carbonate, and 4g of Copper (II) Sulphate to 500 cm^3 of water in a beaker. Stir till completely dissolved.

- Dilute the solution to 1 dm^3 by pouring 100 cm^3 of the Luminol solution into a 1 dm^3 volumetric flask and add distilled water up to the appropriate mark on the flask.[8]

4.2. Method for the Investigation

Before commencing my investigation, I realized that I would have to block out any ambient light that could possibly interfere with my measurements of the light intensity. I enquired with my chemistry facilitator about this, and I discovered there was a room that was completely covered in black wallpaper used by the photography club at my school. I got permission from the school administration and performed my investigation there. Also, before beginning the official trials, I performed a few tests with varied amounts of reactants to get a better understanding of the amount of light that would be produced. I found that observable light would be emitted for around 3 to 4 minutes. I was ready with all the desired amounts of reactants and the measurement devices so that I could start measuring the light as soon as it is

[6] (Biotech)
[7] (Biotech)
[8] (Guy)

emitted. I realized that there were too many devices that needed to be activated simultaneously while performing the reaction, so I arranged for some assistance from a friend.

Lastly, I used a mobile phone application to measure the light intensity produced due to the absence of a light meter in my school laboratory. I was sceptical about the legibility of the application, so to determine the accuracy and reliability of the application I tested it on a tube light that my school used and compared the recorded measurement with a reference that I found on the internet. To my surprise it produced accurate results within the margin of error. A normal stopwatch was used to measure the longevity of the light emitted.

To test the impact of varying concentration of hydrogen peroxide on the light intensity and the longevity of light emitted, 20 vol hydrogen peroxide was used and then varied in concentration. The variations in the concentration of hydrogen peroxide were obtained through various degrees of dilution by water. In the chemistry lab, there were only two concentration strengths of hydrogen peroxide available: 6% and 35%. I performed a trial with the 35% strength hydrogen peroxide; however I did not attain satisfactory results. The emissions of light from the reaction were observed to be often subdued and minimal due to the use of such a high concentration of hydrogen peroxide. Hence, I opted to use the 6% strength variant for my experiment. I varied the concentration by diluting the hydrogen peroxide to obtain lower concentrations. I performed several trails with varying degrees of dilution, I found that using 10 ml of hydrogen peroxide with water in a range of 100 ml to 180 ml leading to hydrogen peroxide to water ratios of: 1:10, 1:12, 1:14, 1:16, and 1:18. I chose this range as in my performed trials I observed that the emitted light was most observable, although varying in intensity, in this range. According to the method that I found for my reaction, the amount of reactants was significant to the results, and I had to make sure that I used equal amounts of both the reactants. Thus, I used the same amount of Luminol solution as the hydrogen peroxide solution in each trial.

Steps:

1. The experiment was performed in a dark room, the Light meter application was kept ready to measure the light intensity.

2. The Stopwatch was kept ready to measure the longevity of the light emitted.

3. Equal volumes of Luminol and H_2O_2 were poured into two separate 500 cm^3 beakers, the volumes were decided according to the concentration ratio being tested. For instance, in the test for the 1:12 concentration ratio test, 120 ml of distilled water was poured into a beaker with 10 ml H_2O_2, and 130 ml of the Luminol solution was poured into another 500 cm^3 beaker.

4. As the two solutions were ready, both the Luminol solution and the H_2O_2 were poured simultaneously into a 1000 cm^3 beaker to initiate the reaction.

5. The LightMeter App was prompted to start recording the light intensity as soon as light was observed, and it was stopped when no more light was emitted. The stopwatch was also started as soon as the reaction began and stopped when no more light was observed.

6. Record the measurements for the desired variables in an excel sheet.

7. Repeat Steps 1-6 three times, for each concentration of Hydrogen Peroxide, the concentration was varied by using different ratios of hydrogen peroxide to water (1:10, 1:12, 1:14, 1:16, and 1:18).

