4 A2 Coursework Individual Investigations

Commentaries are provided on five investigation reports. These have been chosen to cover a range of candidate performance and to illustrate some common features that occur in many reports. They should not be taken as indicating any preferred type of investigation.

4.1 Investigation Topics

The exemplar investigation reports cover the following topics:

Report Number	Topic covered in the investigation
0406	Enzyme catalysed decomposition of hydrogen peroxide
0407	Comparison of ways of finding the concentration of copper(II) ions
0408	Vitamin C in fruit drinks
0409	Iron in audio tapes
0410	Kinetics of the reaction between bromide and bromate(V) ions

4.2 Suggested Marks

Each commentary suggests marks that are appropriate in the four assessed skill areas. These are summarised in the following table.

	Suggested marks in each skill area			
Report Number	**Planning**	**Implementing**	**Analysing**	**Evaluating**
0406	9	9	7	6
0407	7	8	8	4
0408	4	6	4	3
0409	11	11	11	10
0410	9	11	11	9

5.1 Candidate Individual Investigation Report 0406

Enzyme catalysed decomposition of hydrogen peroxide

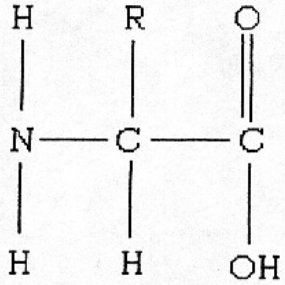
WHAT IS THE MECHANISM FOR THE REACTION BETWEEN CATALASE AND HYDROGEN PEROXIDE?

Aim : To determine the rate equation for the reaction between catalase and hydrogen peroxide in order to propose a mechanism and to investigate the effects of inhibitors and temperature on the reaction.

Hydrogen peroxide is a toxic by-product of many biochemical reactions that occur within organisms. It therefore needs to be broken down into harmless substances so it does not cause any damage within the organism. Catalase is an enzyme which catalyses the break down of hydrogen peroxide. It is found in high concentrations where there are numerous actively metabolizing cells producing hydrogen peroxide, like the liver. The equation for the decomposition of hydrogen peroxide is as follows[1]:

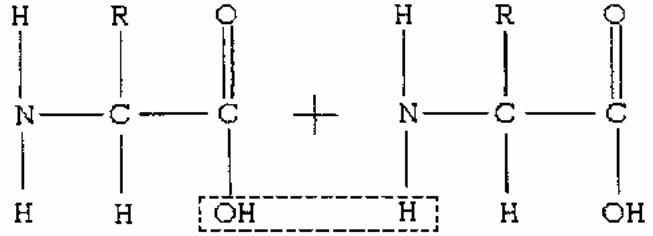
$$2H_2O_2(aq) \xrightarrow{\text{Catalase}} 2H_2O(l) + O_2(g)$$

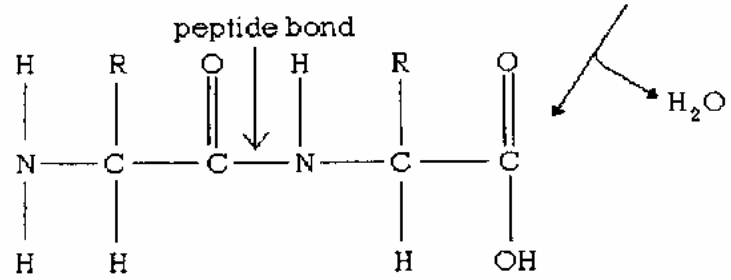
Catalase neither uses up anything, nor is used up in the reaction, it lowers the activation energy of the reaction by providing an alternative route for the reactants to interact. The mechanism an enzyme, like Catalase, uses to catalyze a reaction involves the substrate and enzyme binding to form an Enzyme-substrate complex(ES complex). There are two models for how this happens called the induced fit and lock and key hypothesis. An enzyme is a globular protein, which is formed by the folding of polypeptide chains. An enzymes primary structure consists of a specific linear sequence of amino acids to make a polypeptide chain. The initial folding of this chain as a result of hydrogen bonding gives the secondary structure of alpha helices and beta sheets. The final tertiary structure is the most important as it involves further folding due to ionic bonds, H-bonds and sulphur bridges which make up a 3D crevice where the substrate will bind known as an active site.[2]

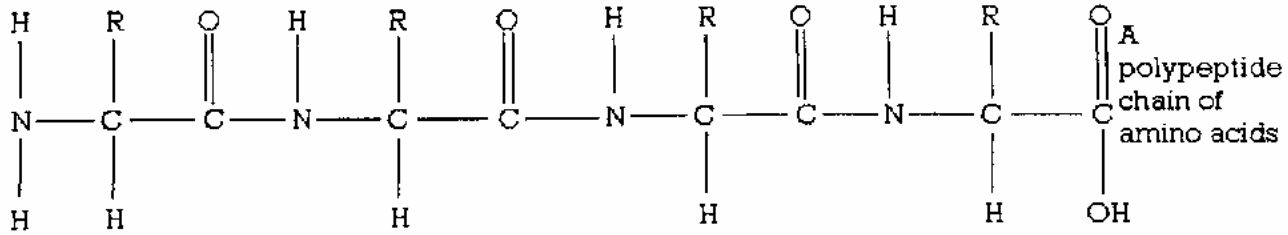


An amino acid

The joining of two amino acids by a condensation reaction to form a dipeptide.

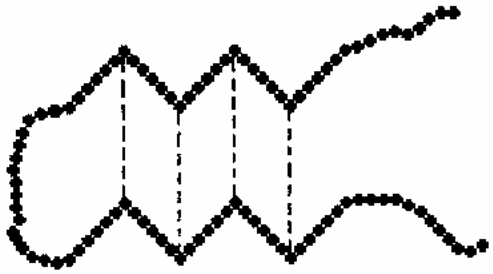






An alpha helix

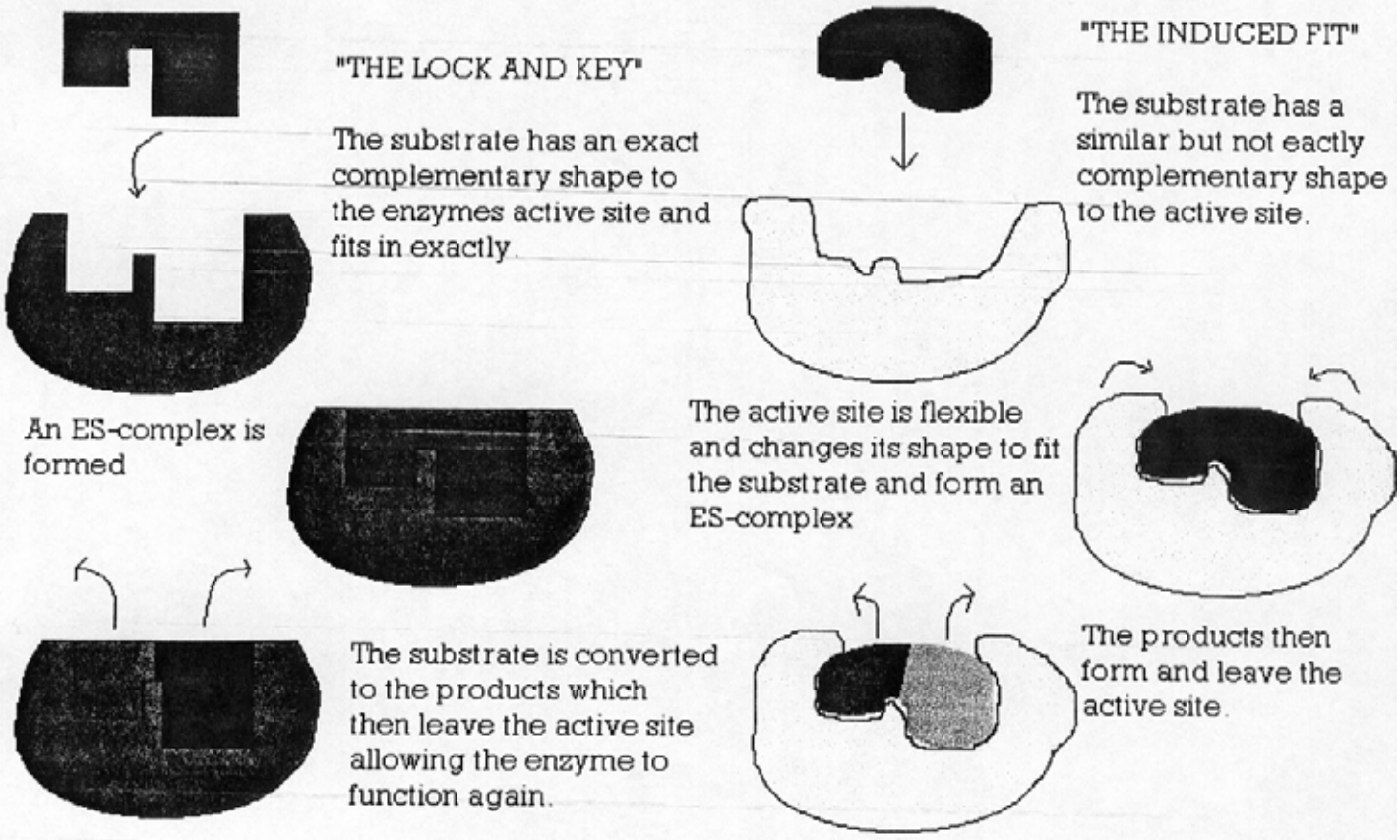
A beta sheet



The two shapes are formed from H-bonds between the NH and CO groups of the peptide bonds from different parts of the chain.

