Enzymes and inhibition

THE EFFECT OF COPPER (II) SULPHATE ON THE ACTIVITY OF CATALSE

Research Question:

What is the effect of increasing the concentration of $CuSO_4$ on the activity of the enzyme catalase as measured by amount of oxygen gas produced in the decomposition of hydrogen peroxide?

Hypothesis:

Increasing the concentration of $CuSO_4$ will decrease the activity of catalase. This is because the Cu^{2+} in $CuSO_4$, like other metallic ions, acts as an inhibitor[1]. As a result, less oxygen will be produced during the breakdown of hydrogen peroxide into oxygen and water the higher the concentration of $CuSO_4$ will be.

Background information:

Catalase is a very important enzyme that is found in nearly all organisms to catalyze the decomposition of toxic hydrogen peroxide into harmless water and oxygen. The enzyme exists in all living tissue to handle the byproduct of oxidative metabolism that is H_2O_2, with particularly high concentrations in the liver because it is the main detoxifying organ of the body. Therefore bovine liver will be used for the purpose of this IA as a source of catalase. The equation for the decomposition of H_2O_2 is:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

Like all enzymes catalase has an active site, the surface of which comes into contact with the substrate. This leads to a change in shape of the enzyme to accommodate the substrate but there are certain substances called inhibitors that affect the active site thus they can slow down or completely stop the catalysis. $CuSO_4$ is specifically a non-competitive inhibitor wherein the inhibitor binds to the enzyme's allosteric site (i.e. any site of the enzyme excluding the active site) because it is not structurally similar to the substrate. This means it is not in competition with the substrate rather it causes a change in the shape of the enzyme's active site, making it non-functional, and this change is often irreversible. Irreversible inhibitors form strong covalent bonds with an enzyme and heavy metals such as Ag^+, Hg^{2+}, Pb^{2+}have strong affinities for –SH (sulfhydryl) groups[2]. Overleaf there is a diagrammatic explanation of the mechanism of non-competitive inhibition.

Apart from inhibitors, pH levels, temperature and substrate concentration also have an impact on enzyme activity. Enzymes have an optimum pH and higher or lower pH conditions might damage the active site by changing its chemical structure. Likewise is also true for high temperatures as enzymes, like other proteins, get denatured. These will we discussed further while explaining the controlled variables in this experiment.

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[1] Ward, W. and Damon, A. 2007. *Pearson baccalaureate*. Harlow, [England]: Pearson Education.
[2] Elmhurst.edu. 2003. *Enzyme Inhibitors*. [online] Available at: http://www.elmhurst.edu/~chm/vchembook/573inhibit.html[Accessed: 10 Jan 2014].

