"How does the concentration of hydrochloric acid affect the rate of reaction for the proton-catalysed hydrolysis of amylose and what order is the reaction with respect to HCl when measured using colorimetry of the amylose-iodine complex over time?"

Introduction and Aim

I chose this topic to investigate human digestion, which relies on both mechanical and chemical breakdown. Starch in everyday food, for example, is broken down by salivary amylase in the mouth, but then mechanically churned and chemically cleaved in the stomach with hydrochloric acid. After learning about kinetics (topic 6) and digestion in biology, I wanted to focus on how increasing acid concentration affects reaction rate to mimic the change in stomach conditions when more protons are secreted upon food intake. To do this, I used starch-triiodide colorimetry to determine the order of hydrolysis with respect to acid concentration. Alternatively, titration with Benedict's quantitative reagent could be used as starch breaks down into reducing sugars, but this was avoided as the mixture would need to be heated which may cause starch degradation regardless of acid concentration.

Background Theories and Existing Studies

When exposed to acid, starch undergoes hydrolysis at a much faster rate than if left to microbial action alone. Wang, Truong & Wang demonstrated that at higher acid concentrations (1.0 mol dm^{-3}) there was more starch degradation than at lower acid concentrations (0.06 and 0.14mol dm^{-3})[1]. Using corn starch, HCl and Fehling's reagent, Bej, Basu & Ash [2] concluded this acid-catalysed hydrolysis of starch occurs via first-order reaction pathways - I will compare this to my own findings. Wang & Copeland[3] suggest acid-catalysed starch hydrolysis occurs in two phases: chains of glucose residues are broken off from the macromolecules, then there is hydrolysis of the shorter, newly formed polysaccharides.

Strictly, this method measures (helical) amylose concentration rather than amylopectin. Assuming the cleavage of alpha 1,4 & 1,6 glycosidic bonds are similar, general decreases in amylose concentrations indicate general decreases in amylopectin concentrations too, so this experiment does represent "starch" hydrolysis as a whole [4].

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[1] Wang, Y.,Truong, V., & Wang, L. (2003). Structures and rheological properties of corn starch as affected by acid hydrolysis. *Carbohydrate Polymers*, *52*(3), 327-333. doi: 10.1016/s0144-8617(02)00323-5

[2] Bej, B., Basu, R., & Ash, S. (2008). Kinetic studies on acid catalysed hydrolysis of starch. *JSIR*, *67*, 295-298.http://nopr.niscair.res.in/bitstream/123456789/789/1/JSIR%2067%284%29%20%282008%29%20295-298.pdf [Accessed 14/11/2020]

[3] Wang, S., & Copeland, L. (2015). Effect of Acid Hydrolysis on Starch Structure and Functionality: A Review. *Critical Reviews In Food Science And Nutrition*, *55*(8), 1081-1097. doi: 10.1080/10408398.2012.684551

[4] Goedecke, C. (2016). Why Does Iodine Turn Starch Blue? :: Education :: ChemistryViews https://www.chemistryviews.org/details/education/10128441/Why_Does_Iodine_Turn_Starch_Blue.html [Accessed 14/11/2020]

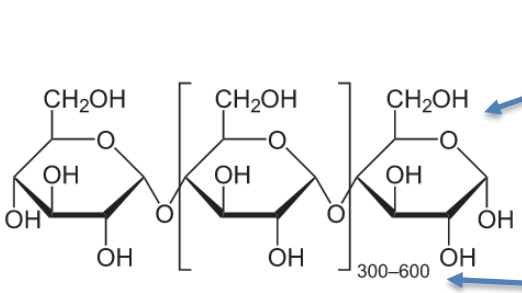

Figure 1 - Amylose Structure [5]