In the lock and key hypothesis the active site of the enzyme has an exact complementary shape to the substrate and so the substrate fits exactly like a key does a lock.[3] The induced fit hypothesis is a development on the lock and key

hypothesis, which focuses on the active site being flexible and changing its shape to fit the enzyme.[4] The diagram below illustrates both models:

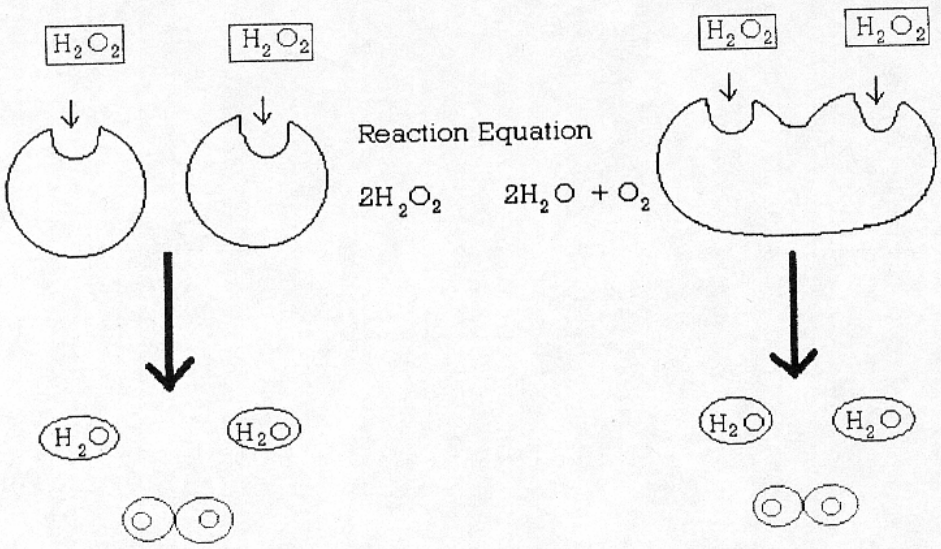


With Both models the substrate binds to the active site of the enzyme and forms the ES complex. The substrate is then changed into the products, which are then released by the enzyme, as they are a different shape, allowing it to function again.

By looking at the equation for the reaction it is possible to deduce two mechanisms, as two molecules of hydrogen peroxide are needed at the start in order to give off the products. The two molecules of hydrogen peroxide could either be catalyzed be two enzymes or one which has two active sites. The only way to determine this is to produce a rate equation.

a substrate molecule each

on 2 substrate molecules



The rate of a reaction is affected by the slowest step, as all other steps are so fast that they are insignificant. Therefore only species that react in the slow step affect the overall rate of the reaction. Since the enzyme catalyses the reaction, the formation of the products must be a fast step, therefore the slow step must be getting the substrate into the active site to form an ES-complex.[5]

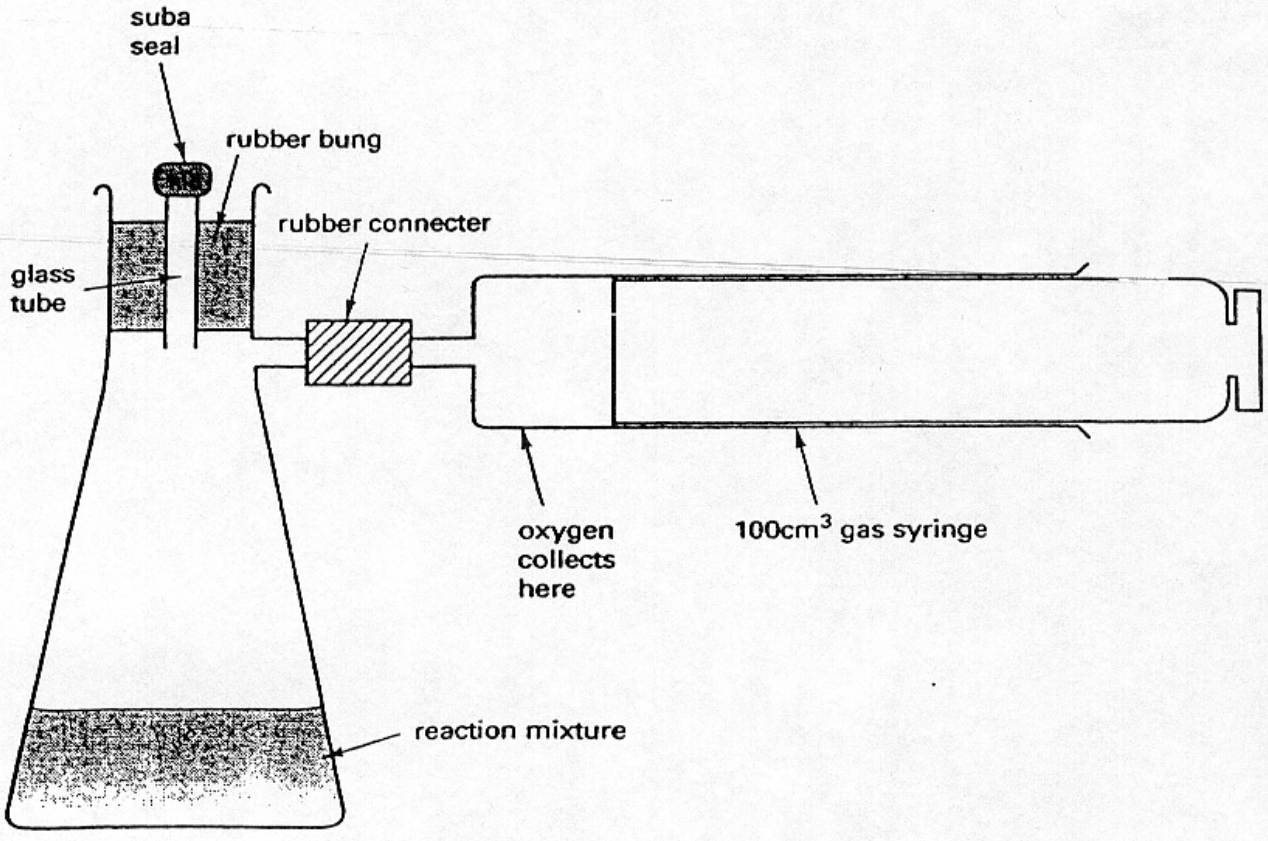
There are a number of factors, which can affect the rate of a reaction. Some of these factors are temperature, concentration of reactants and, where catalysts are concenered, the presence of inhibitors. The way in which these factors affect the reaction can be explained using collision theory. When a molecule is given energy in coverts the energy into three forms: translational, rotational and vibrational. Translational and rotational energy are the movement from one place to another and the spinning round of the whole molecule. Vibrational energy is the stretching and compressing of bonds in the molecule.[6] Collision theory states, that a reaction occurs when reactants collide with a certain amount of energy needed to activate the reaction, the activation enthalpy.[7] It is the activation enthalpy, which gives the reactants molecules energy in order to allow their bonds to stretch and break so that the product molecules can be formed. Varying temperature in the decomposition of hydrogen peroxide can affect the reaction in two ways. Firstly, since heat is a form of energy, increasing the temperature will give the reactants more kinetic energy so more collisions will take place, as the reactants move around faster, with a high proportion colliding with the minimum energy required to activate the reaction. However, since an enzyme is involved this will only occur until a certain point as the enzymes are rapidly denatured. The denaturation of an enzyme is the irreversible loss of its tertiary structure, therefore its active site, leaving it unable to catalyze reactions. Denaturation is a result of vibrational energy increasing so much that it breaks the bonds in the tertiary structure.[8] If low temperatures are used then the reaction will occur at a slow rate as the molecules will only have a

small amount of translational energy and so there will be fewer collisions of which there will only be a low proportion with the activation enthalpy.

Inhibitors are molecules which reduce the rate of reaction of enzymes. There are two types of inhibitors: competitive and non-competitive. Competitive inhibitors are a very similar shape to the substrate and therefore can fit into the active site of the enzyme. However no reaction occurs, as the active site is specific to the substrate so the inhibitor just stays in the active site. As more active sites become occupied by inhibitors, fewer are available to substrates and the rate of the reaction is decreased. Non-competitive inhibitors bind tightly onto anywhere but the enzymes active site and change its overall shape by forming new bonds. The shape of the active site is therefore changed and the substrate no longer fits so the rate of the reaction is decreased.

Many inhibitors are known to be heavy metal ions as they are a similar size and shape to the substrate.[9]

The rate of a reaction can be measured in two ways: how quickly the reactants are used up, or how quickly the products are formed. In the decomposition of hydrogen peroxide, since one of the products formed is a gas and the reactant is a liquid it will be easier to measure how quickly the products are formed by measuring the volume of oxygen produced. The equipment used is shown below:



Pilot Experiments

In order to work out what concentration of catalase and hydrogen peroxide to use in order to carry out controlled experiments a series of pilot experiments was carried out.
The initial concentration of catalase in the liver extract is unknown as it varies since each organism is individual therefore it was taken to be 100%. The concentration of hydrogen peroxide was 20 volume. This means for every $1cm^3$ used $20cm^3$ of oxygen will be produced. A $100cm^3$ gas syringe was used in the experiments with $25cm^3$ of hydrogen peroxide and $10cm^3$ of liver extract. Since $1cm^3$ of hydrogen peroxide would produce 20 cm^3 of oxygen then $25cm^3$ of 20 volume hydrogen peroxide would produce $500cm^3$ of oxygen which is too much oxygen to fit in the syringe so it was clear that the hydrogen peroxide would have to be diluted to a lower concentration. For the experiment to be controlled between 70 and $80cm^3$ of oxygen needed to be produced. $80cm^3$ is 4 times as much oxygen produced than when $1cm^3$ of 20 volume hydrogen peroxide is used so therefore 4 cm^3 of hydrogen peroxide was needed in the $25cm^3$ and the remaining $21cm^3$ was made up with distilled water. The concentration of this was worked out by the following formula:

$$\frac{\text{Amount of } H_2O_2(cm^3)}{25} \times 20$$

This gave the above concentration to be 3.2 volume. This concentration was then used to see what concentration of catalase should be used. The reaction needed to run fast enough to produce the $80cm^3$ of oxygen in a short space of time but also slow enough to be able to note the change at a series of regular intervals. However, as the concentration chosen would be the highest, and all others would be lower, the lowest would to a lot longer to produce the oxygen. Since time was precious it was decided 2 minutes would be an appropriate time for the highest concentration used to catalyze the reaction in. Taking the initial concentration of catalase from the liver extract to be 100% and $10cm^3$ of liver extract for each experiment, $5cm^3$ of liver was taken in a syringe and diluted with $5cm^3$ of distilled water to make 50% concentration. This concentration produced the volume of oxygen required in just 30 seconds making it clear a much lower concentration would be needed. Next 10% concentration was tried by adding $1cm^3$ of liver extract to $9cm^3$ of distilled water. This gave the required amount of oxygen in just under 2 minutes and was therefore suitable to use as the highest concentration, so was now taken to be 100%.