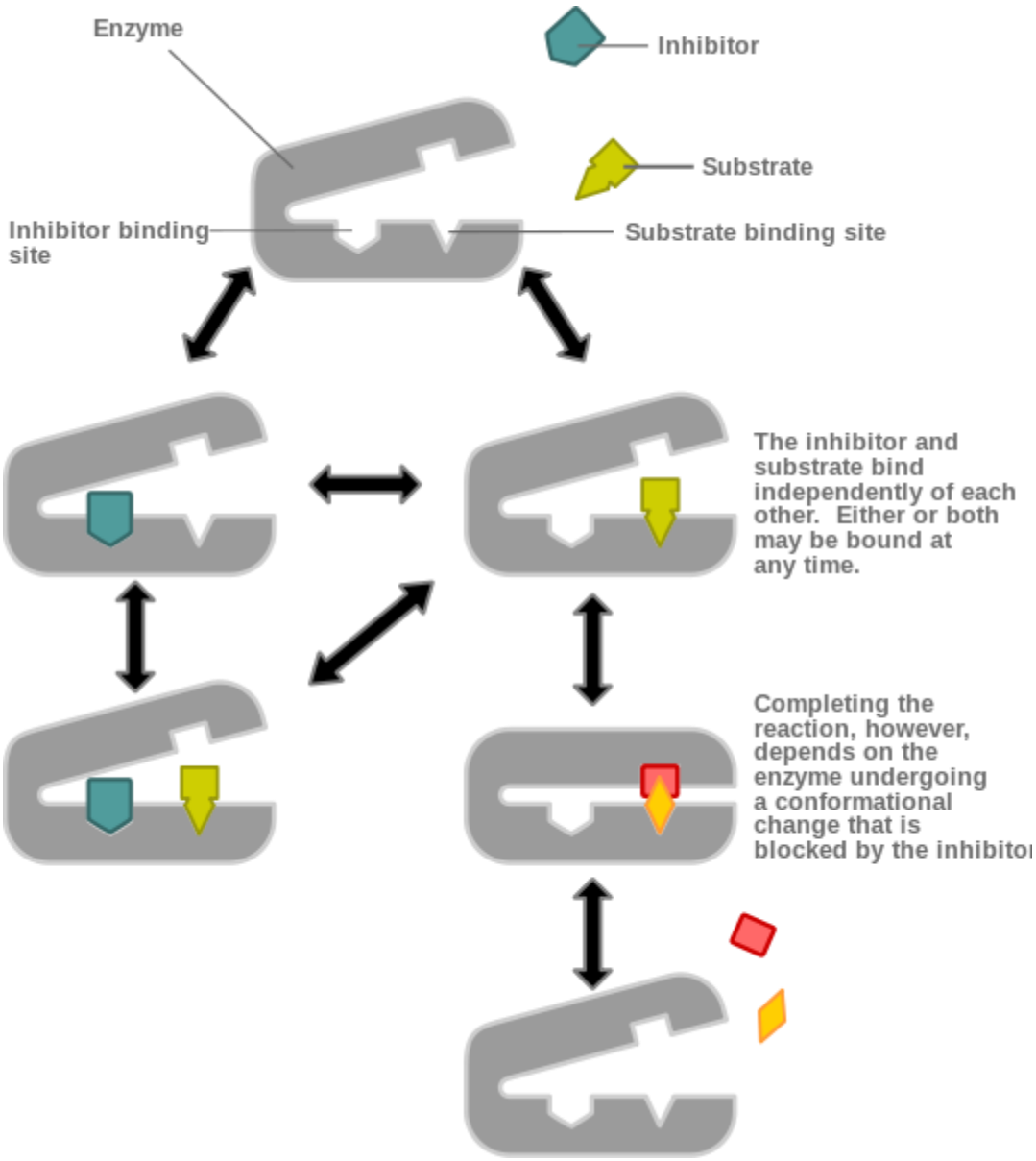


Figure 1: Non-competitive inhibition

3

[3] Wikipedia. 2010. *Non-competitive inhibition*. [image online] Available at: http://en.wikipedia.org/wiki/File:Non-competitive_inhibition.svg [Accessed: 10 Jan 2014]

Variables:

Independent Variable	Dependent Variable
Concentration of $CuSO_4$ solution • 0 mol dm^{-3} • 0.2 mol dm^{-3} • 0.4 mol dm^{-3} • 0.6 mol dm^{-3} • 0.8 mol dm^{-3} • 1 mol dm^{-3}	Activity of catalase in the liver as measured by the amount of oxygen produced in the decomposition of H_2O_2