5. Precautions, Ethics and Environmental Safety Concerns

I made sure to consider the ethicality of my method and followed all precautions that were important. The following are the most significant safety measures that I took:

1. I chose to use the safest possible method, as other methods involved the use of caustic chemicals (sodium hypochlorite) that could be dangerous if used frivolously.

2. Use of protective equipment such as lab coats, gloves, and in some cases protective glasses was a must as some reactants, such as luminol, were irritants.[9]

3. The five concentrations tested were each repeated thrice to ensure relevancy and improve accuracy.

4. The disposal of the solution after no more light was being emitted, was done with consideration for the environment. I recognized that the school drain was connected to a sanitary sewer system and then made sure that a twenty-fold excess of water was used when washing the solution down the drain.

5. The quantity that was disposed of at once was minimal (less than 300 ml).

6. Results and Data Processing

6.1. Raw Data Table

- All the raw measurements are recorded in the raw data table below. Light intensities (lux) are all calculated as an average of the light intensity (lux) recorded throughout the duration of light emitted.

[9] (Science)

Raw Data	Trail 1		Trial 2		Trial 3	
Conc. Of H_2O_2 (Part by part)	Light Intensity (Lux) (±0.1)	Longevity (seconds) (±0.1)	Light Intensity (Lux) (±0.1)	Longevity (seconds) (±0.1)	Light Intensity (Lux) (±0.1)	Longevity (seconds) (±0.1)
1:10	24.3	234.6	23.7	235.0	24.6	234.9
1:12	27.6	234.3	26.9	233.2	28.2	233.6
1:14	23.4	233.1	23.3	231.2	24.1	232.3
1:16	20.7	232.0	21.3	230.3	21.2	230.1
1:18	20.4	230.2	20.3	232.1	21.3	231.0

Table 1

6.2. Calculations

Three trials were conducted for each concentration of hydrogen peroxide. The processed light intensity was calculated by taking an average of the light intensities from the three trials with the use of the following formula:

$$\boldsymbol{Light\ Intensity_{Avg}} = \left(\frac{\boldsymbol{LI_1 + LI_2 + LI_3}}{\boldsymbol{3}}\right)$$

Equation 1.

In the above equation, the $Light\ Intensity_{Avg}$ represents the average of the light intensity throughout the three trials, LI_1 depicts the light intensity recorded during trial 1, LI_2 represents the light intensity recorded during trial 2, LI_3 represents the light intensity during trial 3. The calculated average light intensity throughout the three trials for 1:10 concentration of hydrogen peroxide is shown below as **Example A** (the result shown in the processed data table for concentration ratio 1:10), all other averages for remaining concentrations are calculated by the same process.

$$\left(\frac{24.3 + 23.7 + 24.6}{3}\right) = 24.2\ Lux$$

Example A.

The raw uncertainty for the averaged calculation of the light intensity is propagated by subtracting the minimum (LI_{min}) from the maximum value (LI_{max}) and then dividing the obtained value by 2, as shown by the following equation:

$$\mathbf{Uncertainty_{Avg}} = \left(\frac{LI_{max} - LI_{min}}{2}\right)$$

Equation 2.

In the above equation, $\mathrm{Uncertainty_{Avg}}$ represents the raw uncertainty for the calculated average light intensity, LI_{max} depicts the maximum value of light intensity from the three trials and LI_{min} represents the minimum value of light intensity from the three trials. An example of the calculation in given below in **Example B** for the concentration ratio of 1:12, this was considered as the uncertainty for Light intensity as it was the highest value calculated.

$$\left(\frac{28.2 - 26.9}{2}\right) = \pm 0.7$$

Example B.

As mentioned before, three trials were conducted for each concentration of hydrogen peroxide. The average longevity was calculated by taking an average of the recorded times from the three trials with the use of the following formula:

$$Longevity_{Avg} = \left(\frac{T_1 + T_2 + T_3}{3}\right)$$

Equation 3.

