SASTA OLIPHANT SCIENCE AWARDS

SOUTH AUSTRALIAN SCIENCE TEACHERS ASSOCIATION

Prize Winner

Scientific Inquiry

Year 11-12

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Investigation On the Effect of the Molar Concentration of Aqueous Hydrogen Peroxide and Copper(II) On the Induction Time (seconds) of N-acetyl-L-cysteine-Inhibited Chemiluminescence

1.Introduction

When I was doing figure skating in Beijing, I used to organise glow-stick-making workshops with children at the rink prior to gala performances. In order to develop a variety of creative workshops, I had to do my independent research and attempt to modify the timing and prolong the duration of glowing, which piqued my genuine interest in the elegance of photochemistry, and I wondered what was actually happening on a molecular level. After encountering the Jablonski diagram and learning about the quantization of energy levels during Biology Olympiad Summer School, I was curious and impressed by the fact that chemiluminescence has such significant applications in forensic science, immunoassay, and counter-illumination camouflage, by which controlling the timing of chemiluminescence is highly relevant in these real life applications. Thus, inspired by both my meaningful personal experience and the significance of this topic, I've decided to further explore the factors that play a role in controlling the induction time of chemiluminescence.

During the planning stage, I referred to the self-learnt content of the IB HL kinetics chapter, as well as a number of high quality, peer-reviewed resources on Pubmed and Researchgate. Subsequently, in this experiment, I'm going to determine the dependence of the induction time of chemiluminescence on hydrogen peroxide (H_2O_2) and copper(II) (Cu^{2+}) by calculating the rate constant and the partial reaction order in respect to each species using integrated rate law. Specifically, this will be achieved by varying the concentration of H_2O_2 and Cu^{2+} while keeping the concentration of N-acetyl-L-cysteine constant in order to time their resulting induction time of chemiluminescence (in seconds). The rationale of this approach is that formulating the mixed rate law of N-acetyl-L-cysteine oxidation will allow the induction time of chemiluminescence to be easily predicted for a given amount of reactant (H_2O_2) and catalyst (Cu^{2+}), as N-acetyl-L-cysteine acts as an inhibitor that needs to be fully oxidised for chemiluminescence to take place. A major challenge during data collection was that the induction time of chemiluminescence was increasingly shorter when the concentration of H_2O_2 and Cu^{2+} were increasingly higher, such that it was extremely difficult to time manually. In other words, human reaction time was more of a determining factor of the data registered than the actual cysteine reaction time. Hence, in order to mitigate the percentage error in timing, the duration of the induction time was prolonged by increasing the mass of anhydrous N-acetyl-L-cysteine added to the reaction mixture. Since the major limitation of this investigation is the inherent uncertainty in measurements, this approach has effectively mitigated this.

2. Research question

To what extent does the concentration of aqueous hydrogen peroxide (22mM, 44mM, 88mM, 176mM, 352mM) and aqueous copper (II) (20mM, 40mM, 80mM, 160mM, 320mM) affect the induction time of N-acetyl-L-cysteine-inhibited luminol oxidation, as measured by the duration taken in seconds for chemiluminescence ($\lambda = 425\ nm$) to be first observed by eyes?

3. Background

The phenomenon of chemiluminescence refers to the production of electromagnetic radiation observed when a chemical reaction yields an electronically excited intermediate or product (Christofi, 2005). For instance, chemiluminescence is produced as a result of luminol oxidation by hydrogen peroxide in a weak alkaline solution. Hence, by adding sodium hydrogen carbonate, water-soluble luminol dianion is first produced from water-insoluble luminol via base-catalysed deprotonation (*Diagram 1*). Next, under a suitable redox catalyst (Cu^{2+}), luminol dianion can react with oxidizing agents (H_2O_2), forming 3-Aminophthalate (3-APA*) with electrons in their excited state upon a nucleophilic substitution of nitrogen by oxygen gas. Subsequently, chemiluminescence is produced from the radiative recombination of excited electrons of 3-APA* falling back to their ground state, releasing excess energy in the form of photons.

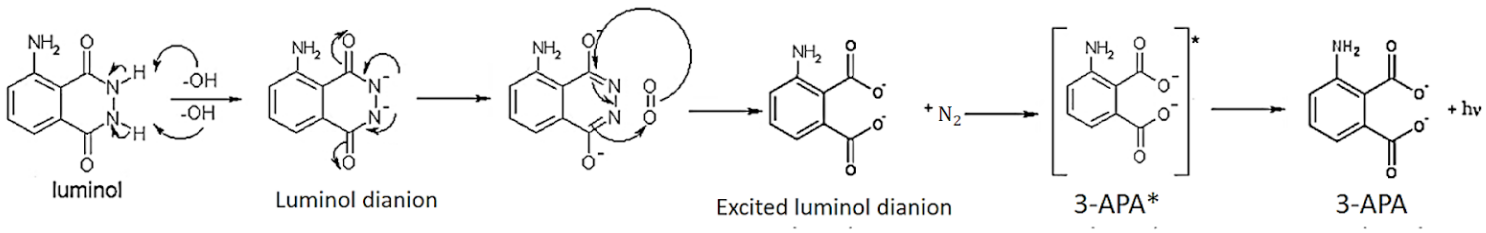


Diagram 1 *- oxidation of luminol by H_2O_2 under a transition metal catalyst to emit 425 nm chemiluminescence via radiative recombination*

This experiment attempts to modify luminol oxidation into a clock reaction, by which a flash of light ($\lambda = 425\ nm$) appears after a certain period of induction time. For the reaction between H_2O_2 and luminol to take place at standard ambient temperature and pressure (STAP), homogeneous catalyst Cu^{2+} is required to lower the activation energy and stabilize the transition state of the reaction. However, by adding N-acetyl-L-cysteine, Cu^{2+} is reduced to Cu^{+} by the lone pairs of the S^{-} group of deprotonated N-acetyl-L-cysteine, and captured in a dimer of Cu(I)–cysteine complex that does not facilitate luminol oxidation (Mandal, 2013). This process is illustrated by *Diagram 2*.

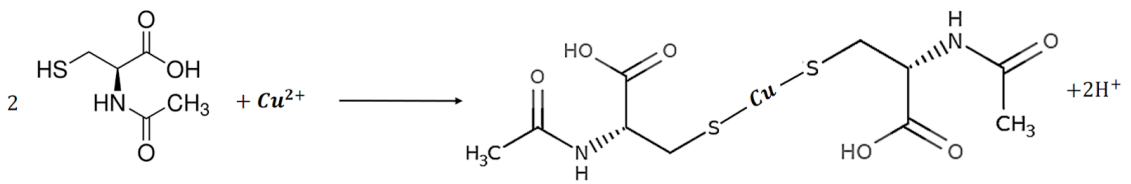


Diagram 2 - *formation of Cu(I)-cysteine complex with Cu(I) covalently bonded to sulfur in a dimer of N-acetyl-L-cysteine*

Nevertheless, this inhibition is only temporary. A cycle of chain reactions fuelled by H_2O_2 leads to gradual oxidation of N-acetyl-L-cysteine, and Cu^+ is also re-oxidized to Cu^{2+} by H_2O_2, restoring its catalytic activity. The oxidation reaction of N-acetyl-L-cysteine is known to follow a chemical chain reaction, which is often associated with reaction orders that are non-integer, or fractional reaction order (Atkins, 2006). One of the mechanisms of N-acetyl-L-cysteine ($C_5H_8NO_3SH$) oxidation and Cu^+ re-oxidation is illustrated by the following three equations according to Rigo (2004) and Xing (2012):

1) $C_5H_8NO_3SH_{(aq)} \rightleftharpoons C_5H_8NO_3S^-_{(aq)} + H^+_{(aq)}$ (fast and reversible)

2) $C_5H_8NO_3S^-_{(aq)} + H_2O_{2\,(aq)} \rightarrow C_5H_8NO_4S^-_{(aq)} + O^{\bullet}_{(aq)} + H_2(aq)$ (slow)

3) $Cu^{+}_{(aq)} + H_2O_{2\,(aq)} \rightarrow Cu^{2+}_{(aq)} + H_2O_{(l)} + O^{\bullet}_{(aq)}$ (slow)

Therefore, the duration taken for chemiluminescence to appear is determined by the rate at which N-acetyl-L-cysteine is consumed, which is in turn, influenced by the concentration of both Cu^{2+} and H_2O_2 due to their involvement as a catalyst and a reactant respectively in the rate determining step (RDS). Hence, the aim of this experiment is to investigate how to precisely control the induction time of N-acetyl-L-cysteine-inhibited chemiluminescence by altering the concentrations of aqueous H_2O_2 and Cu^{2+}. This is done by first calculating the rate of N-acetyl-L-cysteine oxidation (V) using the recorded duration taken for a flash of blue light to appear, upon additions of H_2O_2 (aq) and Cu^{2+} (aq) of increasing concentrations. Hence, by determining the rate constants (k_1 and k_2) and the partial reaction orders (p and q) with respect to each chemical species, the mixed rate law of N-acetyl-L-cysteine oxidation will then be derived, which will serve to predict the induction time of cystine-inhibited chemiluminescence for a given amount of reactant and catalyst.

Beyond the manufacture of glow sticks, being able to precisely predict the timing of chemiluminescence is highly important in various scientific applications, such as immunoassay and biomarkers in imaging, by which it is necessary to ensure that chemiluminescence visibly appears exactly during the interval (or at the instance) of analysis. Additionally, it is also noteworthy that cysteine is specifically chosen to be the inhibitor of Cu (II)-catalyst luminol oxidation as this essential amino acid often serves as the target of bioluminescence probes in diagnostics (Atkin, 2006). Thus, exploring how cysteine inhibits chemiluminescence as well as how the concentrations of H_2O_2 and Cu^{2+} determine the duration of this temporary inhibition may help to minimize the amount of reagents used while still ensuring the desirable scientific and diagnostic outcomes.

4. Hypothesis

It is hypothesized that the induction time (in seconds) of cysteine-inhibited chemiluminescence will decrease, and the rate of N-acetyl-L-cysteine oxidation will increase, as the concentration of H_2O_2 (aq) and Cu^{2+} (aq) increases respectively. This is because the reactant H_2O_2 is involved in the rate determining step of N-acetyl-L-cysteine oxidation (by which increased its concentration would increase the frequency of collisions and shift the position of equilibrium to the right), while the catalyst Cu^{2+} also speeds up this step by lowering the activation energy of N-acetyl-L-cysteine oxidation (to induce a greater fraction of successful collisions). However, the partial reaction orders in respect to H_2O_2 (aq) and Cu^{2+} (aq) cannot be predicted, because they are not equal to the stoichiometric coefficients and can only be determined experimentally (Atkins, 2006).

5. Variables

It is important to note that 22mM (rather than 20mM) was chosen to be the lowest concentration of H_2O_2 (aq) because it was evident from the preliminary tests that 22mM was the minimum concentration that allowed luminol oxidation to proceed, and in fact, the two chemical species do not need to be identical in concentrations for the purpose of formulating the integrated rate law.