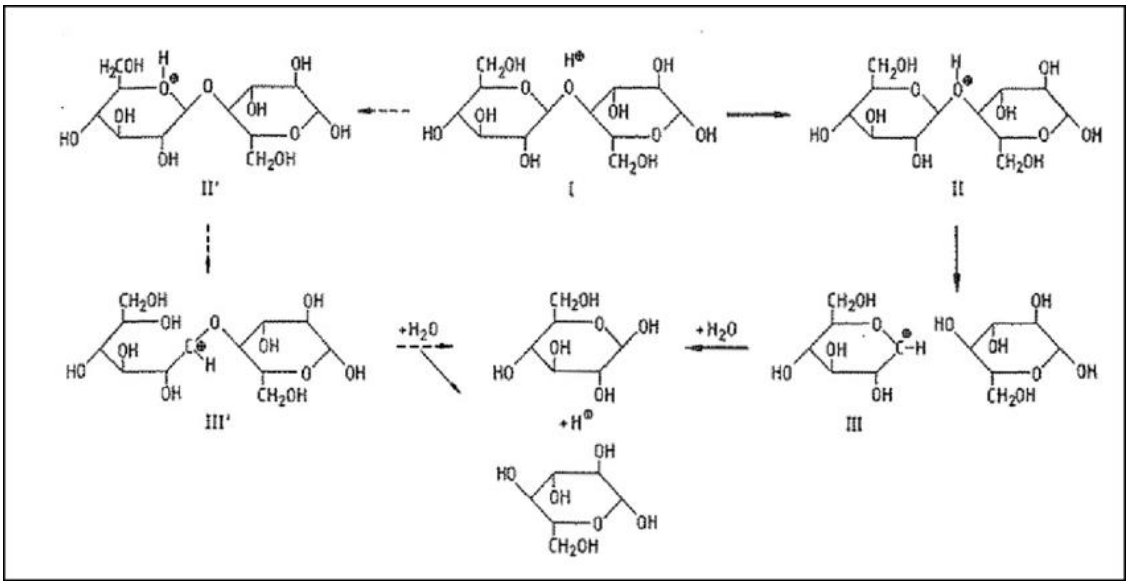

Figure 2 - Glycosidic Bond Cleavage in Amylose. [6]

Fengel and Wegener suggest glycosidic bond cleavage occurs as outlined in Figure 2. The first step is introducing a proton – HCl (aq) is a strong acid so it dissociates completely into protons and chloride ions. The next step involves either the glycosidic (ether) or ring-bound oxygen forming a bond with hydrogen using its lone pairs. Assuming the former occurs, the result is the adjacent rings breaking apart, leaving behind a partially positive carbon susceptible to nucleophilic attack. When water is the nucleophile, a proton is catalytically regenerated as an O-H bond breaks. Acid hydrolysis is usually S_N1 after the proton bonds to the oxygen atom (formation of a carbocation), and as such, the rate determining step is unimolecular and the nucleophile (water) concentration is not expected to affect the rate significantly.

Collision theory states particles only react when they collide with kinetic energies ≥ activation energy - using a catalyst provides an alternative reaction pathway with a lower activation energy and so more bonds will be cleaved per unit time. My hypothesis is reaction rate increases as proton concentration increases.

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[5] NEUROtiker. (2007). *Structure of amylose* [Image]. [Accessed 14/11/2020] https://en.wikipedia.org/wiki/Amylose#/media/File:Amylose2.svg

[6] Fengel, & Wegener. Adapted Fig. 10. "Mechanism of acid hydrolysis of glycosidic bonds in starch." [Retrieved 25 August 2020], from https://www.researchgate.net/figure/Mechanism-of-acid-hydrolysis-of-glycosidic-bonds-in-starch-cellulose-and-hemicellulose_fig8_306059981

According to the Beer-Lambert law, when path length and molar absorptivity coefficient are kept constant, absorbance is directly proportional to the concentration of the 'species' observed, using a complementary-colored filter. Here, the observed species was the amylose-iodine complex. It is suggested triiodide ions insert themselves into the amylose helix to form infinite polyiodide chains[7]. Starch solution changes from colorless to blue-violet when iodine (in KI) solution is introduced – there is charge transfer between the amylose (charge donor) and polyiodide (acceptor), causing an absorbance of different wavelengths compared to amylose/iodide alone[8].