In the above equation, the $Longevity_{Avg}$ represents the average of the longevity throughout the three trials, T_1 depicts the longevity recorded during trial 1, T_2 represents the longevity recorded during trial 2, T_3 represents the longevity during trial 3. The calculated average longevity throughout the three trials for 1:10 concentration of hydrogen peroxide is shown below in **Example C** (the result shown in the processed data table for the concentration ratio 1:10), all other averages for remaining concentrations are calculated by the same process.

$$\left(\frac{234.6 + 235.0 + 234.9}{3}\right) = 235.6\ seconds$$

Example C.

The raw uncertainty for time was calculated by subtracting the minimum (V_{min}) from the maximum value (V_{max}) and then dividing the obtained value by 2.

$$\mathbf{Uncertainty_{Time}} = \left(\frac{L_{max} - L_{min}}{2}\right)$$

Equation 4.

In the above equation, $\text{Uncertainty}_{\text{Time}}$ represents the raw uncertainty for the calculated average longevity, L_{max} depicts the maximum value of longevity from the three trials and L_{min} represents the minimum value of longevity from the three trials. An example of the calculation in given below in **Example D** for the concentration ratio of 1:16.

$$\left(\frac{232.0 - 230.1}{2}\right) = \pm 0.95$$

Example D.

The raw uncertainty value in the example above was considered for all the concentrations as it was the highest value of uncertainty found after calculating uncertainties for all the concentrations.

6.3. Processed Data Table

Averaged values of Light intensity and Longevity corresponding to the different concentrations tested are documented below.

Processed Data	Averaged Results from the 3 trials.	
Conc. Of H_2O_2 (part by part)	Light Intensity (Lux) (±0.7)	Longevity (seconds) (±0.95)
1:10	24.2	234.80
1:12	27.6	233.70
1:14	23.6	232.20
1:16	21.1	230.80
1:18	20.2	231.10

Table 2

As mentioned before, all the calculations done were performed to determine the processed values of the dependent variables which were light intensity (Lux) and longevity (seconds).

7. Interpretation of the Results

7.1. Graphs

I used Microsoft Excel to obtain two graphs, one that depicted the relationship between the hydrogen peroxide concentration and the light intensity recorded and the other depicting the relationship between the hydrogen peroxide concentration and the longevity of the light emitted. The two graphs and their explanations are given below.