Controlled Variables		
	Why was it controlled?	**Method for control**
1. Mass of the liver used	Liver contains catalase. To ensure a fair test it is important that the same amount of enzyme is used in all trials.	0.50 g liver was measured each using a ±0.01 g balance
2. Volume and concentration of H_2O_2	H_2O_2 is the substrate that binds to catalase. Increasing substrate concentration increases the rate of the reaction but after a certain limit, the active site can no longer bind to the substrate; the curve plateaus out. Controlling the substrate's concentration & volume is necessary for a fair test.	A commercially sold 9% solution of H_2O_2 was used and a dropping pipette was used to measure 3 cm^3 of it every time.
3. Volume of $CuSO_4$	The concentration for $CuSO_4$ is changing but the volume has to be kept constant so that only one variable is changing and the same volume of inhibitor is used each time.	Another dropping pipette was used to measure 1 cm^3 of $CuSO_4$ of variable concentrations (these will be explained later in detail)
4. pH	pH is one of the main factors that have an impact on the active site. H_2O_2 is a weak acid (has a low pH) itself[4] and this might have an impact on catalase perhaps because the acidic pH is not optimal for the enzyme. To ensure that *only* the effect of $CuSO_4$ is investigated, it is important to keep the pH constant.	2 cm^3 of buffer solution with pH 7 was used in each trial to ensure it doesn't fluctuate much with the addition of H_2O_2. pH 4 and pH 10 buffer solutions were also available but pH 7 was used because preliminary research before the IA shaped that it was the most optimum pH for catalase[5].

[4] H2o2.com. 2009. *What is the pH of H2O2 solutions? | H2O2.com - US Peroxide - Technologies for Clean Environment*. [online] Available at: http://www.h2o2.com/faqs/FaqDetail.aspx?fId=26 [Accessed: 12 Jan 2014]

[5] Bartoszek, M. and Suákowski, S. 2006. *The Study of pH Influence on Bovine Liver Catalase by Means of UV-VIS Spectroscopy and Spin Labelling Method*. [e-book] Katowice: Polish Journal of Environmental Studies. p. 1. http://www.digitalsilesia.eu/Content/5589/11-041-43.pdf [Accessed: 12 Jan 2014]

5. Temperature	Like pH, temperature has a profound impact on the active sites and high temperatures denature the enzyme because they change the active site irreversibly. Very low temperatures cause the enzymes to work slower.	The lab had an AC on whenever the experiment was carried out and it was kept at a constant 22°C albeit there might have been minor fluctuations.
6. Time	It is important that catalase works on H_2O_2 for the same amount of time each trial and $CuSO_4$ (when used) and the buffer solution are inserted with catalase for the same amount of time too.	A ±0.01 seconds stopwatch was used to keep the buffer solution and $CuSO_4$ with catalase for 30 seconds after which the H_2O_2 solution (substrate) was added and this was allocated a further 90 seconds (1.5 minutes)
7. Apparatus	Each piece of apparatus has its distinctive uncertainty value so it's important to keep it constant during all the trials so that the volumes, mass and concentration of H_2O_2 (some of the controlled variables above) are kept constant.	The same (type of) measuring cylinders, gas syringe, dropping pipettes, balance, stopwatch, boiling tube, delivery tube, etc. were used.

Materials:

- Distilled water
- Commercially sold 9% solutions of H_2O_2
- Solution of 1 mol dm^{-3} $CuSO_4$ that was diluted a using a measuring cylinder in the following way (0 % used no $CuSO_4$ and 1 mol dm^{-3} was utilized as it was)
  - 2 cm^3 1 molar $CuSO_4$ and 8 cm^3 distilled water for 20% or 0.2 mol dm^{-3}
  - 4 cm^3 1 molar $CuSO_4$ and 6 cm^3 distilled water for 40% or 0.4 mol dm^{-3}
  - 6 cm^3 1 molar $CuSO_4$ and 4 cm^3 distilled water for 60% or 0.6 mol dm^{-3}
  - 8 cm^3 1 molar $CuSO_4$ and 2 cm^3 distilled water for 80% or 0.8 mol dm^{-3}
- A portion of the bovine liver cut to 0.5 g pieces
- A buffer solution of pH7
- Gloves
- Safety goggles
- Paper towels

Apparatus:

- Two 10 cm^3 measuring cylinders (±0.05 cm^3)
- Scalpel
- Forceps
- Tile
- Petri dish
- Balance (±0.01 g)
- Three dropping pipettes
- Stopwatch (±0.01 seconds)
- Boiling tube
- Delivery tube in a cork
- A 100 cm^3 gas syringe (±0.50 cm^3)
- Clamp and stand
- Beakers
- Sieve
- Thermometer

Risk Assessment:

Copper (II) sulfate is an irritant[6] and hydrogen peroxide is corrosive[7]. Therefore is important that gloves and safety goggles are worn over the course of the whole experiment to avoid it touching the skin or the eyes which can cause inflammation. Handling raw liver may be an unpleasant experience for some; lab coats should be worn in case the student fears it might spill over on his/her clothes. Scalpels and forceps are sharp and pointy so they should be handled with care.

Uncertainties:

Apparatus	**Percentage uncertainty**
Balance	$\frac{0.01}{0.50} \times 100 = 2\%$
Stopwatch	$\frac{0.01}{120} \times 100 = 0.008\%\ (3\ s.f)$
Measuring cylinder	$\frac{0.05}{10} \times 100 = 0.5\%$
Gas syringe	$\frac{0.5}{x} \times 100 = \frac{5}{x}$ (Where *x* is the volume of oxygen collected, the uncertainty changes depending on each result)
Total % uncertainty:	$\pm 2.508 + \frac{5}{x}\ \%$

[6] Windholz, M., ed. 1983. *The Merck Index*. Tenth edition. Rahway, NJ: Merck and Company

[7] ASTDR. 2002. *H2O2 toxicity and dangers*. [online] Available at: http://www.atsdr.cdc.gov/toxfaqs/tfacts174.pdf [Accessed: 12 Jan 2014]

Photographs of the setup:

Balance weighing 0.50 g of liver in a petri dish

Close up of the boiling tube where oxygen being formed can be seen in the bubbles and the froth on blue $CuSO_4$ containing liver.

Procedure:

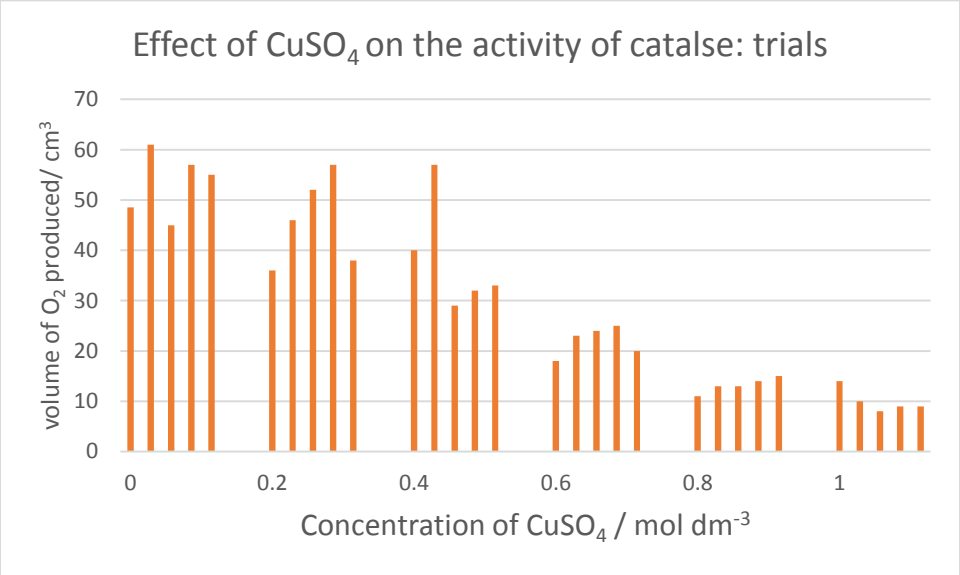
1. Cut out a portion of the liver for your use and place it on the tile. Cut a small piece of it using a scalpel and weigh it on the balance. If the mass is not 0.50 g, make it reach that value by cutting a little more or using a new slightly bigger piece. When it is exactly 0.50g, place it in a boiling tube using forceps.
2. Drop 2 cm^3 of the pH 7 buffer in with the liver while simultaneously starting the stopwatch.
3. Fill the dropping pipette with 3 cm^3 9% H_2O_2 solution and wait for the stopwatch to be near the 30 second mark, and then drop the H_2O_2 in with the liver and buffer solution and immediately close off the boiling tube with the cork that is already connected to the delivery tube and the gas syringe. The plunger will quickly start moving outwards indicating oxygen production. Make sure that no liquid goes through and enters the gas syringe as it makes subsequent readings faulty and if this does happen- use a new gas syringe until the old one dries.
4. Note the reading on the gas syringe when the stopwatch is at 2 minutes (that's 30 seconds for just buffer and 1.5 minutes with H_2O_2) and record it in a table. Remove the cork and empty the contents of the boiling tube in a sink but make sure you use a sieve for filtering out the liver. Bring the plunger of the syringe back to zero.
5. Redo steps 1-4 four other times to complete the 5 trials for this set. Then prepare the different concentrations of $CuSO_4$ using the dilutions method discusses earlier in the Materials section.
6. Steps 1-4 are replicated all the time with the exception that from this point on $CuSO_4$ is added to the liver. 1 cm^3 0.2 mol dm^{-3} $CuSO_4$ is added with the pH buffer and the same 30 second time is allocated for both. The rest of the process remains the same but according to the hypothesis a smaller amount of oxygen should form. Perform four more trials of 0.2 mol dm^{-3} $CuSO_4$.
7. The same as step 6 is done with 0.4 mol dm^{-3}, 0.6 mol dm^{-3}, 0.8 mol dm^{-3} and 1 mol dm^{-3} solutions of $CuSO_4$ with five total trials for each.
8. During this time record all the volumes of oxygen produces as well as some qualitative data.
9. If the investigation occurs over the course of a few days make sure to mark your apparatus, clear the working area and refrigerate liver and H_2O_2 each time and the same at the end of the experiment. Throw all the used liver pieces and used gloves in a bin and wash your hands with soap.

Data collection:

Concentration of $CuSO_4$/ mol dm^{-3}	Trial	Volume of O_2 produced/ cm^3 (±0.05)
0.0	1	48.5
	2	61
	3	45
	4	57
	5	55
0.2	1	36
	2	46
	3	52
	4	57
	5	38
0.4	1	40
	2	57
	3	29
	4	32
	5	33
0.6	1	18
	2	23
	3	24
	4	25
	5	20
0.8	1	11
	2	13
	3	13
	4	14
	5	15
1.0	1	14
	2	10
	3	8
	4	9
	5	9

Table 1: Raw data collection of the volume of oxygen produced with each trial of the 6 different concentrations of $CuSO_4$.

Graphing Raw Data:



This column chart shows the differences in O_2 production not only across the increasing concentrations of $CuSO_4$ but it also shows the variations within each trial.

Qualitative analysis:

Apart from the quantitative analysis above, some other physical changes were observed over the course of this experiment. As soon as the hydrogen peroxide was added to the liver (and/or buffer solution with $CuSO_4$), lots of bubbles would appear within that would soon create a froth-like surface, as seen in one of the photographs a few pages back. The test tube would also become noticeably warmer at the start. The warmth of the test tubes decreased as the concentration of $CuSO_4$ was increased as well. Lastly, on one of the days I cut many 0.50 g pieces of liver to save time but refrigerating these tiny pieces overnight made them very dry and squashy physically and also reduced the enzyme's efficacy, as there was a sharp decrease in the production of O_2 in my results the next day. When I cut a fresh piece of liver and tried again the results followed the previous pattern more closely.