Preliminary Experiment

I added $2cm^3$ of 1% starch and $1cm^3$ of 4 mol dm^{-3} HCl (aq) to 6 test tubes each. I then transferred the test tubes to a 60°C water bath and took a test tube out of the bath every 120s to add excess $NaHCO_3$ (1g) to stop further reaction. I diluted each test tube with $10cm^3$ of water, added a few drops of 0.005 mol dm^{-3} iodine (in KI) and then sampled each tube for colorimetry. I repeated the experiment with $2cm^3$ of acid. The results showed no clear trend and a large scatter between reaction time & absorbance for both acid volumes. Many absorbance readings were so low they weren't detected by the colorimeter. I improved the methodology:

1) Add varying volumes of HCl and water, always creating $50cm^3$ solution to directly control acid *concentration* (rather than volume).
2) Instead of using multiple test tubes, make a larger solution ($50cm^3$) so that initial concentrations of starch, water and acid were the same. This also solves the problem of using very small quantities of each which would create unnecessarily high uncertainties.
3) Control the volume of iodine solution more carefully by using a more dilute solution and a volumetric pipette, rather than "a few drops", to ensure moles added remained constant.
4) Let water, starch and HCl heat up in the water bath individually first so the temperature/rate constant would not change when the reaction started due to the external environment.
5) Alter the range of the independent variable (HCl concentration), such that it does not exceed the ratio of 50:50 (by volume) 1% starch: 4 mol dm^{-3} HCl to ensure colorimetric detection.
6) Another range to be considered in this experiment is at what times I decided to sample the solution. 120s here showed sometimes vast changes and sampling e.g. every 40s showed very little change so I chose to sample every 80s in the final experiment.

The sample mixture also cooled significantly during the reaction, so I needed to observe the temperature more regularly. I decided on maintaining it at 60°C as this was relatively easy to control by adding boiling water to the water bath when needed with a kettle & ensured fast reaction.

[7] Madhu, S et al. (2016). Infinite Polyiodide Chains in the Pyrroloperylene-Iodine Complex: Insights into the Starch-Iodine and Perylene-Iodine Complexes. *Angewandte Chemie International Edition*, *55*(28), 8032-8035. doi: 10.1002/anie.201601585

[8] Goedecke. Why Does Iodine Turn Starch Blue? https://www.chemistryviews.org/details/education/10128441/Why_Does_Iodine_Turn_Starch_Blue.html

Apparatus, Relevant Uncertainties and Chemicals needed for the Final Experiment

Apparatus to measure volumes	Other measuring apparatus	Other equipment for setup
5 ± 0.05cm³ & 10 ± 0.1cm³ Glass Pipettes 100cm³ Measuring Cylinder ± 0.5cm³	Colorimeter with red filter ± 0.001 Au (Absorbance Units) Stopwatch ± 0.01s Thermometer ± 0.5°C Mass scales ± 0.01g	Pipette fillers, Weighing boats, Spatulas, Kettle, 11 Test Tubes, Cuvette, Plastic Pipettes, Small conical flask, Large beaker The kettle is used to boil water and the large beaker for the setup of the water bath.

Figure 3 (Left): Apparatus

Standard Chemicals (assuming no uncertainty in concentration): 4.00 mol dm^{-3} HCl, 0.005 mol dm^{-3} Iodine solution (in KI), 1% Starch Solution (by mass), Water & $NaHCO_3$ powder.

NB: HCl is (aq) or hydrochloric acid during this experiment so the (aq) symbol may be omitted.