5.1. Independent variables

1) Concentration of $CuSO_4$ (aq): 20mM, 40mM, 80mM, 160mM, 320mM
2) Concentration of H_2O_2 (aq): 22mM, 44mM, 88mM, 176mM, 352mM

5.2. Dependent variable

The duration taken for chemiluminescence (a flash of blue light) to be first observed by eyes (in seconds)

5.3. Uncontrolled variables

1) Temperature loss to the surroundings before the final temperature is registered
2) Impurities in the reagents
3) The presence of reverse reactions and side reactions
4) Random fluctuations of room temperature

5.4. Controlled variables

Variables	Impact on data	Method of control
The concentration and amount of cysteine and luminol in the reaction mixture	Variations in the concentration and the amount of these 2 species would result in invalid comparisons across the reaction rates registered. This is because higher cysteine concentration would prolong the induction time to a greater extent as more Cu(II) is reduced to Cu(I), and vice versa. While higher luminol concentration would lead to a longer and brighter duration of glowing, vice versa.	Prepare the stock solutions as well as the diluted solutions using bulb pipettes (1ml) of smallest possible gradation available to mitigate random errors and enhance precision. Use the same measuring instrument throughout the experiment to reduce systematic errors.
The volume of H_2O_2 (aq) and $CuSO_4$ (aq) added to the reaction mixture (for each trial)	Since H_2O_2 is a reactant that is present in the RDS of the reaction mechanism of cysteine oxidation, and Cu^{2+}serves as a catalyst that accelerates the RDS by providing an alternative pathway that requires lower activation energy, thus, under constant concentration, if a higher	All solutions are measured with bulb pipettes (1ml) of smallest possible gradation available. This apt choice of measuring equipment reduces the random error inherent in estimating the last

	volume of H_2O_2 or $Cu^{2+}$is added to the reaction mixture, the induction time of chemiluminescence is likely to decrease. On the other hand, the rate of N-acetyl-L-cysteine oxidation is likely to increase if a greater volume of H_2O_2 or $Cu^{2+}$is applied.	uncertainty digit, which helps to increase the precision of the measurements by minimizing random uncertainty.
The amount of dissolved oxygen in the reaction mixture (in mols)	Dissolved oxygen in the reaction mixture can cause unwanted oxidation that is not induced by the oxidizing agent under investigation (H_2O_2). If there were a significant difference in the amount of dissolved oxygen, then the data collected would be less valid in indicating the extent to which copper ions have restored their catalytic activity solely due to the oxidation of cysteine to cystine.	Unwanted oxygen dissolution is mitigated by shaking the reaction mixture (in a test tube with screw-on cap) instead of stirring it with a glass rod. This reduces the exposure to oxygen gas throughout the mixing process, thus, ensuring the integrity of the solution.
The duration of shaking the reaction mixture	Shaking can transfer kinetic energy to the reaction mixture which increases both the frequency of particle collision as well as the energy upon collision. Longer shaking duration would result in a faster initial rate of cysteine oxidation as well as greater proportion of reactant particles overcoming the activation energy threshold, and vice versa.	Shake the reaction mixture exactly twice for each trial in a standardised manner. Carry out repeated trials and take the mean values of the results registered in order to compensate for potential human differences in shaking.
The initial temperature of all aqueous solutions	Since cysteine oxidation is an exothermic reaction, higher temperature of initial solutions would shift the position of equilibrium to the left, hence, reducing the concentration of cystine converted from cysteine, and vice versa. This would introduce another extraneous variable to the induction time of luminol oxidation.	Place all the stock solutions and distilled water at room temperature in the laboratory (where the experiment is conducted) for at least 2 hours beforehand. Avoid direct sunlight by setting up the work station away from the windows.
Exposure to sunlight	Cu^{2+} can be photochemically reduced to Cu^{+} via free radical mechanism by hydroxyl radicals generated from the photolysis of H_2O_2 (Xing, 2012). Additionally, cysteine-Cu complexes can dissociate, resulting in the generation of Cu^{+} (Xing, 2012).	Close the curtain of the room throughout the entire procedure of the experiment. Carry out all trials with samples in the painted black box and stay away from window benches.

6. Materials

Equipment	Quantity	Uncertainty
5 ml glass bulb pipette	1	± 0.05 ml
1 ml glass bulb pipette	4	± 0.005 ml
Digital stopwatch	1	± 0.01 s
100 ml measuring cylinder	8	± 0.5 ml
Westlab digital scale	1	± 0.01 g
500 ml glass beaker	1	± 25 ml
Mercury thermometer	1	± 0.5°C
10 ml test tubes with screw-on cap	7	± 0.25 ml
4 ml glass vials	7	
A painted black cardboard box	1	

Chemicals	Quantity
1.76 M H_2O_2 (aq)	4.00 ml
Anhydrous N-acetyl-L-cysteine	10.08 g
Anhydrous $CuSO_4$ (s)	2.00 g
Anhydrous luminol	0.10 g
Na_2CO_3(s)	2.00 g
$NaHCO_3$(s)	12.00 g
$(NH_4)_2CO_3$ (s)	0.25 g
Distilled water	1100 ml

7. Method

1. Anhydrous luminol (1.00g), anhydrous N-acetyl-L-cysteine (3.00g), Na_2CO_3(s) (2.00g), $NaHCO_3$(s)(12.00g), and $(NH_4)_2CO_3$(s) (0.25g) were weighed using a digital scale and added to a clearly-labeled 500ml beaker. Distilled water was first added roughly using a funnel to just below the 500ml mark of the 500ml beaker. The solution was then topped up drop-wise using a 5ml bulb pipette until the bottom of the meniscus sat exactly at the 500ml mark when viewed from an eye-level. In the following procedures, all steps involving making solutions to a certain volume were done using this method to mitigate random uncertainties.

2. 10mM, 20mM, 40mM, 80mM, 160mM and 320mM copper solutions were prepared by weighing 0.16g, 0.32g, 0.64g, 1.28g, 2.56g and 5.12g anhydrous $CuSO_4$ respectively. Each of the five weighed $CuSO_4$(s) samples were dissolved in distilled water (100ml) in 5 separate 100ml measuring cylinders.

3. Diluted H_2O_2 solutions of 5 different concentrations were prepared from 6% (1.76M) stock H_2O_2 (aq). This was done by using a 1ml bulb pipette to transfer 5 lots of 1ml 1.76M H_2O_2 into 4 separate 100ml measuring cylinders and topped up using distilled water to 80ml (give 22mM H_2O_2), 40ml (give 44mM), 20ml (give 88mM), 10ml (give 176mM) and 5ml (give 352mM) respectively.

4. To avoid contamination, another 1ml bulb pipette was used to transfer 1ml of the contents in the 500ml beaker to a plastic 10ml test tube with screw-on cap.

5. Referring to *Table 1* below, two separate 1ml pipettes were used to charge a 4ml glass vial with 0.1ml 10mM solution, and 1.0ml 22mM H_2O_2 solution. This made up 'concentration set 1 (**[C] set #1**)'.

Table 1- The concentrations of H_2O_2 (aq) and $CuSO_4$(aq) in each concentration set transferred to the 4ml glass vial

	Concentration (mM)									
Chemicals	**[C] Set #1**	**[C] Set #2**	**[C] Set #3**	**[C] Set #4**	**[C] Set #5**	**[C] Set #6**	**[C] Set #7**	**[C] Set #8**	**[C] Set #9**	**[C] Set #10**
$CuSO_4$ (aq)	10	10	10	10	10	20	40	80	160	320
H_2O_2 (aq)	22	44	88	176	352	22	22	22	22	22

6. The 4ml filled glass vial was carefully inserted into the filled 10ml test tube with screw-on cap without allowing the contents to mix.

7. The room light was switched off and the subsequent procedures were performed with the samples placed in the black cardboard box. The 10ml test tube was sealed and shaken vigorously twice in order to mix in the contents in the 4ml glass vial. The timer was started at the exact instance when shaking began.

8. After shaking, the timer was stopped at the time when a flash of blue light was observed. The duration taken for chemiluminescence to appear (in seconds) in the first trial of concentration set 1 was recorded in the raw data table (*table 2*).

9. Immediately, a mercury thermometer was used to measure the final temperature (°C) of the reaction mixture. The data registered was also recorded in *table 2.*

10. Step **4 - 9** was repeated for another 4 trials for concentration set 1. Hence, 5 trials in total were carried out for each concentration set.

11. Referring to *table 1*, step **4 -10** was repeated for the remaining concentration sets (2-10). All pipettes were rinsed thoroughly with distilled water first, then rinsed with their respective solutions, before the next concentration set was tested. To avoid contamination, a new 4ml glass vial and a new 10ml test tube with screw-on cap were used for each different concentration set.

12. All raw data collected from the procedures documented above were recorded in *table 2* and *table 3.* This data was used to calculate the average induction time (in seconds) according to equation 10 in section 9.4.5, which was then recorded in *table 4* in section 9.3 (processed data). In addition, as described by section 9.4 (data processing method), the temperature data were then used to normalize the recorded induction time to what would be observed at 25°C, before the normalised average induction time and normalised reaction rate (*table 4*) were calculated according to equation 1 (section 9.4.1) and equation 2 (section 9.4.2) respectively.

Adaptation of method: The method used is partially inspired by the 2018 International Chemistry Olympiad (Almássy, 2018), however, it has been modified extensively by conducting trial and error throughout this investigation in an attempt to accommodate factors like unchangeable low room temperature. At first, by adapting the H_2O_2 and Cu^{2+} concentrations used in the 2018 International Olympiad, it was evident that no glowing appeared (after 10 minutes of waiting) and the reaction mixture turned green (due to side reaction). To overcome this issue, the following modifications were made: 1) Cu^{2+} solutions were diluted by increasing dilution factors until reaching a concentration range that was high enough to allow successful catalytic activity while low enough to allow N-acetyl-L-cysteine to outcompete Cu^{2+} for oxidation; 2) The concentration of H_2O_2 was increased by doubling it each time until it was concentrated enough to excite luminol (induce chemiluminescence) to an easily observable extent and to consume N-acetyl-L-cysteine within a viable time frame.

8. Risk assessment

8.1. Safety- handling chemicals: *H_2O_2 , luminol and $CuSO_4$* can cause irritation or damage to eyes, skin and the respiratory system. N-acetyl-L-cysteine is generally safe but may cause nausea and vomiting. *Na_2CO_3, $NaHCO_3$, and $(NH_4)_2CO_3$* are bases that may gelatinize skin tissue that are destructive and hard to remove. Hence, when handling all chemicals, it is compulsory to wear safety goggles, gloves and ensure access to eyewash stations at all times. In the event of accidental exposure, perform first aid (such as flushing eyes/skin, cover irritated skin with an emollient) and seek medical attention immediately. Reacted chemicals should also be disposed safely into the non-halogenated organic waste bottle in the fume hood of the laboratory.