Method

A 500g sheep's liver was taken and chopped into small pieces. It was then placed in a $500cm^3$ beaker with $50cm^3$ of pH7 buffer and liquefied using a

liquidiser. The mixture was then filter through a piece of muslin cloth into a 1000cm^3 beaker to remove any large lumps that still remained in the mixture. Approximately 600cm^3 of pH7 buffer was then added to the liver to make the mixture up to around 900cm^3. This mixture was then poured into ice cube bags and left in the freezer so that the catalase concentration would remain constant for all experiments. When the liver extract needed to be used a few cubes were taken and thawed out in a 100cm^3 beaker by running hot water over the sides of the beaker. For all experiments the gas syringe was read to 90cm^3 since 10cm^3 of oxygen is immediately transferred to the gas syringe when the 10cm^3 is pushed down as there is now an extra 10cm^3 of liquid in the conical flask and not enough space for the air. When the experiment was finished 10cm^3 was taken off all readings.

For constant substrate and varying enzyme concentration experiments:

25cm^3 of 3.2 volume hydrogen peroxide (made up from 4cm^3 of 20 volume H_2O_2 and 21cm^3 of distilled water) was placed in a 250cm^3 side armed conical flask connected to a gas syringe. A rubber bung was placed in the top of the conical flask and an empty 10cm^3 syringe was placed in the suba seal to check the apparatus was airtight by pulling the end of the gas syringe to see if it would move. As it didn't the syringe was removed and was filled with 10cm^3 of the required enzyme concentration. Each enzyme concentration was made up in a 100cm^3 beaker with regards to the following table:

CATALASE CONCENTRATION	VOLUME OF DISTILLED WATER	VOLUME OF LIVER EXTRACT
100%	90cm^3	10cm^3
80%	92cm^3	8cm^3
60%	94cm^3	6cm^3
40%	96cm^3	4cm^3
20%	98cm^3	2cm^3

Each beaker was stirred thoroughly with a glass rod before the 10cm^3 was taken. The reason for making the concentrations up beakers was because it was impossible to measure to 0.1cm^3 in a syringe that could only be read to 0.5cm^3. Once the 10cm^3 of the specific enzyme concentration had been taken up in the syringe, the syringe was placed in the suba seal. As the end of the syringe was pushed down emptying the contents into the conical flask, the stopwatch was started and the conical flask was swirled to evenly mix the enzyme and substrate. The amount of oxygen produced in the syringe was then noted every 30 seconds until the 80cm^3 had been produced. Repeats were carried out as there may have been small differences in the number of enzymes in each 10cm^3 of enzyme solution taken resulting in slower or faster reactions.

For constant enzyme and varying substrate concentration

100% enzyme concentration was used in all experiments in the $10cm^3$ syringe.

Each substrate concentration was made up with regards to the following table:

H_2O_2 CONCENTRATION	VOLUME OF H_2O_2 (cm^3)	VOLUME OF DISTILLED WATER(cm^3)
3.2 Volume	4	21
2.8 Volume	3.5	21.5
2.4 Volume	3	22
2 Volume	2.5	22.5
1.6 Volume	2	23

The experiment was carried out in exactly the same way as the varying enzyme ones expect each experiment was only carried out for 2 minutes to see how much oxygen was produced in that amount of time.

For varying temperature

Both the enzyme and substrate concentrations were kept constant for all experiments at 100% and 3.2 volume. The conical flask containing the substrate and the beaker containing the enzyme were placed in a water bath at a certain temperature and left to acclimatise for 10 minutes. The $10cm^3$ syringe was then filled with the enzyme and placed in the suba seal and the gas syringe was connected to the conical flask. The clock was then started as the syringe was pushed down and the conical flask was swirled. Readings of the gas syringe were taken every 30 seconds until the $80cm^3$ of oxygen had been produced.

For the inhibitor experiments

3.2 volume hydrogen peroxide and 100% catalase were used for all experiments. $2cm^3$ of a 0.1M inhibitor was added to the conical flask with the H_2O_2 and the experiment carried out as before with varying enzyme concentration. A control experiment was done at the start where $2cm^3$ of distilled water was placed in the conical flask in place of the inhibitor. After the first experiment a modification had to be made as a known inhibitor was being added and having little effect as it did not have long enough to make contact with the enzyme. Instead the

inhibitor was added to the syringe containing the enzyme just before it was placed in the suba seal. This was done by only adding 7cm^3 of distilled water to 1cm^3 of enzyme and making the remaining 2cm^3 up with the inhibitor so that the enzyme was still 100% concentration. Each experiment was carried out for 2 minutes to see how much oxygen was produced at 30 second intervals. The following inhibitors were used: Iron (III) Nitrate, Zinc Sulphate, Aluminium Sulphate, Iron (II) Sulphate and Lead Nitrate. They were chosen as they are transition metal ions and the effects of sulphates and nitrates could be compared. Once some inhibitors had be identified by the previous experiment, two inhibitors were taken and their concentrations were altered and further experiments carried out to distinguish which type of inhibitor they were. The concentrations were altered easily. This was because there were quarterly intervals between each concentration, and as 2cm^3 needed to be taken, then 0.5cm^3 of distilled water was added each time. The following table shows this:

Concentration of Inhibitor (M)	Amount of distilled water added	Amount of inhibitor added
0.1	0cm^3	2cm^3
0.075	0.5cm^3	1.5cm^3
0.05	1cm^3	1cm^3
0.025	1.5cm^3	0.5cm^3

Instead of making up the concentrations before, the inhibitors were taken up in the syringe with the enzyme as before but where less inhibitor was needed in the 2cm^3 the rest was made up with distilled water. The experiments were carried out in the same way as with the previous inhibitor experiments for two minutes and the volume of oxygen was noted at 30 second intervals.

Risk Assessment

Chemical	Risk	Protection	Actions to take if spilt on skin, eyes or surfaces, or swallowed
Distilled water	Low risk	N/A	N/A
pH7 buffer	Low risk	N/A	N/A
Hydrogen Peroxide	Irritant	Goggles and gloves	Flood area with water and seek medical attention.
Catalase	irritant	Goggles and gloves	Flood area with water and seek medical attention. In lab scoop up as much as possible

			and wash area with water and detergent.
Iron(III)Nitrate	Irritant	Goggles and gloves	Wash area with water and seek medical a'tention. In lab absorb spillage's with mineral absorbent
Iron(II)Sulphate	harmful	Goggles	Same procedure for Iron(III)Nitrate
Zinc Sulphate	Low risk	N/A	N/A
Aluminium Sulphate	Low risk	N/A	N/A
Lead Nitrate	Toxic	Goggles	Wash area with large quantities of water and seek medical attention.

RESULTS

AMOUNT OF OXYGEN PRODUCED WHEN H_2O_2 CONCENTRATION IS VARIED

	Progress of reaction (seconds)			
Concentration of H_2O_2	30	60	90	120
3.2 Volume	72 cm^3	76cm^3	78cm^3	80cm^3
2.8 Volume	60 cm^3	66cm^3	69cm^3	70cm^3
2.4 Volume	55 cm^3	60cm^3	61cm^3	62cm^3
2.0 Volume	43cm^3	46cm^3	48cm^3	50cm^3
1.6 Volume	34cm^3	36cm^3	38cm^3	39cm^3

TIME TAKEN TO PRODUCE 80CM^3 OF OXYGEN AT VARIED TEMPERATURES

	TEMPERATURE (°c)				
	25	**35**	**40**	**45**	**50**
Time taken (seconds)	120	84	72	86	900

AMOUNT OF OXYGEN PRODUCED(CM^3) WHEN SUBJECTED TO VARIOUS POSSIBLE INHIBITORS

Substance added	PROGRESS OF REACTION (SECONDS)			
	30	60	90	120
Control(H_2O)	60	67	74	80
Iron(III) Nitrate	5	6	9	11
Zinc Sulphate	61	65	68	72
Aluminium Sulphate	60	68	74	80
Iron(II) Sulphate	7	8	9	10
Lead Nitrate	0	2	2	2

AMOUNT OF OXYGEN PRODUCED IN TWO MINUTES WHEN SUBJECTED TO $2CM^3$ OF AN INHIBITOR AT VARIED CONCENTRATION

INHIBITOR	concentration	30	60	90	120
Iron(III) Nitrate	0.1M	1	3	6	11
	0.075M	8	15	19	22
	0.05M	12	17	24	28
	0.025M	19	21	29	36
Lead Nitrate	0.1M	0	0	0	1
	0.075M	0	0	1	1
	0.05M	0	0	1	2
	0.025M	0	1	2	4

INITIAL RATE OF REACTION (CM^3/SECOND) FOR VARYING [CATALASE]