Data processing:

Since each trial produced a slightly different volume of oxygen, it was useful to calculate an arithmetic mean of the results in order to create a more general pattern focused on the increasing concentrations of $CuSO_4$. A sample mean calculation is as follows (the zero concentration of $CuSO_4$):

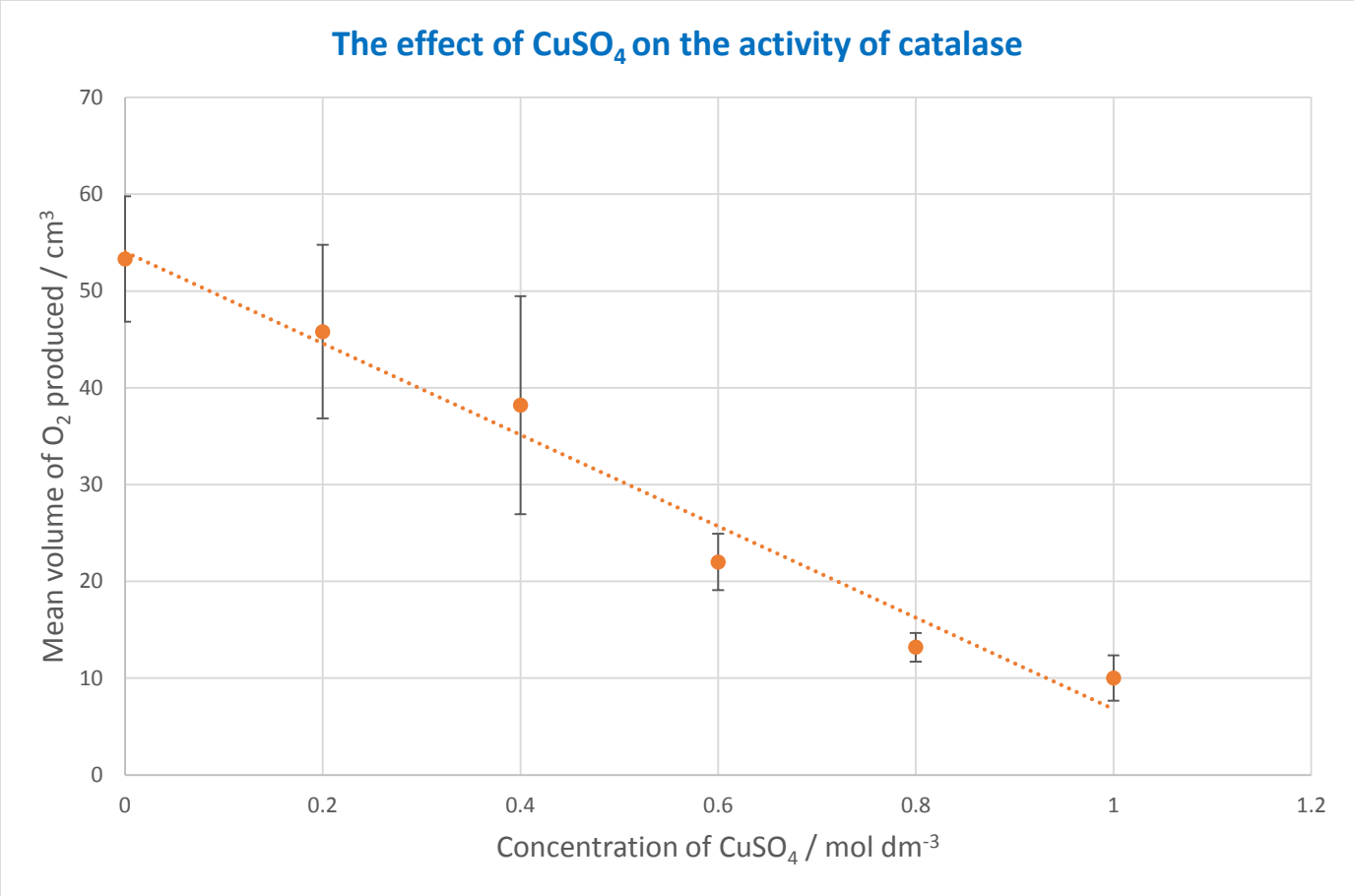
$$\frac{Sum\ of\ values}{Total\ number\ of\ values} = \frac{48.5 + 61 + 45 + 57 + 55}{5} = 53.3$$