8.2. Safety - handling equipment: *Glass pipettes, beakers and the thermometer* may cause accidental cutting and bleeding if broken. Hence, double check for stress lines and chipped rims before use. Additionally, if the mercury thermometer is broken, mercury beads can evaporate into the surrounding and become toxic. Hence, always use two hands to carry the glass equipment, and gloves should be worn when there is a risk of breakage. In the event of an accident, broken pipettes and beakers should be disposed safely into the shared broken-glass box in the laboratory, and wounds should be washed first before treating with bandages. Leaked mercury beads from the thermometer should be collected onto a damp paper towel using an eyedropper. Notify the lab technician immediately.

8.3. Environmental risks and waste disposal: There would be no significant environmental risks associated with this experiment if all wastes were disposed appropriately into the shared organic waste bottle in the laboratory. In addition, stoichiometry is attentively carried out prior to the experiment to estimate the amount of reagents required, thus, minimizing chemical wastes associated with this investigation.

8.4. Ethics: If 8.1- 8.3 were thoroughly followed, then no problematic ethical issues would be present in this experiment due to the exclusion of non-human living organisms, as well as the avoidance of environmental pollution and harm to health. Additionally, there are no ethical considerations in regard to the access of data since no second-hand data is used. All academic literature and resources used are referenced in the bibliography. Lastly, there is no conflict of interest.

9. Results

9.1. Raw data table

It is important to note that two negative controlled samples (without the addition of any H_2O_2 and Cu^{2+}) were also observed. As expected, no detectable evidence indicating chemical changes appeared in the reaction mixture. Hence, the negative controlled sample is excluded from the raw data table. *Table 2* and *table 3* display the raw data collected for concentration set 1-10 throughout 5 trials.

Table 2- the induction time (in seconds) and the final temperature (in°C) of the N-acetyl-L-cysteine-inhibited luminol oxidation reaction registered upon adding H_2O_2 (aq) of increasing concentrations under constant Cu^{2+} concentration (10mM)

[C] Set #	[Cu^{2+}] (mM) ±1mM	[H_2O_2] (mM) ±2 mM	The induction time of chemiluminescence (s) ±0.50s					Final temperature (°C) ±0.7 °C				
			1	2	3	4	5	1	2	3	4	5
1	**10**	**22**	13.63	12.46	13.03	12.57	13.64	18.5	18.5	18.0	18.0	18.0
2	**10**	**44**	10.82	9.85	11.78	10.98	11.60	18.0	18.0	18.0	18.0	18.0
3	**10**	**88**	8.56	9.97	10.27	9.05	9.37	18.0	18.0	18.0	18.5	18.5
4	**10**	**176**	6.48	7.44	6.97	6.88	7.02	18.2	18.2	18.6	18.5	18.5
5	**10**	**352**	4.52	5.23	5.13	3.44	4.98	17.5	17.5	17.5	17.5	17.5

Table 3- the induction time (in seconds) and the final temperature (in°C) of the N-acetyl-L-cysteine-inhibited luminol oxidation reaction registered upon adding Cu^{2+} (aq) of increasing concentrations under constant H_2O_2 concentration (22mM)

[C] Set #	[H_2O_2] (mM) ±2 mM	[Cu^{2+}] (mM) ±1 mM	The induction time of chemiluminescence (s) ±0.50s					Final temperature (°C) ±0.7 °C				
			1	2	3	4	5	1	2	3	4	5
6	**22**	**20**	2.76	3.25	2.22	2.76	3.21	17.5	17.3	17.3	17.3	17.5
7	**22**	**40**	1.24	1.22	1.37	1.02	1.01	18.0	18.5	18.0	18.0	18.0
8	**22**	**80**	1.11	1.06	1.01	1.00	1.01	16.8	16.9	17.0	17.3	17.2
9	**22**	**160**	1.02	0.81	0.69	0.79	0.74	16.4	16.2	16.1	16.8	16.8
10	**22**	**320**	0.67	1.02	0.45	0.15	0.34	16.7	16.7	16.5	16.5	16.5

The upper fence (Q3+1.5 × IQR) and the lower fence (Q1 – 1.5 × IQR) of the 5 trials for each concentration set are calculated. In *table 3,* The two data points highlighted in red are identified as outliers, because 0.15 is less than the lower fence 0.25, and 1.02 is greater than the upper fence 0.85. These two outliers are excluded from all subsequent data processing. It is unsurprising to see outliers for concentration set 10 as chemiluminescence appeared so swiftly such that human reaction time played a greater role in the random uncertainty of timing. Furthermore, the uncertainty of the induction time in *table 2* and *table 3* was determined as follows:

$$\frac{\sum_{n=1}^{10} (max.\ induction\ time\ of\ concentration\ set\ n) - (min.\ induction\ time\ of\ concentration\ set\ n)}{2} \times \frac{1}{10} + \text{stopwatch uncertainty} = \frac{9.62}{20} + 0.01 \times 2 \simeq \pm 0.50\text{s}$$

The uncertainty of the final temperature in *table 2* and *table 3* is calculated via a similar process. However, this method of determining absolute uncertainty clearly possesses limitations as every concentration set has slightly different random uncertainty.

9.2. Qualitative observations

Firstly, when anhydrous N-acetyl-L-cysteine was added to the luminol-containing reaction mixture (step 1 of section 7), gentle effervescence was observed at the surface of the solution. Secondly, after an increasing mass of N-acetyl-L-cysteine was added during experimental modification, it was observed that chemiluminescence appeared increasingly brighter. Meanwhile, it was also evident that the glowing appeared upon the radiative recombination of excited luminol molecules was not uniform throughout the reaction mixture, in other words, glowing tended to start from one corner of the test tube and then gradually spread out. This lack of uniformity in glowing was especially noticeable for lower concentration sets (1-3), by which it raised the question in terms of when the timer should be stopped, as well as providing counter-evidence for the proposition that all N-acetyl-L-cysteine needs to be oxidised for luminol to glow. Additionally, brown copper precipitate was noticed for concentration sets 6-10 after chemiluminescence took place. Through observations, it appeared that the greater the concentrations of H_2O_2 and Cu^{2+}, the larger amount of copper precipitates formed, and the shorter the duration of chemiluminescence (or the glowing was obscured by the copper precipitate more). Lastly, it was clear that the final reaction mixture cooled down very rapidly after glowing due to low room temperature, by which the mercury thermometer appeared to be too insensitive to register its instantaneous temperature accurately. A digital thermometer connected to Logger Pro should have been used to counter this and pursue a greater accuracy in results.

9.3. Processed data

Table 4- the average induction time, average induction time normalized to 25°C, average temperature and standard deviation of the cystine-inhibited luminol oxidation reaction under the treatment of H_2O_2 (aq) and Cu^{2+} (aq) of increasing concentrations

[C] set #	[Cu^{2+}] (mM) ± 1 mM	[H_2O_2] (mM) ± 2 mM	Average induction time (seconds) ± 0.50 s	Average induction time normalized to 25°C (seconds) ± 0.29 s	Average temperature (°C) ± 0.7°C	Standard deviation (seconds)
1	10	22	13.07	8.03	18.2	0.50
2	10	44	11.01	6.64	18.0	0.68
3	10	88	9.44	5.80	18.2	0.62
4	10	176	6.96	4.35	18.4	0.31
5	10	352	4.66	2.91	17.5	0.66
6	20	22	2.84	1.62	17.4	0.37
7	40	22	1.17	0.67	18.1	0.14
8	80	22	1.04	0.57	17.0	0.04
9	160	22	0.81	0.42	16.5	0.11
10	320	22	0.49	0.26	16.5	0.14

In *table 4*, Average induction time and temperature (calculated according to *equation 10* in section 9.4.5), instead of mediums, are calculated for further analysis since there are no data points with extreme values after the outliers are excluded. The highlighted data points are used in subsequent sample calculations. Average induction time normalised to 25°C is calculated according to *equation (1)* in section 9.4.1. The purpose of this step will be discussed in section 9.4.1. The standard deviation is calculated according to *equation 11* in section 9.4.5.

Table 5- the normalised reaction rate of N-acetyl-L-cysteine oxidation under the treatment of each concentration set with increasing concentrations of H_2O_2 (aq) and Cu^{2+} (aq) respectively

	[C] set #1	[C] set #2	[C] set #3	[C] set #4	[C] set #5	[C] set #6	[C] set #7	[C] set #8	[C] set #9	[C] set #10
$CuSO_4$(aq) concentration (mM) ± 1 mM	10	10	10	10	10	20	40	80	160	320
H_2O_2 (aq) concentration (mM) ± 2 mM	22	44	88	176	352	22	22	22	22	22
Normalized reaction rate (mM s^{-1})	4.58 ±0.19	5.54 ±0.29	6.34 ±0.31	8.46 ±0.42	12.7 ±0.62	22.7 ±1.11	54.9 ±2.70	64.6 ±3.18	87.6 ±4.31	141 ±6.94

The normalised reaction rate of N-acetyl-L-cysteine oxidation, and the absolute uncertainty associated with each data point, are calculated using *equation (2)* in section 9.4.2. The uncertainty of $CuSO_4$ concentration is calculated as following for the 10mM sample:

$$[CuSO_4] = \frac{n}{v} = \frac{\frac{0.16\pm0.01g}{Mr(CuSO_4)}}{0.100\pm5\times10^{-4}dm^3} = 10\pm6.75\%\ mM \simeq 10\pm1\ mM$$

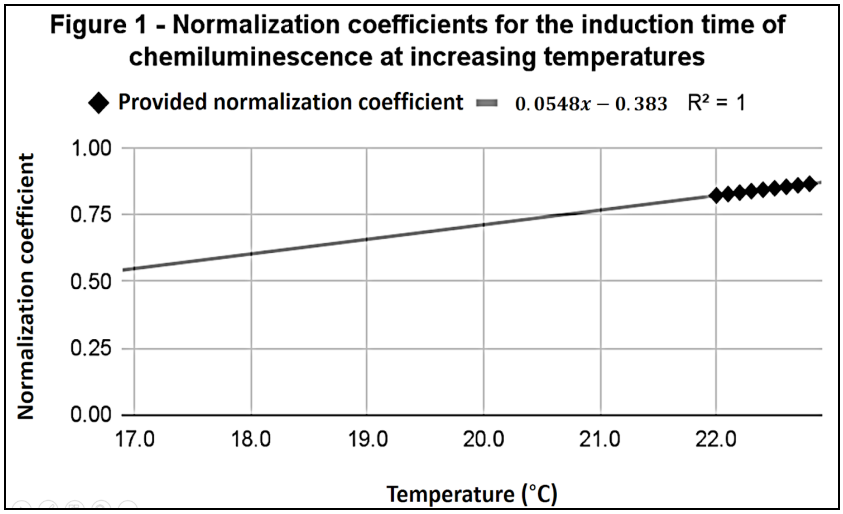
The same process is repeated for the 20mM sample of $CuSO_4$(aq), and the average of their slightly varying absolute uncertainties is calculated to be ± 1.40mM $\simeq \pm 1$mM (1 sig.fig). The uncertainty of H_2O_2 concentration is calculated via the same method.