CATALASE CONCENTRATION	INITIAL RATE
100%	20/2.5 = 8
80%	25/5 = 5
60%	40/10 = 4
40%	39/17.5 = 2.2
20%	29/15 = 1.9

INITIAL RATE OF REACTION (CM^3/SECOND) FOR VARYING [H_2O_2]

H_2O_2 CONCENTRATION	INITIAL RATE
3.2 VOLUME	22/3 = 7.3
2.8 VOLUME	16.5/2.5 = 6.6
2.4 VOLUME	22/4 = 5.5
2 VOLUME	18/4 = 4.5
1.6 VOLUME	18/5 = 3.6

OVERALL RATE OF REACTION(CM^3/SECOND) OF VARIOUS [INHIBITOR]

[INHIBITOR]	Lead Nitrate rate	Iron(III) Nitrate rate
0.1M	0.01	0.09
0.075M	0.01	0.18
0.05M	0.02	0.23
0.025M	0.03	0.3

Analysis

By graphing the results for the varying catalase experiments it could be seen that a series of curves was formed, one for each concentration. All the curves began with a sharp increase in the first 30 seconds before slowly leveling out and finishing at the same point of 80cm^3. This shows the initial rate is the fastest. As the concentration of the catalase solution was decreased the initial rate and overall rate decreased, illustrated by each curve falling beneath one of a higher concentration. The reason for this is because there are less enzyme molecules in the solution as the concentration is lowered so there are less active sites and less chance of collisions between an enzyme molecule and a substrate molecule so it takes longer to form the products. The graph for varying H_2O_2 experiments showed a similar pattern of curves at the beginning of the reactions but the curves fished at different points as less oxygen was produced by lowering concentration. The reason for this is because as concentration decreases there are less substrate molecules so less chance of a substrate molecule and enzyme colliding so less products form in a certain space of time. Fewer products are also formed overall, as there are less substrate molecules to produce the products as the concentration is lowered.

By taking the gradient of the curves at t=o on the graphs for varying enzyme and substrate concentrations the initial rate of the reactions could be found. Each initial rate was plotted on a graph against the concentration to find out the order of the reaction and produce a rate equation. Both varying enzyme and

substrate concentrations against initial rate gave graphs of a diagonal line. This implies that the rate is proportional to the concentration of the enzyme or substrate and the reaction has first order. The order of the reaction is the power, which the concentration is raised to, in the following equation:

$$\text{Rate} = k[A]^m[B]^n$$

Where "A" and "B" are the concentrations of the substances being altered, in this case catalase and H_2O_2, and k is the rate constant which varies for different temperatures and is also related to the activation energy of the reaction. M and n are the order of the reactions.[10] All experiments for varying $[H_2O_2]$ and varying [catalase] were carried out at room temperature of 23°c.

For varying [enzyme] rate $\propto$ [enzyme]

For varying [substrate] rate $\propto$ [substrate]

Since the rate is proportional to both the [enzyme] and [substrate] the two equations can be combined to produce an overall equation for the reaction.

$$\text{Rate} = k\,[\text{catalase}]^1\,[H_2O_2]^1$$

The overall order of the reaction was found by adding "m and n", in this case 1+1 to get 2 which means the reaction has an overall second order.[11]

The gradient of the initial rate of varying $[H_2O_2]$ was obtained in the following way in order to obtain a value of k.

$$\text{Rate} = k[H_2O_2] \quad \text{therefore} \quad k = \text{rate}/[H_2O_2]$$

$$3.4\ (cm^3/\text{second}) / 1.5\ \text{volume} = 2.26666666666 = 2.27\ (2dp)$$

The same formula was used to obtain a value of k for the initial rate of varying [catalase]. However, as the concentration was taken as a percentage because it was unknown, each percentage was expressed as a decimal in the calculations.

$4\ (cm^3/\text{second}) / 0.6 = 2.4$

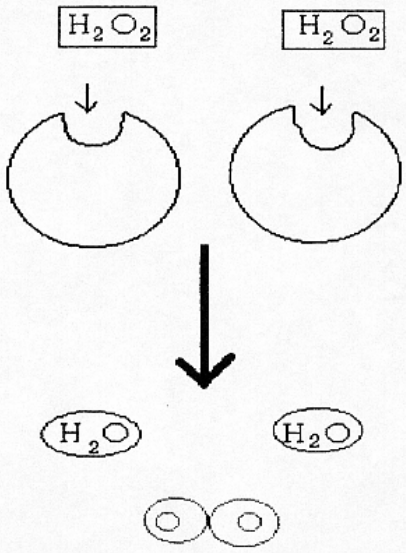
This showed the values of k differed slightly when they should have been the same so there may have been a slight rise in the temperature which couldn't be read on the thermometer between the two experiments which would cause the value of k to rise. By using the combined equation when the enzyme concentration is 100% the rate constant is the same as for varying just $[H_2O_2]$.

5.1 = k [1] [2.25] therefore k = 5.1/2.25 = 2.27 (2dp) at 23°c

Therefore the rate equation for the decomposition of hydrogen peroxide by catalase at 23°c is:

$$\text{Rate} = 2.27\,[\text{catalase}]\ [H_2O_2]$$

By looking at the order of the reaction it is possible to deduce the mechanism of the reaction. Since both [catalase] and $[H_2O_2]$ are first order reactions it means



that in the slow step of the reaction there is one enzyme molecule, with one active site, working on one substrate molecule.

From looking at the graph of the effect of temperature on the rate of the decomposition of hydrogen peroxide and sketching curves to show the rate at which the reaction increases and decreases, it was possible to obtain an approximation of the optimum temperature of catalase. This was done by finding where the lines, showing the rate at which the reaction increases due to increased kinetic energy of the molecules and the rate at which the reaction decreased due to denaturation of the enzyme, crossed which worked out to be 38°C. The catalase molecules began to denature at around 40°C and as the temperature increased more enzymes became denatured. However, the enzymes were not completely denatured at 50°C as the reaction still continued although it had slowed down a lot. The high temperatures needed to denature enzymes show that the bonds that hold the structure together must be very strong. The bonds making up the tertiary structure of an enzyme are ionic bonds, H-bonds and sulphur bridges.

From looking at the graphs of the various inhibitors it could be seen that lead nitrate, iron(II) sulphate and iron (III) nitrate had severe inhibitive effects on the rate of the reaction. In order to see what type of inhibition they were displaying the concentrations of lead nitrate and iron(III) nitrate were altered

and the results graphed. This graph indicated that lead nitrate was a non-competitive inhibitor and iron(III) nitrate was a competitive inhibitor. This was shown by the sharp decrease in the curve for lead nitrate at a low concentration which lowers slightly further until the rate is almost zero. This indicated that the lead nitrate molecules were inactivating the enzymes by attaching themselves to places other than the active site. Since the rate determining step of the reaction is getting the substrate molecules into active sites then a small number of inhibitor molecules would be able to inactivate enzymes very quickly by colliding with them leaving few active sites available for substrate molecules. The curve for iron(III) nitrate decreases gradually as the concentration of iron(III) nitrate increases. This is because the inhibitor can only affect the reaction rate dramatically when there are roughly more or the same number of inhibitor molecules as substrate molecules so there is more chance that an inhibitor will occupy an active site than a substrate molecule.

Error Measurements

All errors from the equipment were calculated using the following formula:

$$\frac{\text{degree of accuracy equipment can be read to}}{\text{reading}} \times 100$$

Gas syringe: 0.5/80 x100 = 0.625%

10cm^3 syringe: 0.25/10 x100 = 2.5%

50cm^3 syringe: 0.5/25 x 100 = 2%

25cm^3 pipette: 0.06/25 x 100 = 0.24%

10cm^3 pipette: 0.05/10 x 100 = 0.5%

These calculations show that the experimental errors are so small that they do not significantly affect the experiments.

Evaluation

Overall the experimental procedure was very accurate as it only involved simple experiments for which any experimental errors were very small and insignificant. However, the results from the experiments would need to be repeated numerous times and have averages taken before they could fully justify any conclusions made. This is because all the sets of results obtained from this investigation

could be anomalous and the only way to check them is to compare them to other results. The only obvious anomalous results produced in the experiments were produced when carrying out the inhibitor experiments. Known inhibitors were not affecting the rate of reaction as much as it should have done so the experiment was modified as stated in the plan so that the enzyme and inhibitor came into contact for a longer period of time. The main sources of error in the experiment arose from room temperature varying slightly from day to day and at different times during the day. This caused the rate constant and activation energy of the reaction to change. The rate constant and activation energy are inversely related by the Arrhenius equation. The Arrhenius equation is:

$$k = Ae^{-E_a/RT}$$

"A", another constant, is the collision frequency.
E_a is the activation energy
R is the gas constant
T is the absolute temperature

By taking natural logs of both sides of the equation and plotting a straight line graph of ln k against 1/T, it is possible to deduce the activation energy E_a since the gradient of the line is $-E_a/R$.[12] A quoted value for the activation enthalpy of liver catalase is 23kJ/mol.[13] Further work on the decomposition of hydrogen peroxide by catalase could include looking at producing the Arrhenius equation in order to calculate the activation energy of catalase from different sources. An improvement to the experiment to reduce errors would be to carry out experiments in a water bath so the temperature could be controlled and constant for all experiments.

Since the tertiary structure of an enzyme is held together by ionic bonds it wold be interesting to see how catalase is affected by varying pH. Ionic bonds involve electron transfer leaving a positively and negatively charged atom and pH is a measure of H^+ ions in solution. Acids are H^+ donors so if an enzyme was placed in a strong acid then the H^+ ions may affect the ionic bonds in the enzymes tertiary structure as they contain charges.[14] This could be another area to investigate.

From looking at the inhibitor experiments it could be seen that some substances seemed to increase the rate of the reaction. Molecules, which work in this way, are called co-enzymes. Further research could also include finding other substances, which seem to increase the rate of the reaction or other catalysts for the reaction.