Once the mean has been calculated, it can be used to calculate the standard deviation which shows the spread of data around the mean. This was done by using the STDEV function in Microsoft Excel and selecting all of the results of one trial (e.g. 48.5, 61, 45, etc.). It will be used later in the error bars on the graph.

The total percentage error can be calculated without any unknown values. Previously, the formula obtained was $\pm 2.508 + \frac{5}{x}\%$, now the *x* can be substituted for the mean values of the results of different concentrations. For example, $2.508 + \frac{5}{53.3}$ gives a value of ±2.60% up to 3 significant figures, which is the total percentage uncertainty for this trial.

Concentration of $CuSO_4$/ mol dm^{-3}	**Mean volume of O_2 produced/ cm^3**	**Standard Deviation**	**Total percentage error**
0	53.3	6.48	±2.60%
0.2	45.8	8.96	±2.62%
0.4	38.2	11.3	±2.63%
0.6	22	2.92	±2.74%
0.8	13.2	1.48	±2.89%
1	10	2.35	±3.01%

Table 2: Processed data



Graph: Mean volumes of O_2 produced with respect to the different concetrations of $CuSO_4$ used, the error bars represent standard deviation

Conclusion:

The results of this experiment seem to agree with the hypothesis that increasing the concentration of $CuSO_4$ will decrease the enzymatic activity of catalase, and that indeed its behavior is characteristic to that of an inhibitor. Consistently less oxygen was produced overall each time the concentration was increased by 0.2 mol dm^{-3} as can been seen in the mean values 53.2, 44.8, 38.2, 22, 13.2 and 10 and in the downward sloping line of best fit in the graph on the previous page.

If higher concentrations of $CuSO_4$ were used, no oxygen will be produced at all since the shape of the active site would be changed completely to interact with hydrogen peroxide and catalyze any decomposition for it[8]. In this case it would have been interesting to investigate whether increasing the substrate concentration, i.e. the volume of hydrogen peroxide restarts the reaction and oxygen begins to form again, which would show that $CuSO_4$ is a reversible inhibitor. However, research online indicated that $CuSO_4$ is an irreversible non-competitive inhibitor; the former because the Cu^{2+} is a heavy metal ion that reacts with sulfhydryl groups and changes the chemical structure such that it's not reversible, and the latter is true because $CuSO_4$ is very different structurally and chemically from catalase's substrate, H_2O_2 to be a competitive inhibitor[8].

It was noted in the qualitative analysis that the boiling tube would become warmer once the reaction started, and that this effect was less in higher concentrations of $CuSO_4$. This happened because the decomposition of H_2O_2 using catalase is an exothermic reaction[9] that causes heat to go out- making the equipment feel warmer. The higher concentrations did not warm as much because less H_2O_2 was decomposed.

Although the results follow the general pattern that catalase activity decreases as concentration of $CuSO_4$ increases- which can be called an inverse relationship- however it is not proportional or linear per se as there were many differences in the individual results of the trials. From the raw data chart on page 9 we can see that the second trial for 0.4 mol dm^{-3} of $CuSO_4$ produced a much higher volume of oxygen compared to other trials. This result can be called an anomaly, as this volume is similar to the highest O_2 volume from the 0.2 mol dm^{-3} concentration, and when this data was processed it probably caused the mean value to be higher than it would have been otherwise. That being said, there were very few anomalies and the overall conclusion is still reliable. The majority of variations in results exist because catalase is not evenly distributed in the liver, and utilizing different portions lead to slightly different results. Others can be accounted for by the uncertainties or random errors which are discussed in detail in the section overleaf.