9.4. Data processing method

9.4.1. Normalization of the induction time

The purpose of monitoring the temperature of the reaction mixture is to normalise the average induction time (t) of chemiluminescence under the treatment of each concentration set to what would be recorded at 25°C. The rationale of this additional step is to ensure fair comparison amongst the induction times recorded by compensating for the fluctuations of room temperature. More importantly, normalisation also warrants a valid comparison between the data obtained from this experiment and the results of existing scientific literature, which are often carried out under STAP. Normalisation is done by multiplying the average induction time observed at x °C with the normalisation coefficient ($n_{x\rightarrow25}$) associated with x °C. The normalisation coefficients for 22°C-23°C (at 0.1°C increments) are taken from the appendix of the 2018 International Chemistry Olympiad Practical Exam and plotted on *figure 1* below. A linear trend line with equation of $f(x) = n_{x\rightarrow25}\ t\ (x\ °C) = 0.0548x - 0.38$ is generated on Excel. Extrapolation was carried out to obtain normalisation

coefficients for the temperature range recorded during this experiment (*table 4*). This extrapolation is highly valid in determining the normalisation coefficients for 17.4°C- 18.4°C since the r^2 value of the trend line is equal to 1.



Sample calculation using the average induction time of concentration set 1 observed at 18.2 °*C* (data highlighted in *table 4*):

$$t\,(25°C) = n_{x \to 25}\; t\,(x\,°C) \qquad \textbf{(1)}$$

Equation for normalisation coefficient:

$$n_{x \to 25}\; t\,(x\,°C) = 0.0548x - 0.383$$

Calculate normalization coefficient for 18.2 °*C* **:**

$$n_{x \to 25}\; t\,(18.2°C) = 0.0548 \times 18.2 - 0.383 = 0.614$$

Normalization of induction time:

$$0.614 \times (13.07 \pm 0.50s) = 8.03s \pm 3.65\% = 8.03 \pm 0.30s$$

Hence, the recorded average induction time 13.07 ± 0.50s is normalised to $8.03\text{s} \pm 0.30s$. The uncertainty of the normalised induction time associated with other data points are propagated using the same method (which slightly varies from $\pm 0.26s$ to $\pm 0.33s$), by which the average of these absolute uncertainties calculated is used as the overall absolute uncertainty associated with the normalised induction time recorded in *table 4* ($\pm 0.29s$).

9.4.2. Normalized rate of N-acetyl-L-cysteine oxidation

The amount of N-acetyl-L-cysteine (Mr =163.20g mol^{-1}) added to the 500ml reaction mixture was 3.00g or 18.4 mmol (section 7 step 1). Thus, 3.68×10^{-2} mmol of N-acetyl-L-cysteine was involved in each trial (1ml sample was used). Since it is assumed that cysteine needs to be completely oxidised for the inhibition of chemiluminescence to be lifted, hence, the normalised average induction time can be used to calculate the rate of N-acetyl-L-cysteine oxidation.

Sample calculation using the normalised induction time of concentration set 1 (data used highlighted in *table 4*):

The normalised oxidation rate of N-acetyl-L-cysteine (v) for concentration set 1, as quantified by the decrease in the concentration of N-acetyl-L cysteine per unit time, is calculated as:

$$v = \frac{\Delta[C]}{\Delta t} = \frac{\frac{n}{v}}{t} = \frac{\frac{18.4 \pm 0.1\ mmol}{0.500 \pm 5\times10^{-5}\ dm^3}}{8.03 \pm 0.29\ s} = 4.58 \pm 4.16\%\ mM\ s^{-1} = 4.58 \pm 0.19\ mM\ s^{-1} \qquad \textbf{(2)}$$

Since the absolute uncertainty associated with each data point of the normalised reaction rate varies tremendously, hence, it is calculated separately for each individual data point according to equation (2) and recorded in *table 4*.

9.4.3. Calculate the partial reaction order (p) with respect to $[H_2O_2]$

The partial reaction order (p) in respect to $[H_2O_2]$ is calculated using the normalised reaction rate of concentration set 1-5 by making use of the data registered for 2 concentration sets at a time (set 1 pair with 2, set 2 and 3, and so on. Hence, 4 pairs in total). Theoretically, there should be no differences between the p values calculated using the different pairs. However, they do vary in reality due to measurement uncertainty and uncontrolled variables. In order to calculate the p values, it is first assumed that the rate of N-acetyl-L-cysteine oxidation in mMs^{-1} (V) can be described by equation (3), where the effect of 10mM $CuSO_4$ on the rate constant k is temporarily omitted in order to make calculations possible:

$$V = k\,[H_2O_2]^p \qquad \textbf{(3)}$$

Sample calculation using the H_2O_2 concentration and the normalised reaction rate for concentration set 1 and 2 (*table 4*) using equation (3):

$$4.58\ mMs^{-1} = k\,[22mM]^p \quad ; \quad 5.54\ mMs^{-1} = k\,[44mM]^p$$

$$\frac{5.62\ mM\ s^{-1}}{4.58\ mMs^{-1}} = \frac{k\,[44mM]^p}{k\,[22mM]^p} \Rightarrow \frac{5.62\ mMs^{-1}}{4.58\ mMs^{-1}} = \left(\frac{44mM}{22mM}\right)^p$$

$$p = log_2 1.23 \simeq 0.3 \;;\; p_{(\,5\ sets\ average)} \simeq 0.4$$

Hence, the generalised formula for calculating the p value using concentration set n and $(n+1)$ can be generalised to $p = log_2 \frac{v_{(n+1)}}{v_n}$, where v_n is the normalised reaction rate (in mMs^{-1}) of concentration set n. This formula is used to calculate the p value for each of the 4 pairs, and the average value is found to be 0.4, in consistent with 2018 International Chemistry Olympiad. As the p value is now known, the rate

constant k can be calculated as follows, where V_n is the normalised reaction rate for concentration set n (*tabel 5*), and $[H_2O_2]_n$ is the H_2O_2 concentration used in set n *(tabel 1):*

$$V = k\,[H_2O_2]^{0.4} \Rightarrow k = \frac{1}{5} \times \left(\sum_{n=1}^{5} \frac{V_n}{[H_2O_2]_n{}^{0.4}}\right) = \frac{1}{5} \times \left(\frac{4.58mMs^{-1}}{22mM^{0.4}} + \frac{5.54mMs^{-1}}{22mM^{0.4}} + \ldots + \frac{12.7mMs^{-1}}{352mM^{0.4}}\right) = \quad 1.18mM^{0.6}s^{-1}$$

$$V = 0.114[H_2O_2]^{0.4} \qquad \textbf{(4)}$$

The same process is repeated to calculate the partial reaction order q and the rate constant k in respect to $[Cu^{2+}]$, but using concentration set 6-10 (where the concentration of H_2O_2 stays constant). Subsequently, the following results are obtained:

$$q = 0.7 \quad ; \quad k = 2.99mM^{0.3}s^{-1}$$

$$V = 3.65[Cu^{2+}]^{0.7} \qquad \textbf{(5)}$$

9.4.4. Formulate the overall mixed law of N-acetyl-L-cysteine oxidation

Formulating the mixed rate law of cysteine oxidation allows the research question to be answered, such that the extent to which the concentration of H_2O_2 and Cu^{2+} affects the induction time of chemiluminescence can be quantitatively described by their partial reaction orders and their rate constants at 25°C. In 2018 International Chemistry Olympiad, the mixed rate law of cysteine oxidation is given by equation (6) (Almássy, 2018):

$$V = k_1\,[H_2O_2]^p[Cu^{2+}]^q + k_2[Cu^{2+}]^q \qquad \textbf{(6)}$$

By substituting in all the information given by equation (4) and equation (5) into equation (6), equation (7) is produced:

$$V = k_1[H_2O_2]^{0.4}[Cu^{2+}]^{0.7} + k_2[Cu^{2+}]^{0.7} \qquad \textbf{(7)}$$

Next, by substituting in the concentration of H_2O_2 and Cu^{2+} and the normalised reaction rate obtained for concentration set 1 and set 2 (*table 5*), the mixed rate law of N-acetyl-L-cysteine oxidation can be generated:

$$4.58mMs^{-1} = k_1[22mM]^{0.4}[10mM]^{0.7} + k_2[10mM]^{0.7}$$

$$5.54mMs^{-1} = k_1[44mM]^{0.4}[10mM]^{0.7} + k_2[10mM]^{0.7}$$

Solve for k_1 and k_2 :

$$V = \ 0.174[H_2O_2]^{0.4}[Cu^{2+}]^{0.7} + 0.314\,[Cu^{2+}]^{0.7}$$

This series of calculations is repeated by substituting the data (*table 5*) associated with concentration set 2 and 3, 3 and 4, and so on, which ultimately, allows the final equation of the mixed rate law of N-acetyl-L-cysteine oxidation to be determined from the average of all real solutions for k_1 (unit: $mM^{-0.1}s^{-1}$) and k_2 (unit: $mM^{0.3}s^{-1}$):

$$V = \ 0.161[H_2O_2]^{0.4}[Cu^{2+}]^{0.7} + 0.563[Cu^{2+}]^{0.7} \qquad \textbf{(8)}$$

It is important to note that theoretical equation presented by Atkin (2006) is $V = k_1\,[H_2O_2]^{\frac{1}{2}}\,[Cu^{2+}] + k_2\,[Cu^{2+}]$. The implications of deviating from the values given by existing literature will be discussed in section 12.2 in the light of equipment restrictions, uncontrolled variables and uncertainties.