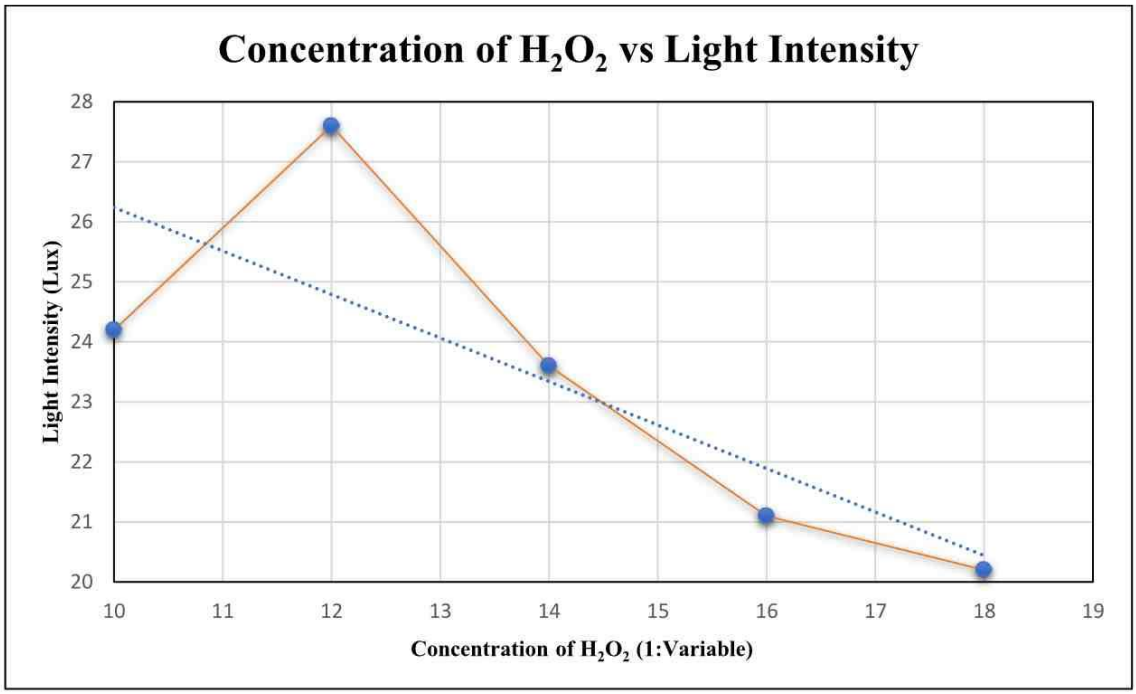

Figure 2

The impact of the concentration of hydrogen peroxide on the light intensity is shown on the graph above in Figure 2. The results that I obtained depicted that the relationship between the concentration of H_2O_2 and light intensity was more complex than what I had hypothesized. It can be observed that the light intensity drops from 27.6 Lux to 23.6 Lux and then further to 21.1 Lux as the concentration of H_2O_2 is increased from 1:12 through 1:16. Therefore, overall the results depicted a negative correlation, as the average light intensity decreased when the concentration was increased. However, the test with the least concentration did not provide the maximum average light intensity, as would be expected. The lesser the concentration of the H_2O_2 the greater the average light intensity, but only until the second concentration of H_2O_2 (1:12), this particular concentration provided the greatest average light intensity. When the concentration decreases further, the light intensity seems to fall again (1:10).