Method for Final Experiment

1) Dilute the iodine solution by adding 0.5cm³ (with 5cm³ pipette) to 100cm³ (using 100cm³ measuring cylinder) of water to create **$2.5 \times 10^{-5} \pm 3 \times 10^{-8}$ mol dm^{-3}** Iodine stock solution (see uncertainty propagation below).
2) Using boiling and cold water, create a 60°C ± 0.5 water bath in a large beaker.
3) Using figure 4, transfer the appropriate volume of starch solution and water to two different test tubes using 10cm³ pipettes. Add the 4 mol dm^{-3} HCl to another test tube using a 5cm³ pipette. Immerse test tubes into the water bath until they all reach ~60°C.

Figure 4 - Volume of Different Chemicals Needed for Each Independent Variable

Overall HCl Concentration (Independent Variable)/ mol dm^{-3}	**Starch (1%) /cm³ ± 0.1cm³**	**HCl (4mol dm^{-3}) /cm³**	**Water / cm³**
0.200 ± 0.006	10.0	2.50 ± 0.05	37.5 ± 0.4
0.400 ± 0.004	10.0	5.00 ± 0.05	35.0 ± 0.4
0.800 ± 0.009	10.0	10.0 ± 0.10	30.0 ± 0.3

4) Add 0.20g ± 0.01 of $NaHCO_3$ to 8 other test tubes (using mass scales & weighing boats).
5) Mix the starch, HCl and water test tubes together into a conical flask and let it sit in the 60°C ± 0.5 water bath - maintaining temperature by adding water to the beaker (outside the flask) from the kettle if it decreases below this point, monitoring using a thermometer.
6) Immediately after addition of the reactants, start the stopwatch and swirl for 5 seconds.
7) Every 80s, use a 5cm³ pipette to extract 2cm³ of solution from the conical flask and add the sample to one of the 8 test tubes with $NaHCO_3$.
8) Once 560s have elapsed, add 5.25cm³ of iodine (in KI) solution to each test tube.
9) Use a plastic pipette to sample into cuvettes and take colorimetry readings with a red filter.
10) Neutralise and dispose of the excess solution (see risk assessment) in the conical flask.

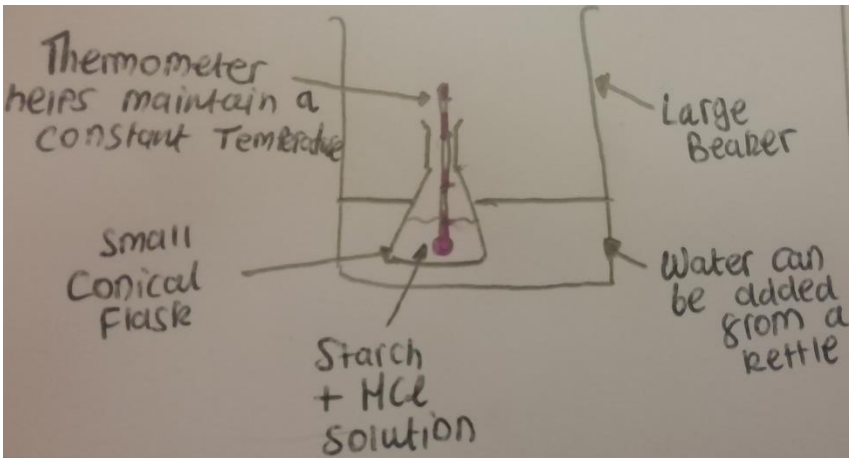


Figure 5 (Left): Experimental Setup (Water Bath with Conical Flask)