9.4.5. Descriptive statistics

Standard deviation and mean values are calculated as a measure of variance and a measure of central tendency respectively for the raw data obtained under the treatment of each concentration set (*table 2* and *table 3*). The arithmetic mean (A) for a set of data points is defined by equation (10), where x_1 refers to each raw data point, and n is the total number of data points. The sample calculation of average induction time for concentration set 1 (highlighted in table 2) is shown as follows:

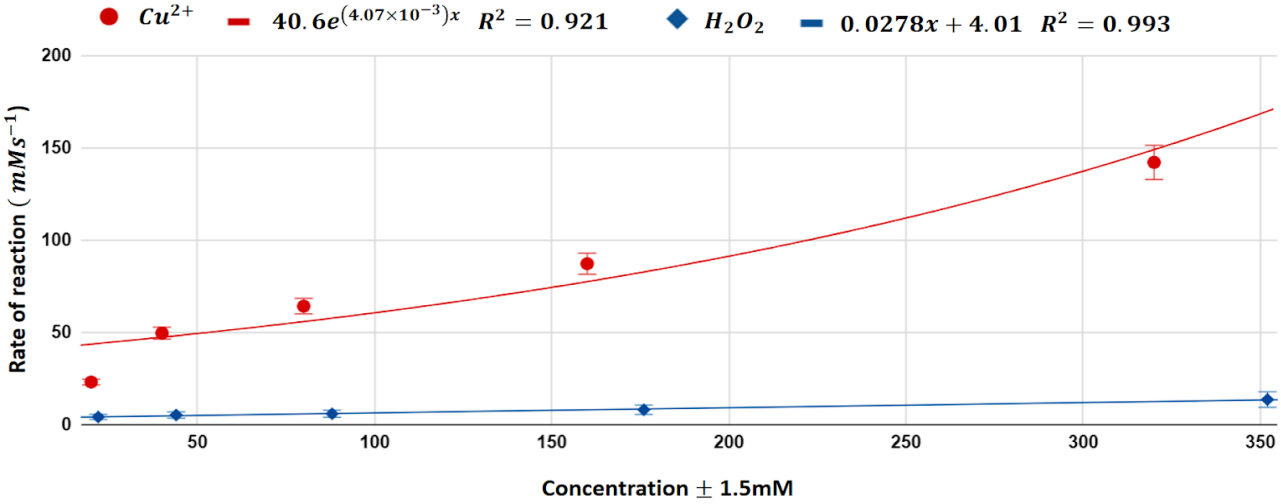
$$A = \frac{1}{n}\sum_{i=1}^{n} x_i = \frac{13.63+12.46+13.03+12.57+13.64}{5} \simeq 13.07\ \text{s} \qquad \textbf{(10)}$$

The standard deviation (σ)for a set of data is defined by equation (11), where x_1 is each of the five raw data points obtained for a given concentration set, $\bar{x}$ is the mean of the data set (as calculated according to equation 10), N is the total number of data points (5). The sample calculation for concentration set 1 (highlighted in table 2) is shown as follows:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{N}(x_i-\bar{x})^2}{N}} = \sqrt{\frac{(13.63-13.07)^2+(12.46-13.07)^2+(13.03-13.07)^2+(12.57-13.07)^2+(13.64-13.07)^2}{5}} \simeq 0.50\ \text{s} \qquad \textbf{(11)}$$

9.5. Graphical representations and analysis

***Figure 2*-Increase in the rate of N-acetyl-L oxidation oxidation against increasing concentration of H_2O_2 (concentration set 1-5) and Cu^{2+} (concentration set 6-10) respectively**



The error bars generated for *figure 2* represent the associated absolute uncertainty for each data point of the reaction rate (*table 5*). It is important to note that some error bars for H_2O_2 are too small to be visible as H_2O_2 results in much slower reaction rate compared with equimolar Cu^{2+}, thus, reducing the percentage error in timing. The absolute uncertainty labeling the *x*-axis of *figure 2* is a representation of the average of the absolute uncertainty of H_2O_2 ($\pm$2mM) and Cu^{2+} ($\pm$1mM). Trend lines are generated using the least square regression method for the purpose of maximizing the Pearson correlation coefficient (r^2) values. Hence, it is found that an exponential trend line for Cu^{2+} provides a r^2 value (0.921) that is closest to 1, while a linear trend fulfills the same purpose for H_2O_2 ($r^2 = 0.993$).

Figure 2 illustrates that the reaction rate of N-acetyl cysteine oxidation (by the oxidizing agent H_2O_2) increases as the concentration of H_2O_2 increases and as the concentration of Cu^{2+} increases. This is evident from the positive gradient of both trend lines, indicating a positive correlation between the rate of reaction and the concentration of these two chemical species. Hence, this demonstrates that H_2O_2 is indeed involved in the rate-determining step of the chain reaction of N-acetyl-L cysteine oxidation. Meanwhile, it is also evident that the addition of Cu^{2+} always results in a faster rate of the N-acetyl cysteine oxidation compared with the addition of equimolar H_2O_2. For instance, according to interpolation, the addition of 40mM Cu^{2+} results in the reaction of 50.0 mMs^{-1}, compared with the reaction rate of 5.60 mMs^{-1} that would be shown under the treatment of 40mM H_2O_2. This is almost a 10-fold difference. Hence, *figure 2* suggests that the homogeneous catalyst Cu^{2+} tends to affect the rate of N-acetyl-L-cysteine oxidation and reduce the induction time of chemiluminescence to a greater extent compared with equimolar H_2O_2. This statement is further supported by the evidence that the best-fit trend line for H_2O_2 is closer to that of a first order reaction, such that its r^2 value of 0.993 suggests a fairly strong correlation with a linear trend line. In comparison, this observation contrasts with the curve of Cu^{2+}, which possesses a reaction order that is greater than first order, and is showing the characteristic of a pseudo-second order reaction. This means that despite the graph showing an exponential increase in the rate of reaction as the concentration of Cu^{2+} increases (resembles a second order reaction), however, it can be seen from the mixed rate law $V = 0.161[H_2O_2]^{0.4}[Cu^{2+}]^{0.7} + 0.563\,[Cu^{2+}]^{0.7}$ that the a second-order artifact is created by the addition of the term $k_2\,[Cu^{2+}]^{0.7}$.

Figure 3 - Decrease in the induction time of chemiluminescence under the treatment of 10mM Cu^{2+} and increasing concentrations of H_2O_2 (concentration set 1-5)

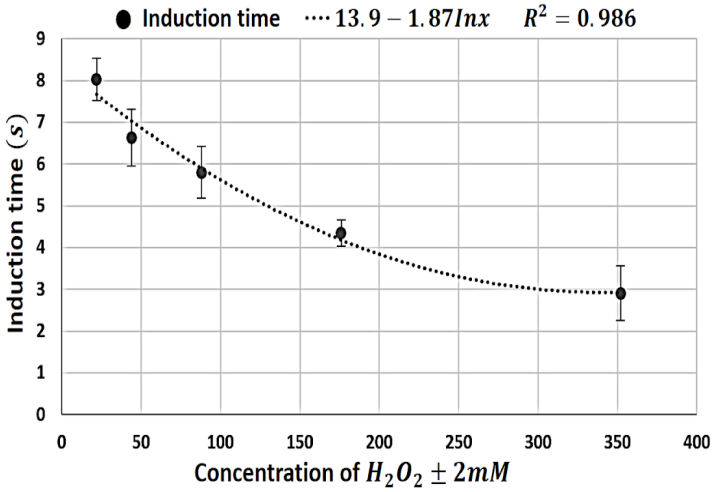
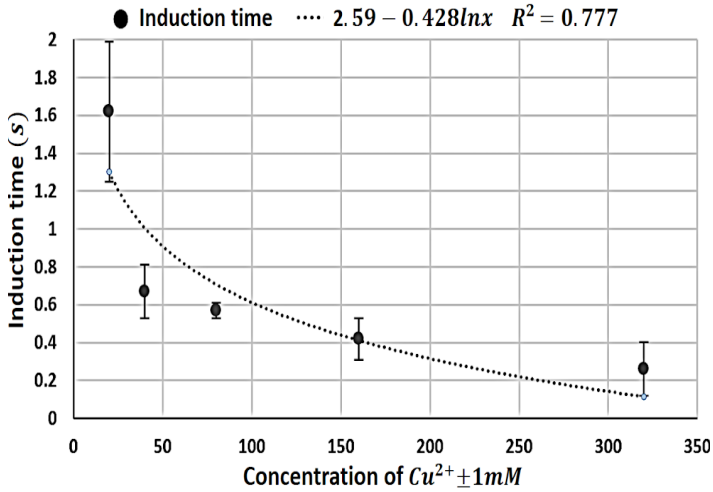


Figure 4 - Decrease in the induction time of chemiluminescence under the treatment of 22mM H_2O_2 and increasing concentrations of Cu^{2+} (concentration set 6-10)



Each error bar in *figure 3* and *figure 4* represent the absolute standard deviation for each data point, as displayed in *table 4*. It is evident that the standard deviation values vary toward both positive and negative direction as the concentration of H_2O_2 and Cu^{2+} increases respectively, indicating that random errors were present throughout the measurement process. The influence of random error in timing appeared to result in further spread of data from the mean for higher concentration conditions compared with lower concentration conditions, as shown by the standard deviation of 0.14 (relative STD=53.8%) for the 320mM Cu^{2+} condition compared with the relative STD of 22.8% for the 20mM Cu^{2+} condition. The presence of relatively large variations between replicates, as indicated by their comparatively high standard deviation and data range, again implies insufficient replicability as well as the presence of random errors in the identification of chemiluminescence and the timing of the induction period. This observed data variation combined with the fact that only five replicates were carried out for each condition, further challenges the reliability and the credibility of the results.

Furthermore, from both figures, it is evident that the induction time of chemiluminescence decreases in a logarithmic manner as the concentration of H_2O_2 and Cu^{2+} increases, as shown by their logarithmic trend line of $t = 13.9 - 1.87\, In[H_2O_2]$ and $t = 2.59 - 0.428\, In[Cu^{2+}]$ respectively that provide the r^2 values closest to 1. Additionally, for both chemical species, it is also observed that the induction time of chemiluminescence tends to decrease more rapidly (steeper slope of the curve) as the concentration of H_2O_2 and Cu^{2+} increases at low concentrations (<50mM), by which a greater percentage decrease in the induction time (in seconds) is exhibited when increasing the concentration of either species from 20mM to 40mM, compared with the percentage decrease shown as the concentration increases from 280mM to 300mM (where the trend line smoothes out). Lastly, it is noteworthy that the trend line of best-fit for the data points in *figure 4* possesses a much lower r^2 value (0.777) compared with *figure 3* (r^2 =0.986), as the second data point in *figure 4* deviates significantly from the expected trend, implying how a very slight increase in the concentration of the homogeneous catalyst Cu^{2+} (10mM increase) can significantly reduce the induction time of chemiluminescence (by 1.54 seconds or 44 %).

10. Evaluation

10.1. Conclusion

The aim of this investigation has been achieved, which is to investigate the extent to which increasing the concentrations of H_2O_2 and Cu^{2+} affects the induction time of chemiluminescence through formulating the mixed rate law N-acetyl-L-cysteine oxidation. Prior to the experiment, it was hypothesized that the induction time of cysteine-inhibited chemiluminescence will decrease, and the rate of N-acetyl-L-cysteine oxidation will increase, as the concentration of H_2O_2 (aq) and Cu^{2+} (aq) increases respectively. This hypothesis is supported by the experimental results, as a positive correlation is evident between the concentration of these two chemical species and the rate of N-acetyl-L-cysteine oxidation (*figure 2*), while a negative correlation is shown between the induction time of chemiluminescence and the concentration of H_2O_2 and Cu^{2+} respectively (*figure 3* and *figure 4*). These experimental findings are generally in line with the Almássy (2018), however, the results in Almássy (2018) indicate a less rapid decrease in the induction time of chemiluminescence as the concentration of the reactant and the catalyst doubled successively. This discrepancy is believed to be associated with differences in the temperature to which the experiments were carried out, as the rate constant is proportional to the absolute temperature in Kelvin (Atkins, 2006).