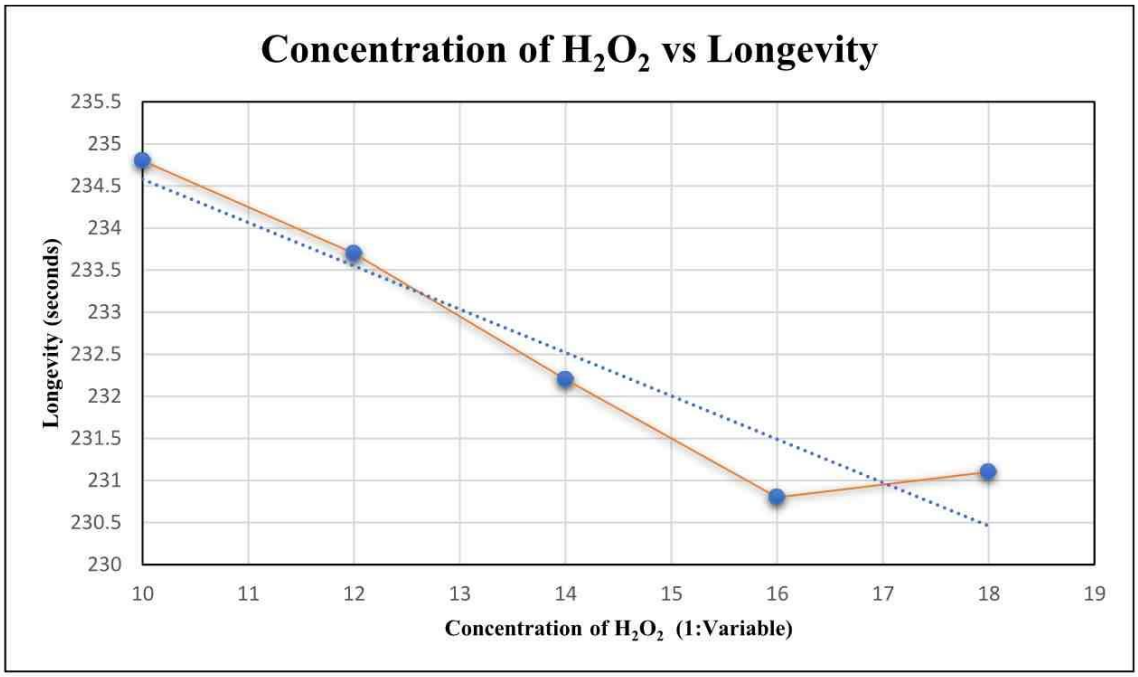

Figure 3

The impact of concentration of H_2O_2 on the longevity of light emitted was relatively simpler and also the results that I obtained supported my hypothesis. The results are depicted above in Figure 3. It can be observed that the longevity of the light emitted drops from 234.80 seconds to 233.70 seconds and further to 232.20 seconds as the concentration of H_2O_2 is increased from the ratio of 1:10 through 1:14. Therefore, the overall results display a negative correlation between longevity and concentration of H_2O_2, as the average longevity of the light emitted is seen decreasing as the concentration of H_2O_2 is increased. Hence, the data reinforces what was hypothesized. However, there is a slight variation when the concentration is increased from the ratio 1:16 to 1:18, as the longevity increases and does not follow the trend. In conclusion, the greater the concentration of H_2O_2 the lesser the longevity of the light emitted.

7.2. Explanation

I had hypothesized that the light intensity would prove to be directly proportional to the concentration of H_2O_2 because I thought that an increase in the number of oxidizing molecules of H_2O_2 would definitely prompt an increase in the number of luminol particles that react successfully to create more 3-APA*(refer to the background information section), 3-aminophtaltate molecules that are in the electronically excited state, thus increasing the

intensity of light emitted by the reaction. The results that I obtained suggest the opposite of my hypothesis, I believe this could be because when forming my hypothesis I assumed that the luminol was in excess and therefore increasing the concentration of H_2O_2 would prompt a greater light intensity. After the investigation, I think that that it is the H_2O_2 that is in excess rather than luminol. This is because during the creation of the luminol solution, a very little amount of luminol was used. I was still perplexed by my results as even if the hydrogen peroxide was in excess an increase in the concentration of it should not have resulted in a decrease in the light intensity as the amount of luminol particles that react would still be the same.

The only other possibility that I could think of was that the copper (II) sulphate present in the luminol solution was already evolving excess oxygen from the H_2O_2, thus increasing the concentration of H_2O_2 only disturbed the reaction as there would be more H_2O_2 molecules that are not involved in any collisions hindering the collisions of the molecules that actually do react. If true, this would support my findings. Nevertheless, even if what is proposed above was true it does not compensate for the anomalous nature of the light intensity when the concentration was lowered from 1:12 to 1:10, here the light intensity did not follow the trend that it displayed of increasing as the concentration was decreased. I tried to understand why this was occurring by looking for any information or similar readings in any research papers that I could find, I also consulted my supervisor about it. Unfortunately, I could not find any explanation for the anomaly, but this only further emphasizes the importance of this investigation as if studied further an explanation could be developed.

In the case of longevity, the results obtained validated my hypothesis that a stronger and more rigorous reaction with higher concentration will result in a higher rate of reaction. Evidently, increasing the concentration of H_2O_2 resulted in a decrease in longevity.

8. Conclusion

My original hypothesis stated that as the concentration of H_2O_2 is increased the intensity of the light emitted will also increase. The results that I obtained show that my hypothesis was incorrect, as it is shown in Figure 1, the light intensity decreases as the concentration of H_2O_2 is increased. However, the light intensity did increase when the concentration was increased from the ratio of 1:10 to 1:12. My second hypothesis stated that the longevity of light emitted

would decrease as the concentration of H_2O_2 is increased. Figure 2 clearly depicts that as the concentration of H_2O_2 is increased the longevity of light emitted decreases. Hence, this hypothesis was validated. However, there is a slight variation in the last increase of concentration as the longevity increases and does not follow the trend that it displayed before. I concluded this increase to be an anomaly as I could not determine a reason for it, I tried to look for similar results in various research papers, but I could not find any.