Justification and Propagating of Measurement Uncertainties

1) For a control variable (Iodine concentration after diluting to create a stock solution):
   Assuming there is no uncertainty in the original standard (Iodine) solutions:
   Adding 0.50cm^3 ± 0.05 of 0.005 mol dm^{-3} Iodine solution to 100cm^3 ± 0.5 of water:
   Uncertainty in Iodine moles = ± ((0.05/0.5) x 100%) = ± 10%
   Uncertainty in volume of new solution = ± ((0.55 / 100.5) x 100%) = ± 0. 547263... %
   [0.55cm^3 due to measuring cylinder (± 0.5cm^3) and pipette uncertainties (± 0.05cm^3)]
   Total uncertainty in concentration = ± (10 + 0. 547263...) % = 10.547263…%
   Final Iodine Concentration = 2.4875… x 10^{-3} ± (0.10547263… x 2.4875… x 10^{-3})
   Concentration to 2sf = 2.5 x 10^{-3} **mol dm^{-3} ± 3 x 10^{-4}**

2) Independent Variable (HCl Concentration): Volume uncertainties are different because of how many times the associated pipette was used. When 5cm^3 of hydrochloric acid was added, the pipette was used once, with 10cm^3 the pipette was used twice (figure 4).

   Example: 10.0 ± 0.1cm^3 1% starch, 37.5 ± 0.4cm^3 water, 2.5 ± 0.05cm^3 4 mol dm^{-3} HCl.
   Percentage uncertainty in moles of acid added: (0.05 / 2.5) x 100% = 2%.
   Percentage uncertainty in total volume of mixture: (0.55/50) x 100% = 1.1%.
   Therefore, HCl Concentration = 0.200 ± 0.006 mol dm^{-3}

3) Dependent Variable (Rate of reaction): Although the ultimate goal is to measure change in concentration (rate) using absorbance, which has an uncertainty of ± 0.001 Absorbance Units (Au), in order to gauge the rate of reaction, a sample of the solution must be neutralised every 80s and then sampled for colorimetry. Considering uncertainty, though the stopwatch and reaction time play a role, the largest source of uncertainty is the time taken to transfer the solution in the conical flask to the test tube with $NaHCO_3$ to stop the reaction at a certain time. I estimate this took up to 5 seconds, **so the uncertainty is given as ± 5s.** This is not strictly an uncertainty associated with the apparatus itself and is a systematic error (as explained in the *Evaluation*) but is treated as uncertainty for the purpose of rate calculations.

Variables in the Final Experiment

Figure 6 – Control Variables

	Value & Units	Why must it be controlled?	How was it controlled?
Temperature reaction takes place at	60.0°C ± 0.5	Temperature affects the rate of reaction - a higher temperature results in more collisions and a greater proportion of them would be successful as more particles have kinetic energy ≥ activation energy.	All reactants are individually heated in a beaker to 60°C before reacting. If temperature decreases (before or during reaction), enough hot water from a kettle is added at needed time to maintain 60°C. (Monitored with thermometer)
Mols (volume) of 1% starch solution added	10.00cm^3 ± 0.05	Adding more or less to different experiments would be unfair as it could change the absorbance readings regardless of the acid concentration. Moreover, total mixture volume needs to stay constant to control acid concentration - the total volume of the solution is 50cm^3.	Use a 10cm^3 graduated, glass pipette to transfer this amount to the conical flask before adding the acid.
Volume of 2.5 x 10^{-3} mol dm^{-3} ± 3 x 10^{-4} Iodine (in KI) solution added post-reaction	5.25cm^3 ± 0.05	Even if a solution with the same concentration of amylose is used, if the total moles of iodine solution added is different the intensity of the blue colour will be vastly different. Also, this must stay within the range detectable by the colorimeter.	*A very dilute (2.5 x 10^{-3} mol) stock solution is made beforehand (outlined above).* Use a 10cm^3 graduated, glass pipette to transfer this amount to each sample after 80s and neutralisation.
Moles of $NaHCO_3$ added to each sample after 80s increments	0.20g ± 0.01	All the acid must be neutralised as soon as possible after the specified time is reached to ensure no further acid-catalysed starch hydrolysis occurs.	$NaHCO_3$ (s) + HCl (aq) → NaCl (aq) + CO_2 (g) + H_2O (l) *Equation 1* Even at the highest acid concentration (0.8 mol dm^{-3}), only 0.13g of $NaHCO_3$ is needed (1.6 x 10^{-3} moles) to neutralise the solution that is extracted from the flask, so 0.2g is well in excess.
Colorimetry Settings	Red Filter	Wavelength of light (filter) used affects the absorbance reading.	Calibrated (with water) to reduce systematic error.