Furthermore, prior to the experiment, the partial reaction order in respect to H_2O_2 and Cu^{2+} were unable to be predicted since reaction orders are empirical in nature. According to the results of this experiment, the integrated rate law of N-acetyl-L-cysteine oxidation is found to be $V = k_1[H_2O_2]^{0.4}[Cu^{2+}]^{0.7} + k_2[Cu^{2+}]^{0.7}$, where $k_1 = 0.161 mM^{-0.1}s^{-1}$ and $k_2 = 0.563 mM^{0.3}s^{-1}$. Hence, it is also concluded that the dependence of the rate of cysteine oxidation (V) has fractional dependence on both species, and the concentration of Cu^{2+} affects the rate of N-acetyl-L-cysteine oxidation to a greater extent than H_2O_2. This conclusion is further supported by *figure 2* in the sense that the effect of Cu^{2+} on the reaction rate shows the characteristics of a pseudo-second order reaction, while the effect of H_2O_2 concentration on the rate of N-acetyl-L cysteine oxidation is closer to that of a first order reaction. Fractional reaction orders imply the presence of complex chain reaction mechanisms, competing reactions or side reaction reactions. However, it is highly important to note that the final equation of the mixed integrated rate law derived is inconsistent with current literature. Namely, $V = k_1 [H_2O_2]^2 [Cu^{2+}] + k_2 [Cu^{2+}]$ is derived by Atkin (2006). This deviation from existing literature is not surprising as this experiment was conducted in a high-school laboratory, by which human errors were inherent in timing and dissolved oxygen could have competed with H_2O_2 for reduction.

All in all, this investigation provides some insights and real-life applications in controlling the timing of chemiluminescence by adjusting the concentration of H_2O_2 and Cu^{2+}. However, the presence of random errors including the estimation of the last digit in measurements, as well as systematic errors inherent in potential side reactions and the measuring instrument used, may collectively limit the reliability and the validity of the conclusions drawn. Hence, in order to check the validity of the findings, further research needs to be done using spectrophotometer to pursue a greater precision in timing and a greater accuracy in the identification of chemiluminescence, as well as using inert gas to purge dissolved oxygen in the reaction mixture. Strength, limitations and improvements of this investigation will be further discussed from section 10.3.

10.2. Scientific context

The recorded increase in the rate of N-acetyl-L-cysteine oxidation as the concentration of H_2O_2 or Cu^{2+} increases is well-explained by the kinetic molecular theory of matter and the Maxwell-Boltzmann distribution curve respectively. In regards to H_2O_2 which is involved as a reactant in the rate-determining step, an increase in its concentration increases the frequency of collisions between the reactant particles, resulting in greater kinetic energy and greater entropy of the system. As a result, a higher fraction of reactant particles are able to overcome the activation energy threshold and successfully convert into product per unit of time, leading to a faster rate of reaction and shifting the position of equilibrium to the product side (increase the reaction quotient Q_c). Subsequently, as N-acetyl-L-cysteine is being oxidized more rapidly, the induction time of chemiluminescence shortens. Secondly, the fact that increasing concentration of Cu^{2+} also increases the rate of reaction can be interpreted using the Maxwell-Boltzmann distribution curve in the light of the catalytic property of transition metals. Specifically, transition metal cation Cu^{2+} can have variable oxidation state due to small differences between its successive ionisation

energy. This property allows Cu^{2+} to act as a homogenous catalyst by facilitating electron exchange when reactants are brought to close proximity. As a result, the transition state of the reaction is stabilized and its activation energy threshold is lowered (shifted to the left hand side of the Maxwell-Boltzmann distribution curve), thus, the probability of successful collisions is again enhanced and the induction time of chemiluminescence is subsequently shortened. Further, the fractional reaction order in the mixed integrated rate law of the N-acetyl-L-cysteine oxidation reaction can be interpreted in terms of the presence of complex chain reaction mechanisms, side reactions and competing reactions. This statement is evident from the fact that the reaction mixture turned light green when solutions with higher Cu^{2+} concentrations were added, indicating that the added amorphous $CuSO_4$ powder has reacted with species other than H_2O_2. Since inert gas pumps were not available in the laboratory, it is postulated that dissolved oxygen gas might have been involved in the described side reaction, which may in turn compete with H_2O_2 for oxidising Cu^+ back to Cu^{2+}, hence, affecting the induction time of chemiluminescence.

In order to carry out cross-comparison between the conclusions drawn from this experiment and existing scientific literatures, key words including "N-acetyl-L-cysteine oxidation", "mixed rate law" and "luminol oxidation" were searched on Pubmed and Research gate. Unfortunately, no existing peer-reviewed papers directly relevant to the specific conditions involved and methods employed in this investigation were found (other than 2018 International Chemistry Olympiad), which serves as a limitation of this investigation in the sense that the reproducibility and the reliability of the results obtained are unable to be justified sufficiently by using a sole resource. Nevertheless, there are a few pieces of indirect evidence collected via extensive research. For instance, in Adari (2006), the mixed rate law describing the oxidation of L-cysteine by the oxidizing agent pyridinium chlorochromate (PCC) was found to have an overall fractional order. However, it is important to note that Adari (2006) obtained this result at 30°C (instead of below 20°C), and cysteine solution with concentration of 0.3M was used. More importantly, it is imprudent to generalize the findings on oxidation of L-cysteine by PCC to the mixed rate law of N-acetyl-L-cysteine oxidation by H_2O_2, hence, further research is needed to confirm the findings of this investigation.

Additionally, it is also noteworthy that there are inconsistencies between the findings of existing literature and this experiment. For instance, Millero (1991) found that 1×10^{-7}M H_2O_2 is sufficient in reducing Cu^{2+} to Cu^+. Similarly, Moffet and Zika (1983) also concluded that $< 1 \times 10^{-8}$ M of H_2O_2 can still result in significant reduction of Cu^{2+}. However, the statements from Millero (1991) and Moffet and Zika (1983) appear contradictory to what was observed throughout the trial tests. Furthermore, Sharma and Millero (1989) found that the re-oxidation of Cu^+ by H_2O_2 was unsuccessful when H_2O_2 concentration exceeded 1×10^{-5} M (since Cu^+ would become competitive with oxygen). However, their findings are again highly contradictory to the results of this experiment, by which it was evident that chemiluminescence has indeed appeared upon the re-oxidation of Cu^+ to Cu^{2+} in the presence of H_2O_2 with concentration as high as 3.52×10^{-1} M. These inconsistencies again raise questions on the reproducibility and the validity of the results obtained by this experiment.

10.3. Strength

Preliminary trial-and-error testings were carried out rigorously in order to enhance the effectiveness of the methodology used, which constituted a few stand-out strengths of this investigation. During preliminary trials, multiple methods of mitigating the fluctuations in the room temperature were attempted, such as using a water bath and placing the reaction mixture inside an insulating container. However, it appeared that light refraction through the water bath as well as the semi-transparent nature of the insulation materials tended to interfere with the identification of weak chemiluminescence. Hence, it was finally decided to monitor the temperature of the reaction mixture at the end of each trial, which allowed the uncontrolled variable of fluctuations in room temperature to be compensated by normalising the average reaction time and reaction rate to 25° C. Since it is evident from the data points in *table 2* and *table 3* that the room temperature appeared to decrease at the end of the experimental day, normalisation allowed reaction rates that took place at different temperatures to be compared together in a valid manner. Furthermore, the design of this experiment is apt and creative in terms of how it made use of the phenomenon of chemiluminescence as an indicator to determine the end point of the N-acetyl cysteine oxidation reaction, instead of employing more conventional methods such as using indicators and plotting titration curves — which would have resulted in lower accuracy, more effort and time. Meanwhile, this experimental design also served to provide dual understandings in a single experiment — namely, methods of predicting and control the timing of chemiluminescence in real life (photochemistry), as well as the mixed rate law of N-acetyl-L cysteine oxidation in the light of fractional reaction order (kinetics).

10.4. Weakness

10.4.1. Random errors and improvements

Firstly, for some samples that were treated with low $[H_2O_2]$ and $[Cu^{2+}]$, it was observed that the glow was very subtle. As a result, due to the dimness of glowing as well as the nature of human reaction time, there was a certain degree of random errors in determining the exact instance when chemiluminescence appeared in the visible spectrum, and thus, resulted in lower precision of the data collected. This uncertainty was particularly significant for the trials with relatively concentrated H_2O_2 or Cu^{2+} solutions added to the reaction mixture (concentration set 9 and set 10), such that the induction time of chemiluminescence was too short for humans to react (less than 1 second), which manifested in the presence of outliers (*table 3*). Despite repeated trials being conducted to counterbalance this random error in this experiment, it is important to note that random errors are theoretically unable to be fully eliminated. In order to counter this issue, a spectrophotometer connected to the LoggerPro software can be used in the future in an attempt to obtain more reliable readings, as well as providing quantitative descriptions of the brightness and the duration of chemiluminescence. With that being said, it should be noted that this proposed improvement method may transfer random errors into systematic errors if the spectrophotometer is not correctly calibrated. Secondly, a 500ml beaker with an absolute uncertainty as large as ±25ml was used to prepare the stock solution (section 7 step 1). However, in reality, the uncertainty was highly unlikely to be indeed ±25ml as the solutions were topped up drop-wise using a 5ml pipette to reach the bottom of the gradation. Nevertheless, in order to reduce the random error associated with the estimation of the significant last digit, a 500ml measuring cylinder (which was not available in school) should be used instead in order to reduce the absolute uncertainty from ±25ml to ±0.5ml. This improvement is highly crucial as all subsequent trials and results, without any exceptions, were potentially affected by the uncertainty associated with the stock solution.