References

Understanding Biology for advanced level, second edition, G and S Toole

1. pg 47
2. pg 26 - 29
4. pg 304
9. pg 307 -308

Chemical Ideas, salters advanced chemistry, G Burton, J Holman, G Pilling, D Waddington

6. pg 57
7. pg 173
11. pg 176

Chemical Storylines, Salters Advanced Chemistry, G Burton, J Holman, G Pilling, D Waddington, J Lazonby

3. pg 166
8. pg 161
14. pg 161

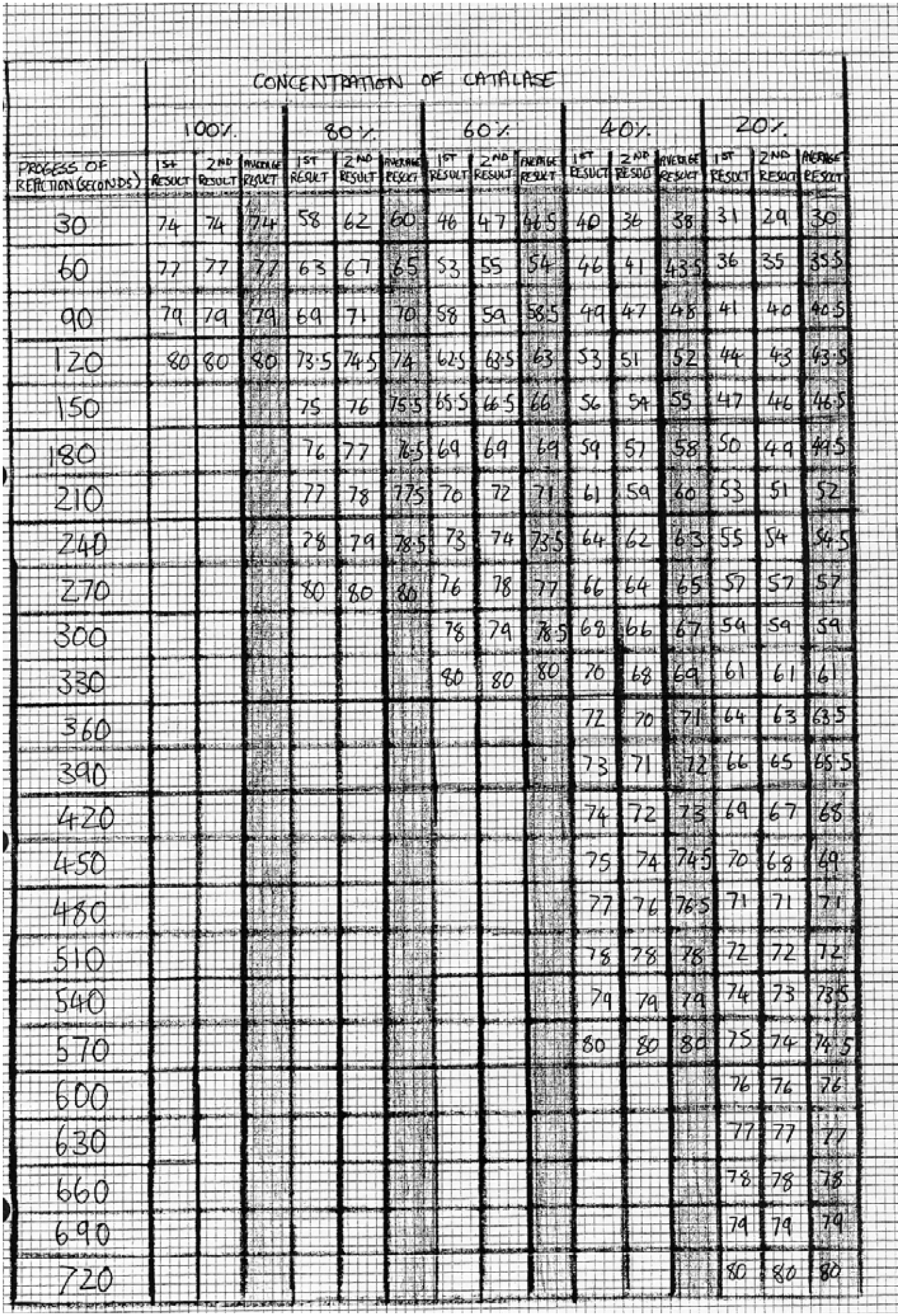
Principles of Physical and Organic Chemistry, Alan Jarvis

10. pg 27 - 30

13. http://antoine.frostburg.edu/chem/sense/101/kinetics/faq/Ea-catalysed-h2o2-decomposition