Figure 7 – Independent Variable

	Final HCl concentrations in mixture (mol dm^{-3})	*How was it achieved?*
Hydrochloric Acid Concentration	0.200 ± 0.006, 0.400 ± 0.004, 0.800 ± 0.009	Using 4 mol dm^{-3} stock HCl (aq), I added 2.50, 5.00 and 10.0 cm^3 of it to form a 50cm^3 mixture of starch, water and acid.

Figure 8 – Dependent Variable

	How was it measured?
Rate of Reaction measured by Change in Absorbance of Red Light (to determine concentration of helical amylose-iodine complex) Au ± 0.001 (Au s^{-1} for rate)	Every 80s neutralise the extracted solution with $NaHCO_3$ to fix the concentration of the complex (stopping further reaction) and add the iodine (in KI) solution. Then, using a red filter (complementary wavelength to blue-violet solution) take a sample. Use cuvette to take absorbance readings.

Risk Assessment, Environmental and Ethical Considerations:

In the format *{Chemical Name [Maximum Concentration used]; Hazards; Control Measures}:*

- **Hydrochloric Acid [4 mol dm^{-3}]**; Irritant to eyes, skin and respiratory system if fumes are breathed in [9]; Goggles worn, care is taken to avoid breathing in fumes, hands washed thoroughly after experiment.
- **Iodine in Potassium Iodide Solution [0.005 mol dm^{-3}]**; Respiratory hazards are minimal at such low concentrations potential to stain skin[10]; Lab coat worn, and solution used sparingly.
- **Sodium Hydrogen Carbonate, $NaHCO_3$ [pure powder];** Powder may cause irritation of respiratory system, When reacting with HCl, the liberated CO2 may cause splattering of the acidic solution and some heat will be generated as it's exothermic; Goggles worn, test tubes secured in a rack away from experimenter when reacted with acid.
- **1% starch solution and hydrolyzate products** (polysaccharides) are very low risk.

Other risks include using boiling water to maintain the water bath temperature - the beaker is only partially filled initially to allow more volume to be added without spilling and direct contact with hot water is avoided. Glassware can also cause injury, so care is taken adding fillers to pipettes to prevent slipping and are screwed in tight to prevent solutions leaking.

Environmentally, neutralising unreacted acid with $NaHCO_3$ creates a solution which can be safely disposed of in a chemical sink alongside the other chemicals. Iodine solution can be harmful to aquatic organisms but as it is so dilute here it is also safe to dispose of in this way[11]. As I was not exhausting the school's resources supply, there are no major ethical implications.

[9] CLEAPSS. (2019). *Student Safety Sheets - Hydrochloric Acid* [Ebook]. CLEAPSS. Retrieved from http://science.cleapss.org.uk/Resource/SSS020-Hydrochloric-acid.pdf [Accessed 14/11/2020]
[10] CLEAPSS, Retrieved from http://science.cleapss.org.uk/Resource/SSS056-Iodine.pdf (pp1-2).
[11] CLEAPSS, Retrieved from http://science.cleapss.org.uk/Resource/SSS056-Iodine.pdf (pp1-2).