10.4.2. Systematic errors and improvements

It was noticed throughout the trials that the mercury thermometer appeared to be too insensitive to register the instantaneous temperature of the reaction mixture after each trial, as suggested by the fact that the temperature registered tended to sit at whatever the room temperature was despite the reaction mixture being obviously warmer when felt by hands after luminol oxidation (*table 2*). This choice of measuring instrument has lowered the validity of the subsequent induction time normalisation carried out. Therefore, in order to enhance the accuracy of the temperature readings, a digital thermometer connected to the Logger Pro program should be used in the future. This would also allow the temperature curve to be extrapolated back to the *y*-axis to find out the initial temperature while accounting for the temperature loss. Furthermore**,** side reactions were noticed for some samples under the treatment of high [Cu^{2+}], such that the reaction mixture turned light green and dark copper precipitates were formed at the bottom of the test tube. This means that there was actually less amount of H_2O_2 and electrons involved in the reaction under investigation than expected. The 'loss' of reactants served as a systematic error that could potentially reduce the rate of reaction and the product yield. A possible improvement is to use titration to determine the purity of the reagent prior to the experiment and to determine the amount of oxidised luminol at the end of the reaction. This improved method would allow stoichiometry to be carried out using the molar coefficients of balanced equations to calculate the amount of reagents that were actually involved in the reaction under investigation, by referencing the amount of expected product produced. Moreover, even though the amount of dissolved oxygen in the reaction mixture was mitigated to some extent by avoiding stirring, this method of control was arbitrary, by which a better control can be established using deoxygenated water by bubbling N_2 into distilled water. This methodological improvement is highly important because Cu^+ can be oxidised by dissolved oxygen in the reaction mixture via a free radical mechanism (Xing,2012): $Cu^+ + O_2 \rightarrow Cu^{2+} + O_2^{\bullet -}$. As a result, assuming the catalyst Cu^{2+} was not in excess initially, this extraneous variable was highly likely to amplify the effect of H_2O_2 and reduce the induction time of chemiluminescence, which in turn may contribute to the deviation of the final integrated rate law equation from what is proposed in existing literature.

10.4.3. Methodological issues and assumptions

In reality, the concentration of cysteine itself also affects the rate of the oxidation reaction as it is involved in the RDS. This proposition is supported by Adari (2006), which provided the rate equation $V = \frac{k[Cys][PCC]}{1+k[Cys]}$. However, due to time constraints and the complexity of the serial reaction mechanism, it was decided to keep the concentration of N-acetyl cysteine constant throughout the course of the experiment, and derive the rate equation without considering the involvement of cysteine. Subsequently, it is suspected that this simplification of the reality might have led to decimal partial reaction orders before rounding-up was carried out. Nevertheless, justifying this speculation is beyond the scope of this experiment. Furthermore, the principle assumption of the methodology is that all N-acetyl-L cysteine needs to be consumed for chemiluminescence to occur. However, this proposition contradicts with the qualitative observations during this experiment, by which it was noticed that the blue glow spread out across the reaction mixture gradually in a non-uniform manner. This observation suggests that some luminol was able to be oxidised when the cysteine molecules surrounding it were partially consumed. Hence, there is a lack of rigor associated with this assumption. Lastly, referring to *figure 2*, it is important to note that the methodology used possesses another limitation in the sense that the series of diluted $Cu^{2+}$and H_2O_2 solutions were not equimolar with their associated counterpart (for instance, 20mM Cu^{2+} was compared with 22mM H_2O_2). This difference was necessary as 20mM Cu^{2+} and 22mM H_2O_2 were tested to be the minimum concentrations that facilitate luminol oxidation. Despite this limitation was compensated via interpolation using trend lines, however, it should be noted that extrapolation has shown an artifact in *figure 2* in terms of how 0mM $Cu^{2+}$and H_2O_2 concentrations (at the origin of the *x*-axis) were shown to be associated with a specific reaction rate. This tends to be misleading as it was evident from the two negative controlled samples prepared that the absence of either $Cu^{2+}$and H_2O_2 led to failures for the reaction to proceed.

10.5. Extensions

In the future, it would be valuable to extend the investigation in chemiluminescence to other types of radiative relaxation such as phosphorescence and fluorescence, as well as the subtype of chemiluminescence known as bioluminescence. These investigations will allow more in-depth understanding into photomestry, including the role of intersystem crossing, spin multiplicity, singlet versus triplet state excited state, and Pauli exclusion principle in the control of induction time. More importantly, these further investigations will be highly relevant to many practical applications, such as mineralogy, dyes and clock dials. Furthermore, using spectrophotometry to monitor the change in the absorbance of the reaction mixture over a period of time would also provide additional insights. This would allow a graph of $\frac{1}{[C]}$ verse time to be plotted by converting the absorbance recorded to solution concentration using Beer Lambert's law (Atkins, 2006). This extension will help to evaluate the dependence of the rate of N-acetyl-L cysteine oxidation more comprehensively via multiple parameters and visualization methods, which would ultimately result in more accurate and reliable derivation of the mixed rate law.

11. Bibliography

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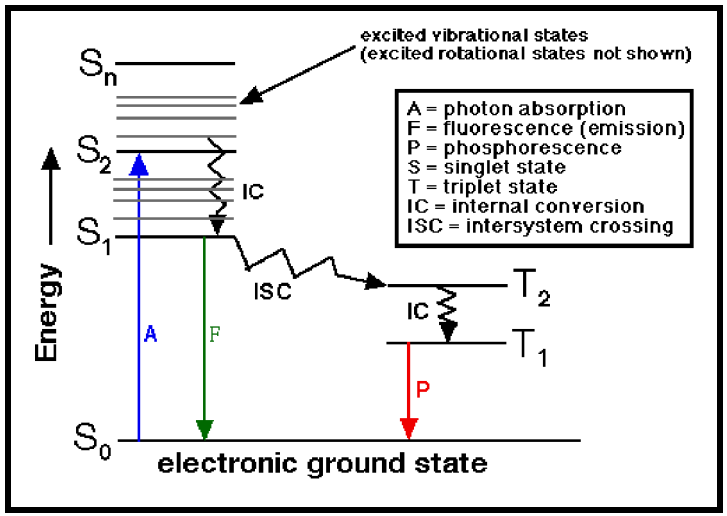
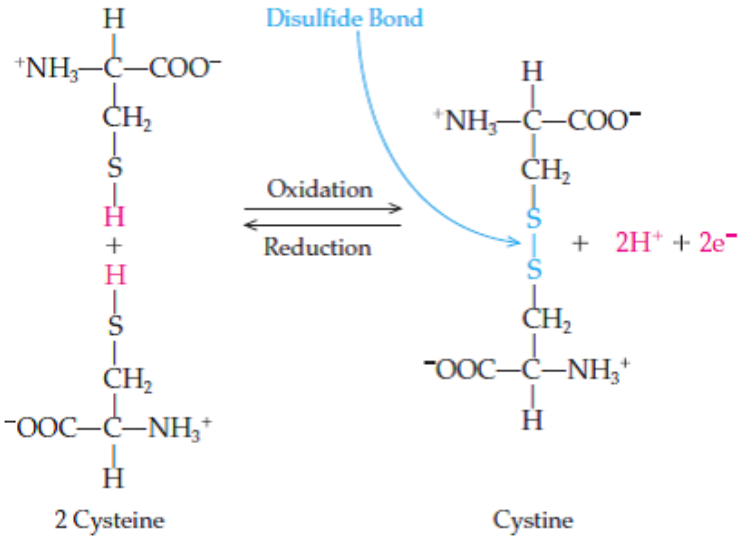
2021 Log Book

Data	Record and notes
April 30th	**Fascinated by the phenomenon of chemiluminescence and carried out independent research.** **To understand the underlying mechanisms of chemiluminescence on a molecular level, it is essential to define a few key concepts. Hence, I took notes below:** **Spin quantum number (ms):** The ms value describes both the magnitude ($\frac{1}{2}$) and direction (+/-) of an electron, by which it is assigned '+ $\frac{1}{2}$ ' for a up-spined electron and ' - $\frac{1}{2}$ ' for a down-spined electron. **Total spin quantum number (S):** Total spin quantum number is determined by the sum of the spin quantum number (ms) of each individual electron. For a pair of electrons with opposite spin, $S = (+\frac{1}{2}) + (-\frac{1}{2}) = 0$. Similarly, S value of 1 is obtained for a pair of electrons orienting toward the same direction. **Spin multiplicity(Ms):** Spin multiplicity describes the number of possible orientations of the spin angular momentum of a given total quantum number (S), as calculated by 2S+1. Hence, a pair of electrons in an orbital oriented according to the Hund's rule would give: $Ms = 2 \times [(+\frac{1}{2}) + (-\frac{1}{2})] + 1 = 1$; while a pair of electrons oriented toward the same direction would give a Ms value of 3. **Singlet (S_n) and triplet (T_n) spin state:** The spin states of electrons refers to their spin multiplicity (Ms). A Ms value of 1 means that the electrons are in a singlet spin state. Without changing the orientation of spin, an electron at its ground state (S_0) can be promoted to a vibrational state within the same electron state or get boosted to a new electronic state with higher energy (such as S_1) upon absorption of photons. This is known as the singlet excited state with a net spin of 0. On the other hand, if the down-spined electron flips its spin direction upon absorption of a photon, then the electron pair (with Ms value of 3) is said to be in triplet excited state (T_n**).** **Jablonski diagram:** The electronic states in a Jablonski diagram are arranged vertically by increasing energy and grouped horizontally by spin multiplicity, separating singlet and triplet states. **Radiative recombination:** Radiative recombination is achieved by vibrational relaxation, resulting in chemiluminescence in the visible spectrum.

	Intersystem-crossing (ISC): ISC refers to the process where a pair of electrons transfer from the singlet excited state (on the left hand side of the Jablonski diagram) to triplet excited state (on the right hand hand), or vice versa.
May 1st - May 4th	 **I started to write the introduction of my scientific inquiry report:** The phenomenon of chemiluminescence refers to the production of electromagnetic radiation observed when a chemical reaction yields an electronically excited intermediate or product (Christofi, 2005). Chemiluminescence is produced from the radiative recombination of excited electrons falling back to singlet ground state (S_0) from singlet excited states, releasing excess energy in the form of photons. In this experiment, water-solution dianion is first produced from water-insoluble luminol upon the addition of alkaline through an elimination reaction. Under a suitable redox catalyst (Cu^{2+}), dianion may react with oxidizing agents (H_2O_2), forming 3-Aminophthalate (3-APA) with electrons in their excited triplet state (T_1) upon the substitution of N_2 by O_2. Due to the Pauil exclusion principle, these electrons in T_1 are unable to fall back to the ground state. Thus, intersystem crossing takes place to transfer triplet 3-Aminophthalate (3-APA) to singlet excited state (S_1), which allows the excited electron to transfer back to S_0, realising the quantized energy difference as a flash of blue light. **Additionally, I also looked into the oxidation of N-acetyl-L-cysteine:**

	
May 5th - May 10th	**I finished planning for materials and considering the controls that need to be established (see section 6 and 5.4 of the scientific inquiry report).** **Ideas and process of approach:** I referred to the self-learnt content of the IB HL kinetics chapter, as well as a number of high quality, peer-reviewed resources on Pubmed and Researchgate. Subsequently, in this experiment, I'm going to determine the dependence of the induction time of chemiluminescence on hydrogen peroxide (H_2O_2) and copper(II) (Cu^{2+}) by calculating the rate constant and the partial reaction order in respect to each species using integrated rate law. Specifically, this will be achieved by varying the concentration of H_2O_2 and Cu^{2+} while keeping the concentration of N-acetyl-L-cysteine constant in order to time their resulting induction time of chemiluminescence (in seconds). The rationale of this approach is that formulating the mixed rate law of N-acetyl-L-cysteine oxidation will allow the induction time of chemiluminescence to be easily predicted for a given amount of reactant (H_2O_2) and catalyst (Cu^{2+}), as N-acetyl-L-cysteine acts as an inhibitor that needs to be fully oxidised for chemiluminescence to take place.
May 11th -	**The following procedures were carried out:**

May 12th

Two full days of experiment completion

1. Anhydrous luminol (1.00g), anhydrous N-acetyl-L-cysteine (3.00g), Na_2CO_3(s) (2.00g), $NaHCO_3$(s)(12.00g), and $(NH_4)_2CO_3$(s) (0.25g) were weighed using a digital scale and added to a clearly-labeled 500ml beaker. Distilled water was first added roughly using a funnel to just below the 500ml mark of the 500ml beaker. The solution was then topped up drop-wise using a 5ml bulb pipette until the bottom of the meniscus sat exactly at the 500ml mark when viewed from an eye-level. In the following procedures, all steps involving making solutions to a certain volume were done using this method to mitigate random uncertainties.