[8] Elmhurst.edu. 2003. *Enzyme Inhibitors*. [online] Available at: http://www.elmhurst.edu/~chm/vchembook/573inhibit.html [Accessed: 10 Jan 2014].

[9] Sites.google.com. n.d. *Endothermic and Exothermic Reactions - LMS Room 225*. [online] Available at: https://sites.google.com/a/wvde.k12.wv.us/lms-room-225/endothermic-and-exothermic-reactions [Accessed: 12 Jan 2014].

Evaluation:

The experimental design was suitable for this experiment, but it was very time consuming as initially it was hard to get one method right and the method in this IA was achieved after many preliminary trials. The time meant that we had to refrigerate pieces of liver again re-set all of the equipment again which may have been one of the causes of relatively larger differences in the earlier trials as compared to the later ones (the ones with lower concentration of $CuSO_4$, they also have larger standard deviations which demonstrate this fact).

Out of all the controlled variables, the one that was probably not controlled effectively was temperature. It was possible to heat water and use it in a big beaker in which the boiling tube would be immersed. This would have also been able to be heated until 45°C which is the optimum temperature for catalase[10] but it was decided that the AC controlled room temperature would be sufficient so long as it remained constant. The problem is that the weather and sunlight conditions outside the lab would change, specifically because this experiment was performed over a period of about 6 days.

The percentage uncertainties as seen in table 2 were not very high, however in some instances I forgot to push back the plunger before starting the new trial so it was necessary to subtract the old reading at the end from the new one, this could lead to a double uncertainty for the gas syringe. The reaction time may be another source of random errors, because there were often too many things to handle at once, for example, the boiling tube, the dropping pipette with H_2O_2, and the cork all at once and using them quickly also because the time had to be close to the 30 second mark and oxygen had to be prevented from escaping. Some oxygen still escaped but this is an error that cannot really be improved apart from having long term practice with the equipment or working with a lab partner.

Sometimes the boiling tube with the buffer, $CuSO_4$ solution, catalase and H_2O_2 would be shaken lightly after the cork was closed to promote the oxygen gas to travel to the gas syringe. This was done on most trials but it was not controlled in a precise way, yet it should've probably been controlled.

The liver itself, as mentioned earlier, was one of the primary reasons for fluctuations in the results, especially the earlier ones. Apart from the fact that catalase is not spread uniformly across the liver, the earlier results were carried out on different days with different state of being refrigerated. The last 3 concentrations, 0.6 mol dm^{-3} 0.8 mol dm^{-3} and 1.0 mol dm^{-3} were all carried out on the same day during the same lab session and the effect of this can be noticed on the results as there is much less variation in these results (can be seen on the column chart and standard deviation values)

[10] Sobe K, Inoue N, Takamatsu Y, Kamada K, Wakao N (January 2006). "Production of catalase by fungi growing at low pH and high temperature". *J. Biosci. Bioeng.* **101** (1): 73–76

Overall the results of this experiment are quite reliable because there isn't a huge total percentage uncertainty and the results are backed up by scientific literature elsewhere. Having said that, overleaf is a brief table of two of the main weaknesses of this investigation with suggested improvements.

Weakness	**Improvement(s)**
1. The experiment was too time consuming, spanned over several days which may have had an impact on the result.	One of the most time consuming aspects was weighing the liver to be 0.50 g each time (and overall this was done 30 times). If there was a stencil that helped cute 0.50 g pieces, that would have saved a lot of time.
2. Control of the temperature	Lab temperature would fluctuate even despite the AC being on the same temperature. It would have been better controlled if the water was heated in the kettle and then used. Letting the boiling tube be in a water bath would also spread an optimum temperature more uniformly around it.

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123helpme.com. n.d. *Investigating the Effect of Copper Sulphate on Amylase Activity: Papers*. [online] Available at: http://www.123helpme.com/view.asp?id=148488 [Accessed: 12 Jan 2014].

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