Qualitative Results and Observations

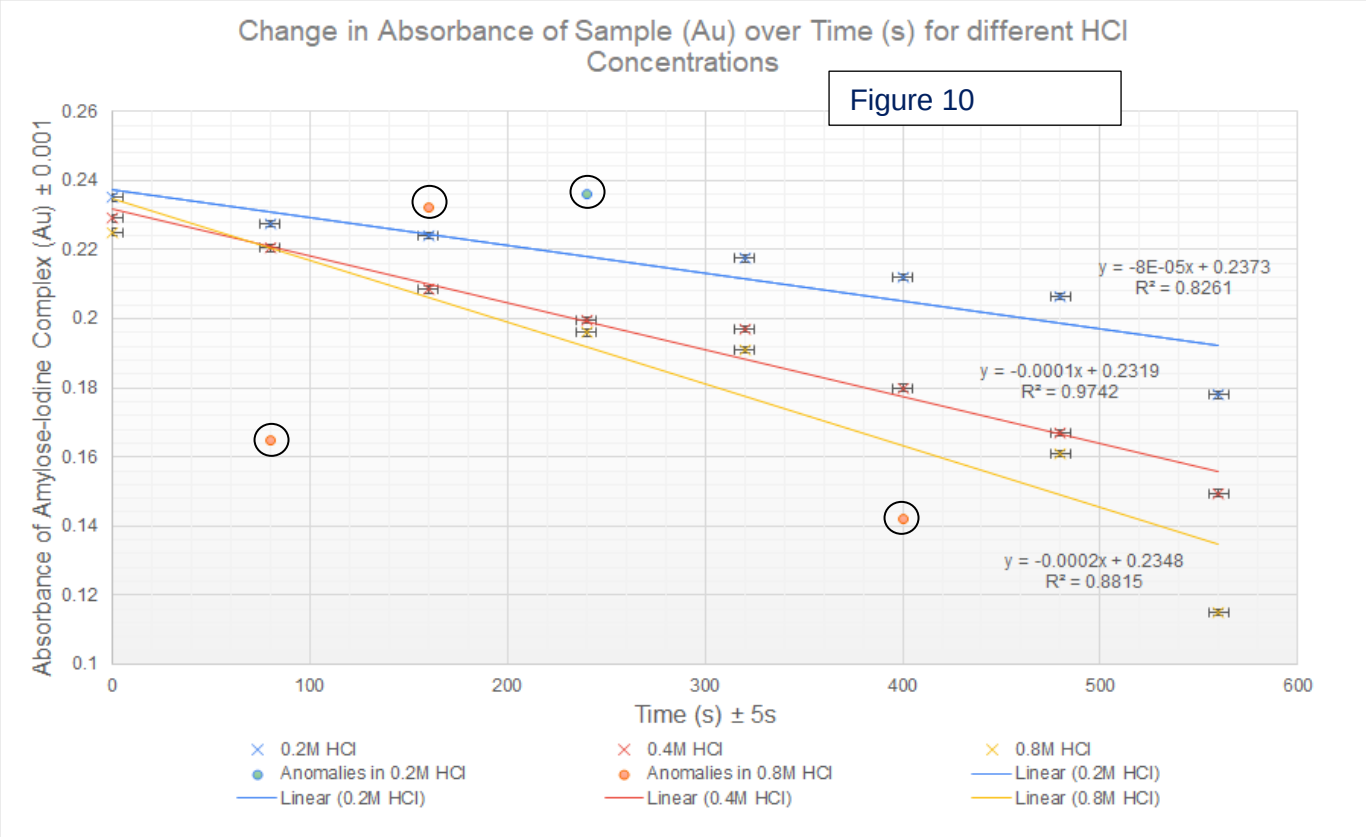
In most repeats I could visibly see the intensity of the violet complex faded as the reaction continued over time and the change was quicker for the highest acid concentration. Within the experiment, I noticed rapid effervescing of the solution when added to $NaHCO_3$ due to the liberation of CO_2 from neutralization before excess base settled to the bottom of the test. The mixture in the flask itself remained colourless.