2. 10mM, 20mM, 40mM, 80mM, 160mM and 320mM copper solutions were prepared by weighing 0.16g, 0.32g, 0.64g, 1.28g, 2.56g and 5.12g anhydrous $CuSO_4$ respectively. Each of the five weighed $CuSO_4$(s) samples were dissolved in distilled water (100ml) in 5 separate 100ml measuring cylinders.

3. Diluted H_2O_2 solutions of 5 different concentrations were prepared from 6% (1.76M) stock H_2O_2 (aq). This was done by using a 1ml bulb pipette to transfer 5 lots of 1ml 1.76M H_2O_2 into 4 separate 100ml measuring cylinders and topped up using distilled water to 80ml (give 22mM H_2O_2), 40ml (give 44mM), 20ml (give 88mM), 10ml (give 176mM) and 5ml (give 352mM) respectively.

4. To avoid contamination, another 1ml bulb pipette was used to transfer 1ml of the contents in the 500ml beaker to a plastic 10ml test tube with screw-on cap.

5. Referring to *Table 1* below, two separate 1ml pipettes were used to charge a 4ml glass vial with 0.1ml 10mM solution, and 1.0ml 22mM H_2O_2 solution. This made up 'concentration set 1 (**[C] set #1**)'.

Table 1- The concentrations of H_2O_2 (aq) and $CuSO_4$(aq) in each concentration set transferred to the 4ml glass vial

	Concentration (mM)									
Chemicals	**[C] Set #1**	**[C] Set #2**	**[C] Set #3**	**[C] Set #4**	**[C] Set #5**	**[C] Set #6**	**[C] Set #7**	**[C] Set #8**	**[C] Set #9**	**[C] Set #10**
$CuSO_4$ (aq)	10	10	10	10	10	20	40	80	160	320
H_2O_2 (aq)	22	44	88	176	352	22	22	22	22	22

6. The 4ml filled glass vial was carefully inserted into the filled 10ml test tube with screw-on cap without allowing the contents to mix.

7. The room light was switched off and the subsequent procedures were performed with the samples placed in the black cardboard box. The 10ml test tube was sealed and shaken vigorously twice in order to mix in the contents in the 4ml glass vial. The timer was started at the exact instance when shaking began.

8. After shaking, the timer was stopped at the time when a flash of blue light was observed. The duration taken for chemiluminescence to appear (in seconds) in the first trial of concentration set 1 was recorded in the raw data table (*table 2*).

9. Immediately, a mercury thermometer was used to measure the final temperature (°C) of the reaction mixture. The data registered was also recorded in *table 2.*

10. Step **4 - 9** was repeated for another 4 trials for concentration set 1. Hence, 5 trials in total were carried out for each concentration set.

11. Referring to *table 1*, step **4 -10** was repeated for the remaining concentration sets (2-10). All pipettes were rinsed thoroughly with distilled water first, then rinsed with their respective solutions, before the next concentration set was tested. To avoid contamination, a new 4ml glass vial and a new 10ml test tube with screw-on cap were used for each different concentration set.

12. All raw data collected from the procedures documented above were recorded in *table 2* and *table 3.* This data was used to calculate the average induction time (in seconds) according to equation 10 in section 9.4.5, which was then recorded in *table 4* in section 9.3 (processed data). In addition, as described by section 9.4 (data processing method), the temperature data were then used to normalize the recorded induction time to what would be observed at 25°C, before the normalised average induction time and normalised reaction rate (*table 4*) were calculated according to equation 1 (section 9.4.1) and equation 2 (section 9.4.2) respectively.

Adaptations:

The method used is partially inspired by the 2018 International Chemistry Olympiad (Almássy, 2018), however, it has been modified extensively by conducting trial and error throughout this investigation in an attempt to accommodate factors like unchangeable low room temperature. At first, by adapting the H_2O_2 and Cu^{2+} concentrations used in the 2018 International Olympiad, it was evident that no glowing appeared (after 10 minutes of waiting) and the reaction mixture turned green (due to side reaction). To overcome this issue, the following modifications were made: 1) Cu^{2+} solutions were diluted by increasing dilution factors until reaching a concentration range that was high enough to allow successful catalytic activity while low enough to allow N-acetyl-L-cysteine to outcompete Cu^{2+} for oxidation; 2) The concentration of H_2O_2 was increased by doubling it each time until it was concentrated enough to excite luminol (induce chemiluminescence) to an easily observable extent and to consume N-acetyl-L-cysteine within a viable time frame.

Raw data collected:

Table 2- the induction time (in seconds) and the final temperature (in°C) of the N-acetyl-L-cysteine-inhibited luminol oxidation reaction registered upon adding H_2O_2 (aq) of increasing concentrations under constant Cu^{2+} concentration (10mM)

[C] Set #	[Cu^{2+}] (mM) ± 1mM	[H_2O_2] (mM) ± 2 mM	The induction time of chemiluminescence (s) ± 0.50s					Final temperature (°C) ± 0.7 °C				
			1	2	3	4	5	1	2	3	4	5
1	10	22	13.63	12.46	13.03	12.57	13.64	18.5	18.5	18.0	18.0	18.0
2	10	44	10.82	9.85	11.78	10.98	11.60	18.0	18.0	18.0	18.0	18.0
3	10	88	8.56	9.97	10.27	9.05	9.37	18.0	18.0	18.0	18.5	18.5
4	10	176	6.48	7.44	6.97	6.88	7.02	18.2	18.2	18.6	18.5	18.5
5	10	352	4.52	5.23	5.13	3.44	4.98	17.5	17.5	17.5	17.5	17.5

Table 3- the induction time (in seconds) and the final temperature (in°C) of the N-acetyl-L-cysteine-inhibited luminol oxidation reaction registered upon adding Cu^{2+} (aq) of increasing concentrations under constant H_2O_2 concentration (22mM)

[C] Set #	[H_2O_2] (mM) ± 2 mM	[Cu^{2+}] (mM) ± 1 mM	The induction time of chemiluminescence (s) ± 0.50s					Final temperature (°C) ± 0.7 °C				
			1	2	3	4	5	1	2	3	4	5
6	22	20	2.76	3.25	2.22	2.76	3.21	17.5	17.3	17.3	17.3	17.5
7	22	40	1.24	1.22	1.37	1.02	1.01	18.0	18.5	18.0	18.0	18.0
8	22	80	1.11	1.06	1.01	1.00	1.01	16.8	16.9	17.0	17.3	17.2
9	22	160	1.02	0.81	0.69	0.79	0.74	16.4	16.2	16.1	16.8	16.8

10	**22**	**320**	0.67	1.02	0.45	0.15	0.34	16.7	16.7	16.5	16.5	16.5

Qualitative observations

Firstly, when anhydrous N-acetyl-L-cysteine was added to the luminol-containing reaction mixture (step 1 of section 7), gentle effervescence was observed at the surface of the solution. Secondly, after an increasing mass of N-acetyl-L-cysteine was added during experimental modification, it was observed that chemiluminescence appeared increasingly brighter. Meanwhile, it was also evident that the glowing appeared upon the radiative recombination of excited luminol molecules was not uniform throughout the reaction mixture, in other words, glowing tended to start from one corner of the test tube and then gradually spread out. This lack of uniformity in glowing was especially noticeable for lower concentration sets (1-3), by which it raised the question in terms of when the timer should be stopped, as well as providing counter-evidence for the proposition that all N-acetyl-L-cysteine needs to be oxidised for luminol to glow. Additionally, brown copper precipitate was noticed for concentration sets 6-10 after chemiluminescence took place. Through observations, it appeared that the greater the concentrations of H_2O_2 and Cu^{2+}, the larger amount of copper precipitates formed, and the shorter the duration of chemiluminescence (or the glowing was obscured by the copper precipitate more). Lastly, it was clear that the final reaction mixture cooled down very rapidly after glowing due to low room temperature, by which the mercury thermometer appeared to be too insensitive to register its instantaneous temperature accurately. A digital thermometer connected to Logger Pro should have been used to counter this and pursue a greater accuracy in results.

May 13th - July 1st

Report writing

Reflections:

Firstly, for some samples that were treated with low [H_2O_2] and [Cu^{2+}], it was observed that the glow was very subtle. As a result, due to the dimness of glowing as well as the nature of human reaction time, there was a certain degree of random errors in determining the exact instance when chemiluminescence appeared in the visible spectrum, and thus, resulted in lower precision of the data collected. This uncertainty was particularly significant for the trials with relatively concentrated H_2O_2 or Cu^{2+} solutions added to the reaction mixture (concentration set 9 and set 10), such that the induction time of chemiluminescence was too short for humans to react (less than 1 second), which manifested in the presence of outliers

(*table 3*). Despite repeated trials being conducted to counterbalance this random error in this experiment, it is important to note that random errors are theoretically unable to be fully eliminated. In order to counter this issue, a spectrophotometer connected to the LoggerPro software can be used in the future in an attempt to obtain more reliable readings, as well as providing quantitative descriptions of the brightness and the duration of chemiluminescence. With that being said, it should be noted that this proposed improvement method may transfer random errors into systematic errors if the spectrophotometer is not correctly calibrated. Secondly, a 500ml beaker with an absolute uncertainty as large as ±25ml was used to prepare the stock solution (section 7 step 1). However, in reality, the uncertainty was highly unlikely to be indeed ±25ml as the solutions were topped up drop-wise using a 5ml pipette to reach the bottom of the gradation. Nevertheless, in order to reduce the random error associated with the estimation of the significant last digit, a 500ml measuring cylinder (which was not available in school) should be used instead in order to reduce the absolute uncertainty from ±25ml to ±0.5ml. This improvement is highly crucial as all subsequent trials and results, without any exceptions, were potentially affected by the uncertainty associated with the stock solution.

Further research:

[1] Adari, K., Nowduri, A. and Vani, P., 2006. Kinetics and mechanism of oxidation of l-Cysteine by Corey's reagent. Transition Metal Chemistry.

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