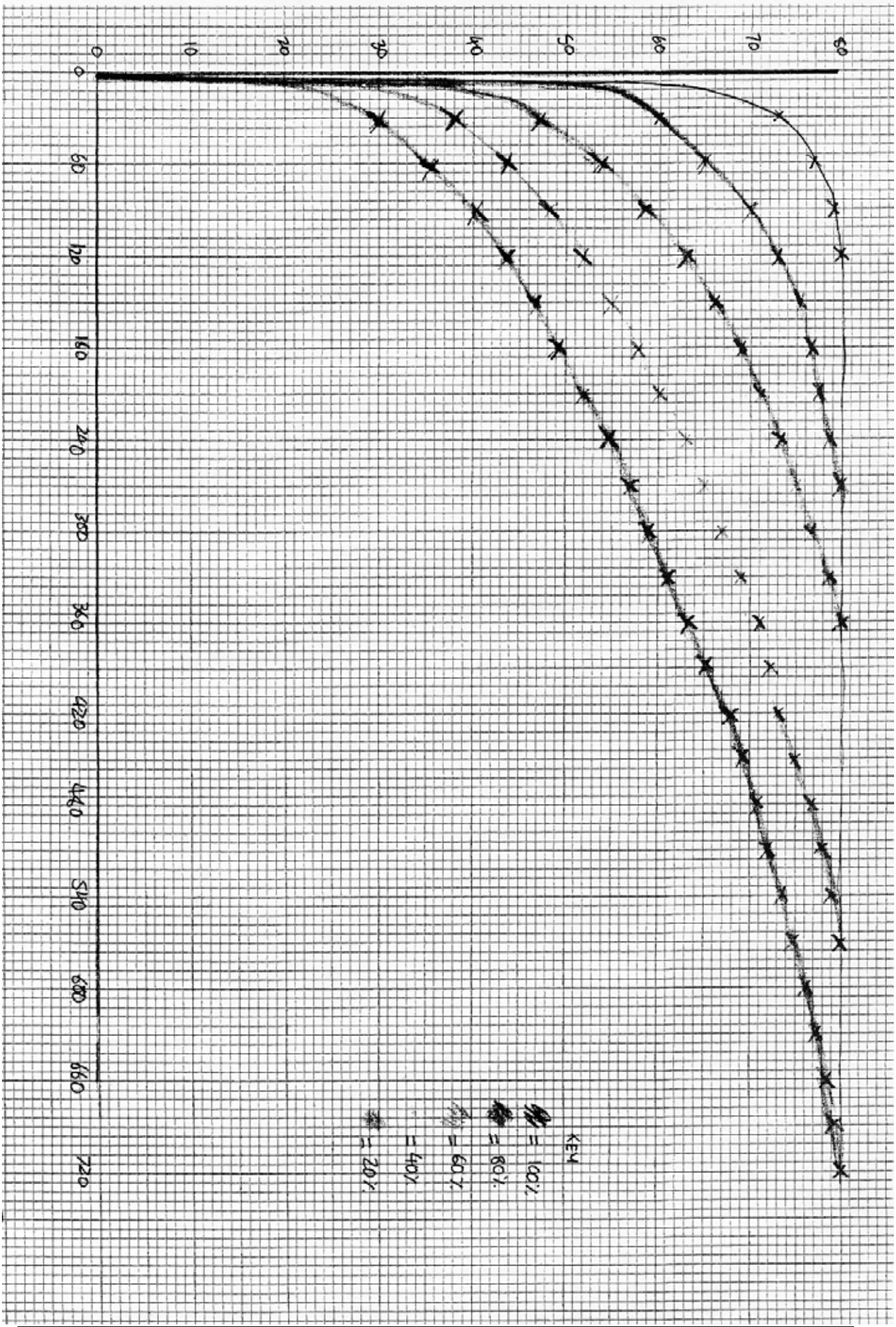
Chemistry

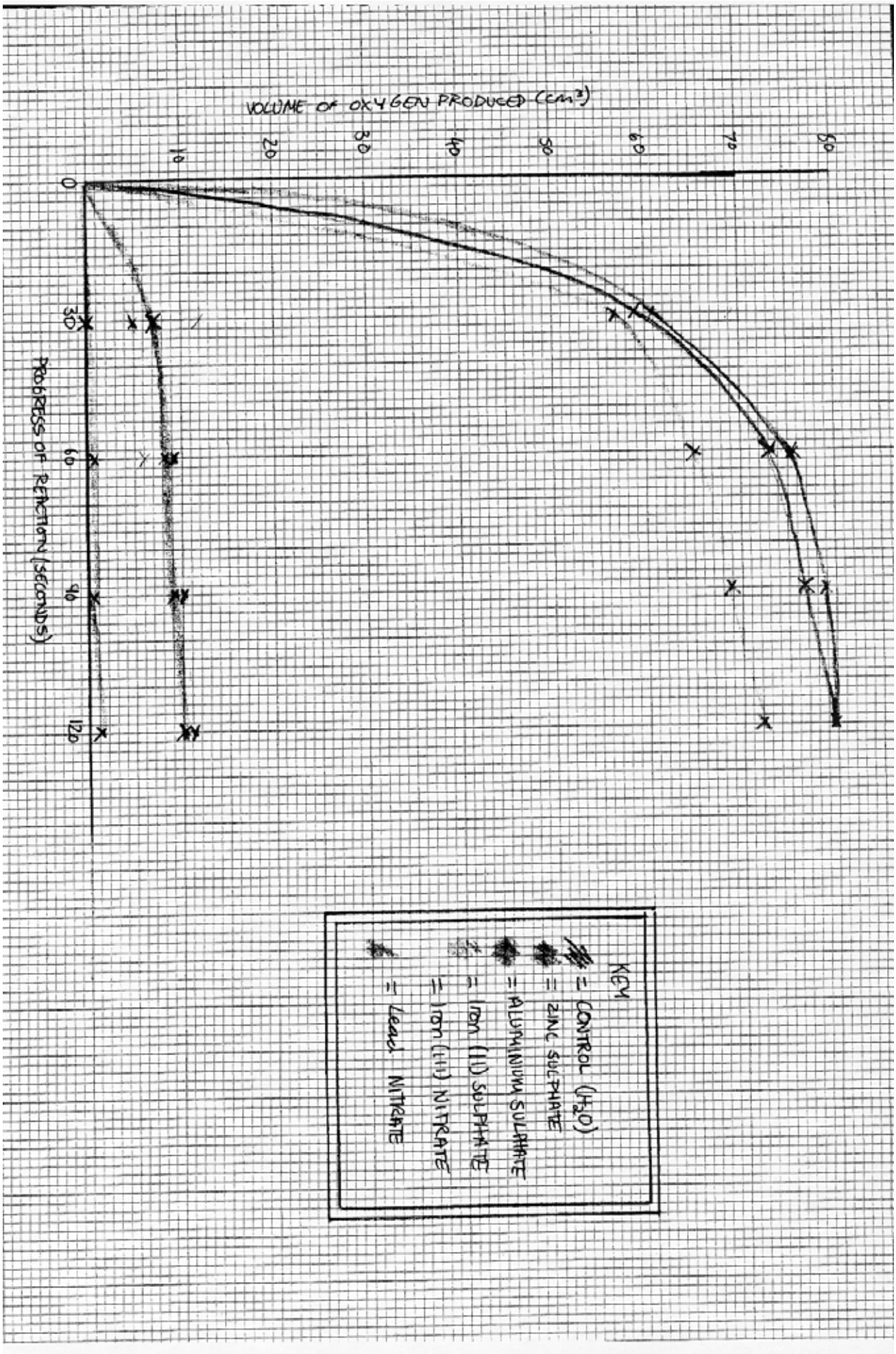
5. pg 591
12. pg 598

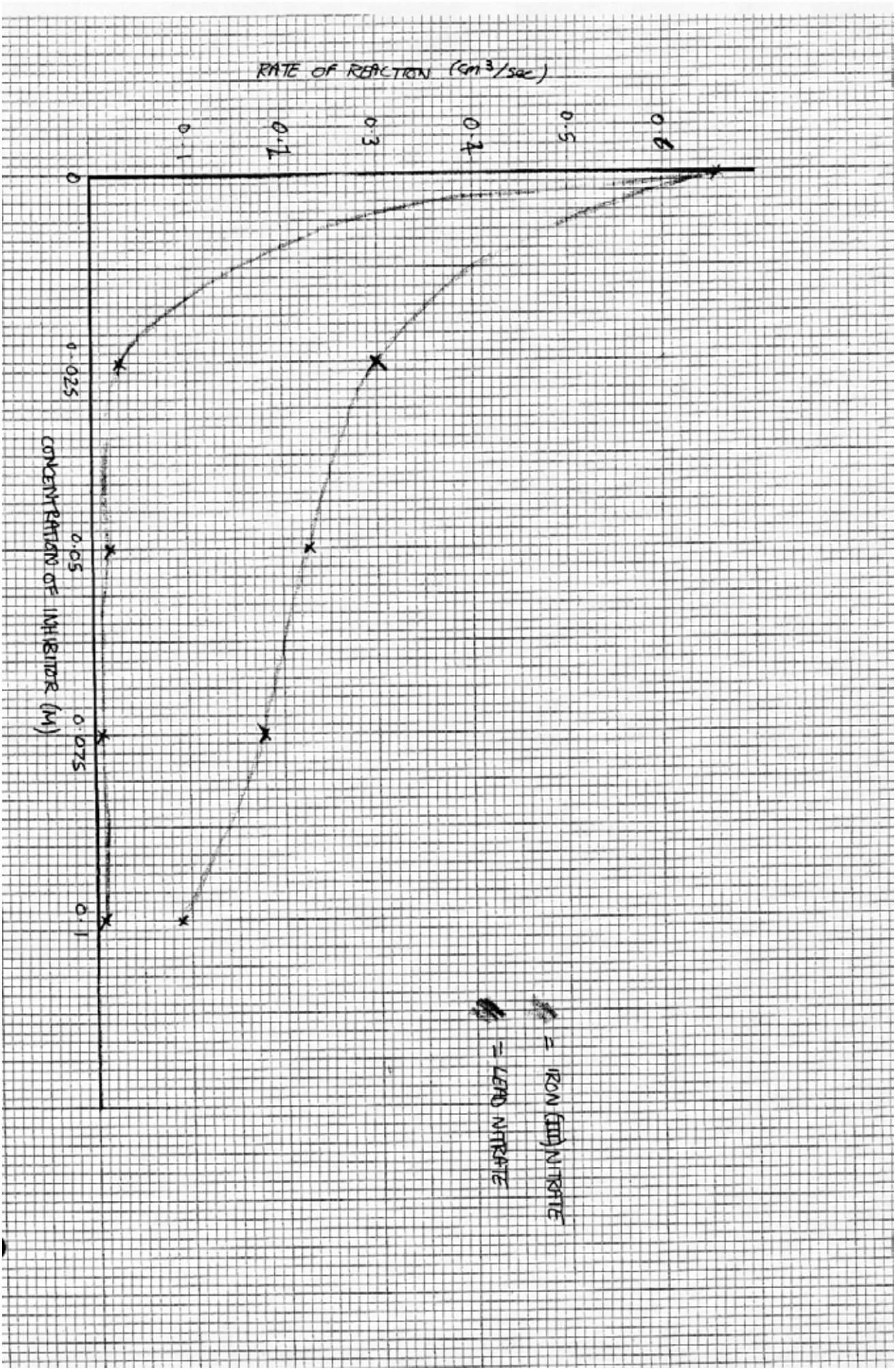


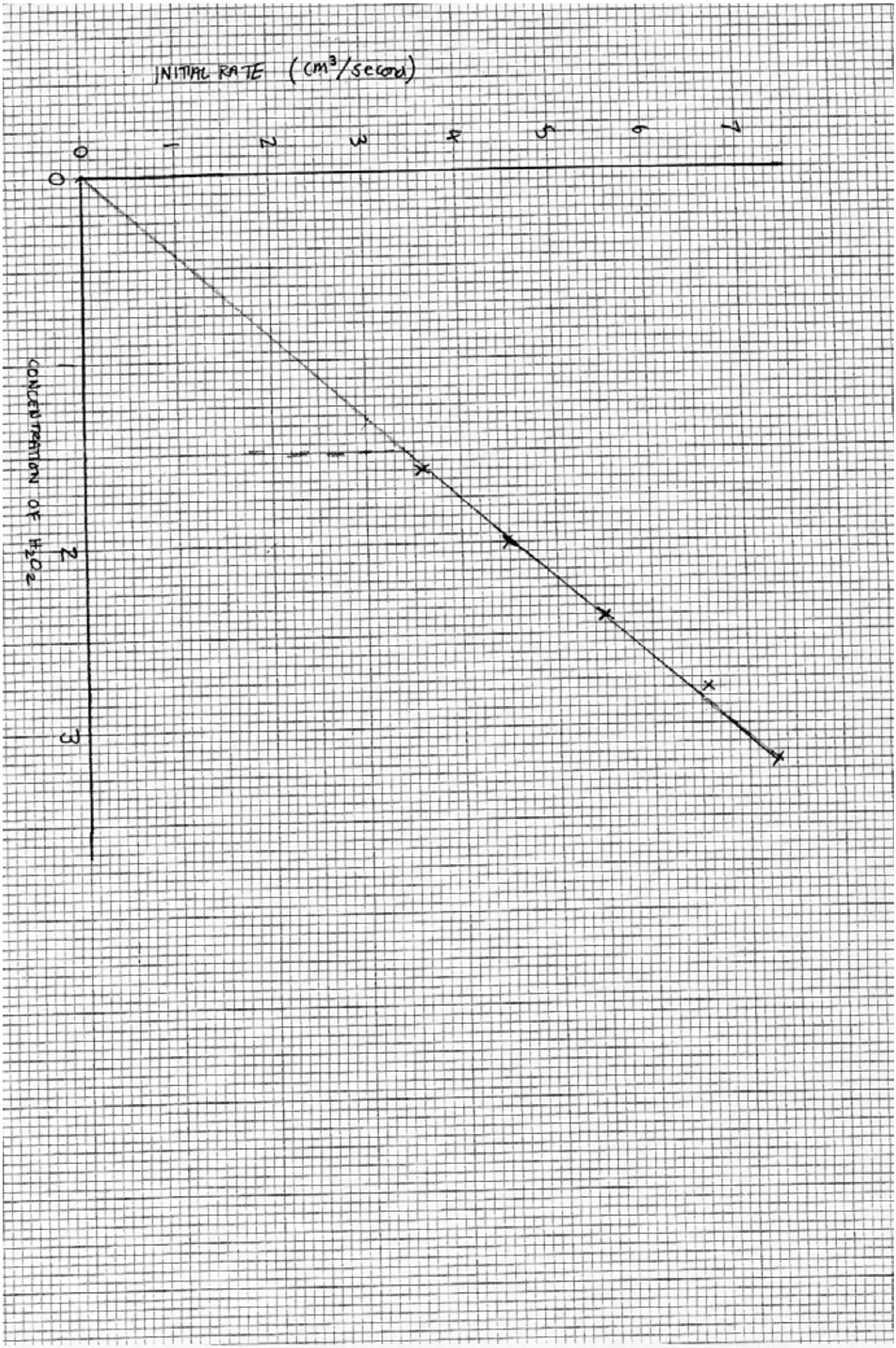
CONCENTRATION OF CATALASE

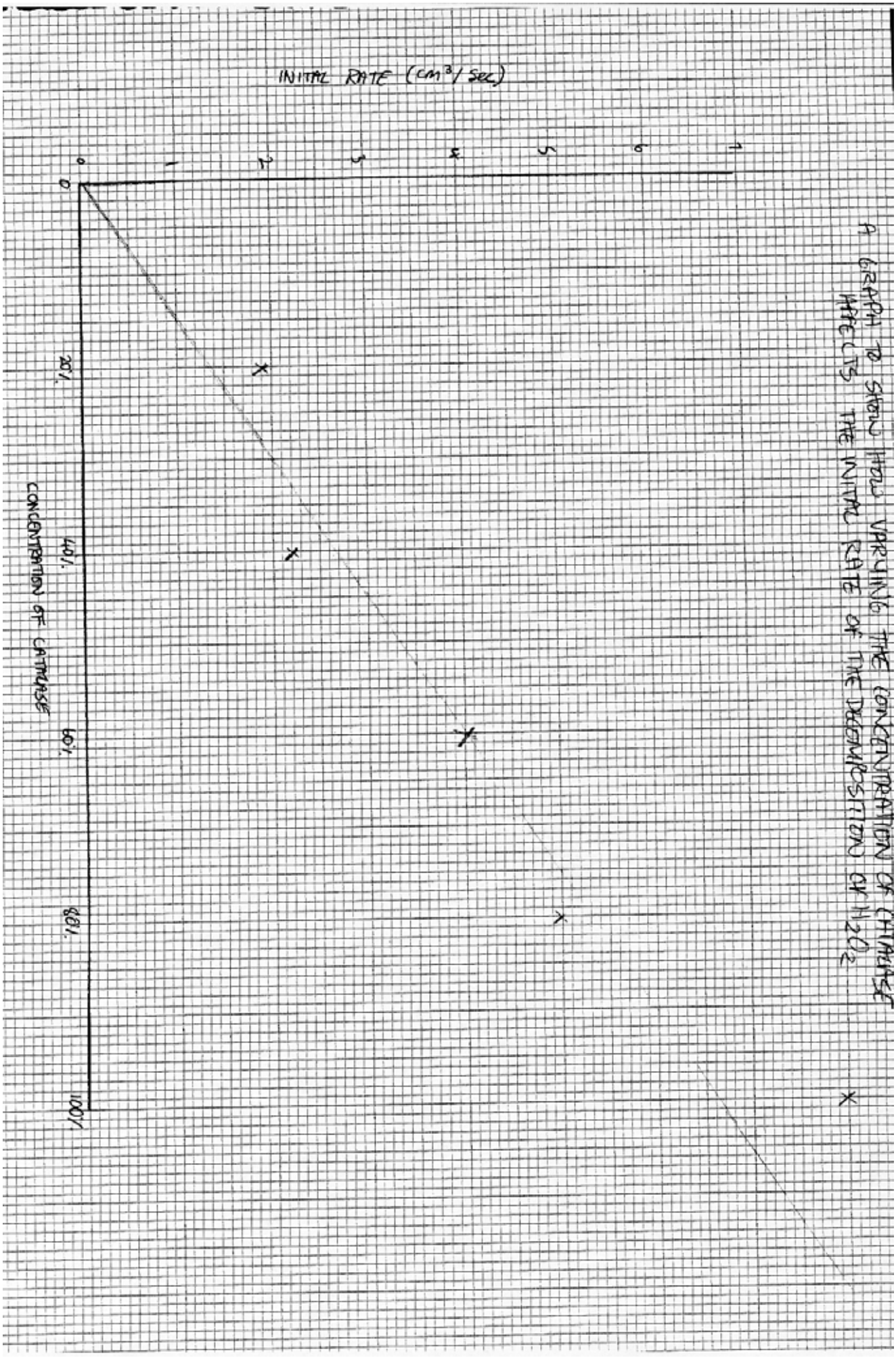
PROGESS OF REACTION (SECONDS)	100%			80%			60%			40%			20%		
	1ST RESULT	2ND RESULT	AVERAGE RESULT	1ST RESULT	2ND RESULT	AVERAGE RESULT	1ST RESULT	2ND RESULT	AVERAGE RESULT	1ST RESULT	2ND RESULT	AVERAGE RESULT	1ST RESULT	2ND RESULT	AVERAGE RESULT
30	74	74	74	58	62	60	46	47	46.5	40	36	38	31	29	30
60	77	77	77	63	67	65	53	55	54	46	41	43.5	36	35	35.5
90	79	79	79	69	71	70	58	59	58.5	49	47	48	41	40	40.5
120	80	80	80	73.5	74.5	74	62.5	63.5	63	53	51	52	44	43	43.5
150				75	76	75.5	65.5	66.5	66	56	54	55	47	46	46.5
180				76	77	76.5	69	69	69	59	57	58	50	49	49.5
210				77	78	77.5	70	72	71	61	59	60	53	51	52
240				78	79	78.5	73	74	73.5	64	62	63	55	54	54.5
270				80	80	80	76	78	77	66	64	65	57	57	57
300							78	79	78.5	68	66	67	59	59	59
330							80	80	80	70	68	69	61	61	61
360										72	70	71	64	63	63.5
390										73	71	72	66	65	65.5
420										74	72	73	69	67	68
450										75	74	74.5	70	68	69
480										77	76	76.5	71	71	71
510										78	78	78	72	72	72
540										79	79	79	74	73	73.5
570										80	80	80	75	74	74.5
600													76	76	76
630													77	77	77
660													78	78	78
690													79	79	79
720													80	80	80