My goal throughout this investigation was to determine an optimum concentration of H_2O_2 where the maximum light intensity and longevity would be attained, and I believe I have gained enough insight into the effect that the concentration of H_2O_2 has on the oxidation of luminol to identify an optimum concentration. From my results, I conclude that a concentration in the ratio of 1:12 (H_2O_2: Water) is the best to obtain the maximum light intensity and almost the maximum longevity. An image of the oxidation of Luminol with concentration of H_2O_2 in the ratio of 1:12 (H_2O_2: Water) is depicted below in the Figure 4.

Figure 4: *The oxidation of Luminol with concentration of H_2O_2 in the ratio of 1:12*

The concentration in the ratio of 1:10 (Water: H_2O_2) did last longer but I believe this difference in longevity does not outweigh the gain in light intensity when the concentration is increased. Therefore, an optimum concentration of H_2O_2 was determined.

9. Evaluation and Improvements

9.1. Random Error

The masses of the materials used in the synthesis of Luminol were susceptible to random error. This was chiefly due to the physical and amorphous nature of most of the materials, as moving the materials after weighing them proved to be a difficult task. Most of the materials such as Sodium carbonate and Ammonium carbonate tended to stick to the filter paper that was used in the weighing machine possibly leading to a miniscule loss of mass. This source of random error was exacerbated when it came to the Sodium Hydrogen Carbonate, as it was in the form of lumps, and I had to crush it into an amorphous form and then weigh it. But the amount require of it was 24 grams which was much higher than the required amount of the other materials, this also meant that there was a lot of the substance on the filter paper when moving it after weighing, thus resulting in a loss of mass.

An improvement to the investigation would be to increase the number of trials performed for each concentration, as this would further reduce any random error. However, to implement this into my investigation I would have to reduce the number of concentrations being tested because if not, the essay would exceed the word limit. I opted to not implement this change because if there were only three concentrations being tested, I would not have reached a satisfactory conclusion.

9.2. Systematic Error

A source of systematic error could have been the light emitted by the phone screen when it was measuring the light intensity. This could have been possibly fixed by finding the average intensity of light emitted by the model of the phone that I owned and then subtracting it from the recorded value.

10. Scope of Investigation

The results and conclusions obtained in the experiment prompted a better understanding of the relations between Luminol and the factors tested above. However, there is much more scope for this investigation to evolve and discover much more than what has already been found. As mentioned before in the background information, Luminol is chiefly used in the forensic field

to test for blood stains on crime scenes. Luminol conveniently enhances blood stains, enabling the interpretation of blood stain patterns. Haemoglobin, which has iron atoms, is a component of blood. These iron atoms can serve as a catalyst to speed up the reaction between hydrogen peroxide and luminol. However, my experiments and tests were done with Copper (II) Sulphate acting as a catalyst. Therefore, I think that if similar tests are conducted using a catalyst that contains iron atoms, the results obtained would benefit the forensic field as a known optimum for the reaction and concentration of H_2O_2 could aid the forensic specialists to better prepare the luminol solutions and set up the reactions at crime scenes.

11. Appendix I

11.1. Apparatus and Chemicals

11.1.1. Chemicals

- 0.4g of Sodium carbonate.
- 0.2g of Luminol.
- 24g of Sodium hydrogen carbonate.
- 0.5g of Ammonium carbonate.
- 0.4g of Copper (II) sulphate.
- 50ml of 30 vol Hydrogen peroxide.

11.1.2. Apparatus for synthesizing Luminol and for the Investigation

- Distilled Water – 1 Litre
- Beakers (2) – 1000 cm^3
- Beakers (2) – 300 cm^3
- Spatula.
- Weighing Scale.
- Stopwatch.
- LightMeter App.

Note: All the chemical quantities mentioned above are required to perform 1 trial of the experiment.

11.2. Additional Images relevant to the Investigation.

Figure 5: An image of the room that I performed the investigation in, as seen all the walls are covered with black wallpaper to ensure no ambient light is recorded.

Figure 6[10]: A screenshot of the mobile application "Light Meter". I used it to record the light intensities of the reaction throughout the investigation.

[10] (Adams)

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