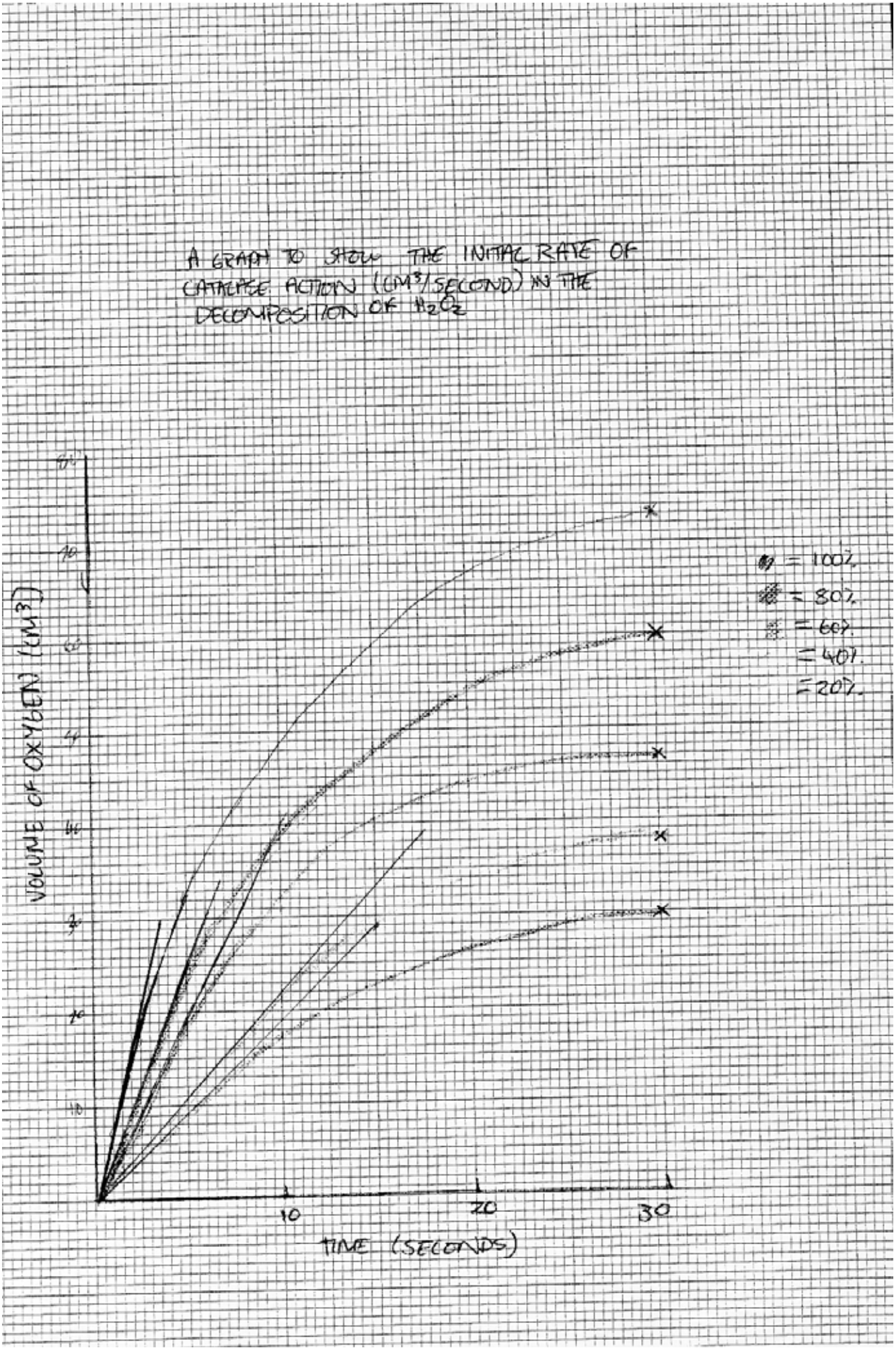

0
10
20
30
40
50
60
70
80
0
60
120
180
240
300
360
420
480
540
600
660
720
KEY
= 100%
= 80%
= 60%
= 40%
= 20%

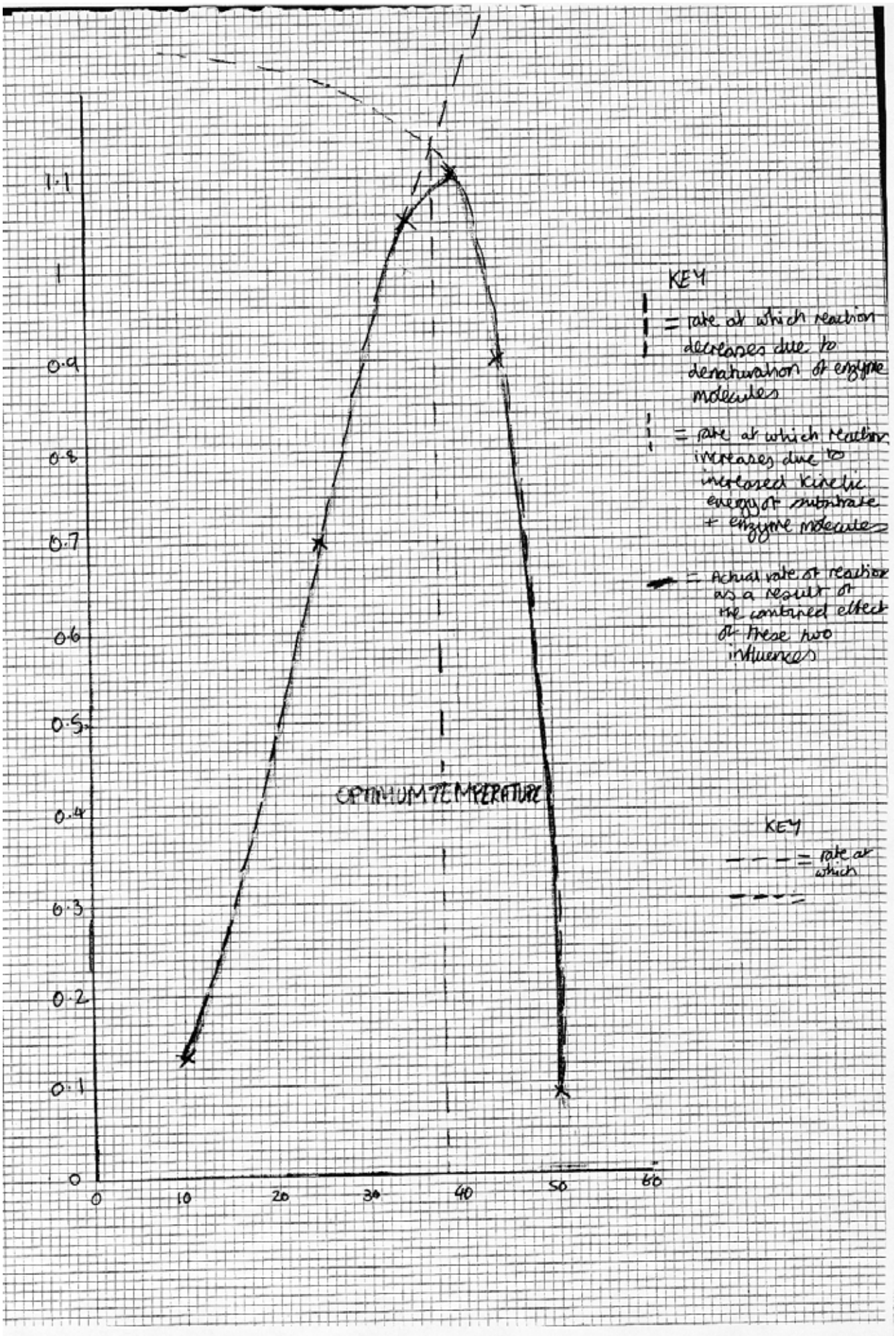



RATE OF REACTION (cm³/sec)
0
0·1
0·2
0·3
0·4
0·5
0·6
0·025
0·05
0·075
0·1
CONCENTRATION OF INHIBITOR (M)
= LEAD NITRATE
= IRON (III) NITRATE


INITIAL RATE (cm³/second)
0
1
2
3
4
5
6
7
0
1
2
3
CONCENTRATION OF H2O2


INITIAL RATE (cm³/sec)
0
1
2
3
4
5
6
7
0
20%
40%
60%
80%
100%
CONCENTRATION OF CATALASE
A GRAPH TO SHOW HOW VARYING THE CONCENTRATION OF CATALASE AFFECTS THE INITIAL RATE OF THE DECOMPOSITION OF H2O2




1·1
1
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
0
10
20
30
40
50
60
OPTIMUM TEMPERATURE
KEY
= rate at which reaction decreases due to denaturation of enzyme molecules
= rate at which reaction increases due to increased kinetic energy of substrate + enzyme molecules
= Actual rate of reaction as a result of the combined effect of these two influences
KEY
= rate at which

A GRAPH TO SHOW THE VOLUME OF OXYGEN PRODUCED (CM³) BY VARYING H_2O_2 CONCENTRATIONS IN 2 MINUTES

5.2 Commentary on Individual Investigation Report 0406

Enzyme catalysed decomposition of hydrogen peroxide

Introduction

This is an example of a very common type of individual investigation. Candidates who choose to explore enzyme kinetics need to take care that they focus on the chemistry of their reaction and do not spend too much of their time on biological aspects of the system. They also need to take care that the conclusions that they draw are not simply a superficial re-statement of sections of textbook. In this example the candidate has opted for the fairly safe option of spending most time investigating the effects on reaction rate of concentration of enzyme and substrate and of temperature where the outcome is well documented. An alternative approach to explore the effect of potential inhibitors in detail or to compare the relative effectiveness of enzyme and inorganic catalysts where the outcome is much less predictable might be more motivating to some candidates.

Overall, this is an effective investigation that is let down a bit by some of the detail. It is a good illustration of the need for quality data to meet the high level descriptors in implementing. It also illustrates the need to identify limitations in experimental procedures, as well as uncertainties associated with measurements, in the evaluation section.

Planning

The aim of the investigation is clearly stated and provides a useful framework for the rest of the report. The expected background theory about enzymes is included but the section on inhibitors is not relevant to the use of heavy metal ions in this project. Some of the expected theory about reaction kinetics is included in this section and additional material is included in the analysing section and should be given credit under planning. The theoretical treatment of temperature effect is basic. Enthalpy profiles are not included and the Arrhenius equation is only mentioned in the evaluation section. The risk assessment is adequate for the task involved. The list of references which have been consulted are linked to the text and include appropriate page number detail, although one lacks a clear title. This aspect of the plan satisfies coursework descriptor requirements up to level P8b but only partially satisfies the requirements at P11b.

The pilot experiments and subsequent calculations help the development of an appropriate strategy. The use of ice cube bags to ensure consistency of enzyme extract is ingenious. The plan includes precise detail of quantities used but the glassware used for measuring is not always clear. The choice of number and range of measurements is not well explained and the plan to study the effect of inhibitor is likely to lead to uncertain results. This aspect of the plan satisfies the coursework descriptors up to level P8a but only partially satisfies the requirements at P11a.

Overall, planning meets the requirements of the descriptors at level P8 but only partially meets the requirements at level P11. A mark of 9 or 10 is therefore appropriate, with 9 being most suitable in this case.

Implementing

Most data is recorded in an expected format, although the temperatures might have been recorded to one decimal place, even if this was a five or a zero. The table of results from experiments that explore the effect of catalase concentration on reaction rate, are poorly presented on graph paper.

The main problem in this section, however, concerns the quality of the data recorded. In some experiments, most of the reaction is over and most of the oxygen gas has already been produced before the first measurement is taken after 30 seconds. This could have been spotted and the opportunity taken to repeat the experiment with additional readings taken during the early stages of the reaction which would meet the needs of the descriptor at level I11b.

Overall, the recording strand of implementing only just meets the requirements at level I8. A maximum mark of 9 is available for this skill area if there is sufficient achievement in the manipulation strand of the skill area. Since all of the requirements at level 11 have not been met, a mark of 12 in this skill area is not possible.

Analysing

Graphs are poorly drawn using a thick pointed pencil. Some graphs lack headings, some lack axis labels and some axis labels lack units. Graphs used to show the effect of catalase concentration appear to have been drawn with just two points. There is no attempt to draw a graph from which the activation enthalpy might be calculated. This aspect of this section does not meet the descriptors at level A8a since most graphs are not of suitable quality and format.

While the conclusions drawn about the order of reaction are sound, those about the effect of temperature on reaction rate are superficial. The discussion about the effects of inhibitors on reaction rate illustrates a lack of understanding of chemical ideas about bonding and catalysis. This aspect of this section just satisfies the descriptors at level A8b but only partially meet the needs of the descriptors at level A11b.

Overall, the analysing section does not meet the requirements of descriptors at level A8 so a mark of 7 is just reached.

Evaluating

The limitations arising from fluctuating temperature between experiments are identified. The unexpected results arising from experiments designed to explore the effect of inhibitors on reaction rate are noted but the cause of the problem, which lies in the experimental method, is not seen. The actual experimental procedures receive little attention. This aspect of this section meets the requirements of the coursework descriptors at level E5a but the descriptors at level E8a are not met.

The uncertainty associated with specific measurements is evaluated which meets the requirements of descriptors at level E5b and E8b.

Overall, the evaluation meets the requirements of the descriptors at level E5 but only partially meets the needs of the descriptors at level 8. A mark of 6 is therefore appropriate