Quantitative Results

Figure 9 – Table of Raw Data for the Final Experiment (Anomalies Highlighted with Asterisks)

Absorbance (Au)	0.2 *mol dm^{-3}* HCl/ Au (±0.001)			0.4 *mol dm^{-3}* HCl/ Au (±0.001)			0.8 *mol dm^{-3}* HCl/ Au (±0.001)		
Time/s ± 5	Repeat 1	Repeat 2	Mean	Repeat 1	Repeat 2	Mean	Repeat 1	Repeat 2	Mean
0	0.217	0.253	0.235	0.215	0.243	0.229	0.240	0.210	0.225
80	0.209	0.246	0.228	0.209	0.232	0.221	0.208*	0.165	0.165*
160	0.206	0.242	0.224	0.189	0.228	0.201	0.232	0.155*	0.232*
240	0.212*	0.236	0.236*	0.176	0.223	0.200	0.229	0.163	0.196
320	0.203	0.232	0.218	0.175	0.219	0.197	0.221	0.161	0.191
400	0.201	0.223	0.212	0.167	0.193	0.180	0.225*	0.142	0.142*
480	0.198	0.215	0.207	0.167	0.201*	0.167	0.186	0.136	0.161
560	0.178	0.216*	0.178	0.117	0.182	0.151	0.171	0.058	0.115

The major limitation of this experiment is that only two repeats were taken and sometimes values of two repeats (for the same time and concentration) varied so greatly that this decreases the reliability of the results for some readings significantly and even the means are anomalies from the expected trend - anomalies are circled on figure 10. As the repeats had similar trends, though, it adds to the reliability and increases the validity of any conclusions drawn. If amylose, the substrate, begins to run out I expected a gradual curve as successful collisions would become less frequent as there are less reactant particles per unit volume. However, figure 10 follows a linear model more closely, hence any rate calculations will also deal with the overall gradient of the trend line, rather than taking a tangent to find initial rate of reaction. The graph shows absorbance change over time at different acid concentrations with error bars representing the measurement uncertainties in absorbance and an uncertainty associated with the time/reaction length. In most cases the vertical error bars are too small to be seen:



Conclusion(s): Figure 10 shows that as acid concentration increases so does the rate of reaction, indicated by steeper gradients. According to collision theory, this makes sense when amylose is in excess, as the greater the concentration of protons (a catalyst) the more frequently collisions occur per unit volume (hence more successful collisions) and more amylose is cleaved per unit time. The one exception to this is that cleavage of the larger amylopectin molecules takes place first to form amylose, so higher absorbance values are temporarily expected (formation of starch-triiodide complex), but this was not observed in this experiment because the increment between 0s and 80s was too great to make this noticeable.

High R^2 values show the trendlines fit the data well and there is a negative linear correlation between the absorbance of the sample (due to amylose-triiodide complex) and the duration of acid hydrolysis at 60°C ± 0.5 for all concentrations. However, 3 of 8 data points (for 0.8 mol dm^{-3} HCl) were anomalous and this decreases the reliability of my results. Bej, Basu & Ash [12] concluded the acid hydrolysis of starch likely occurs with parallel first order reaction pathways (and order 0 with respect to starch at any given temperature) so if my data is to match this I expect the gradients to have doubled as I doubled the HCl concentration.

[12] Bej, Basu & Ash. Kinetic studies on acid catalysed hydrolysis of starch. http://nopr.niscair.res.in/bitstream/123456789/789/1/JSIR%2067%284%29%20%282008%29%20295-298.pdf

To investigate whether my data fits this observation I drew lines of minimum and maximum gradients to ascertain the slope gradient (and, thus, the rate) and its associated uncertainty for each acid concentration. Typically, such lines go through each data point's respective error bar. However, I am less confident in my data's accuracy, so the lines sometimes miss the data point, giving a larger estimate of the uncertainty, which is appropriate. I have illustrated the construction of such lines for 0.2 mol dm^{-3} HCl below, though identical processes were used for the others and the results of this data processing are collectively represented in figure 12.

Figure 11 - Example Minimum-Maximum Uncertainty Processing for Absorbance over Time with Different HCl Concentrations

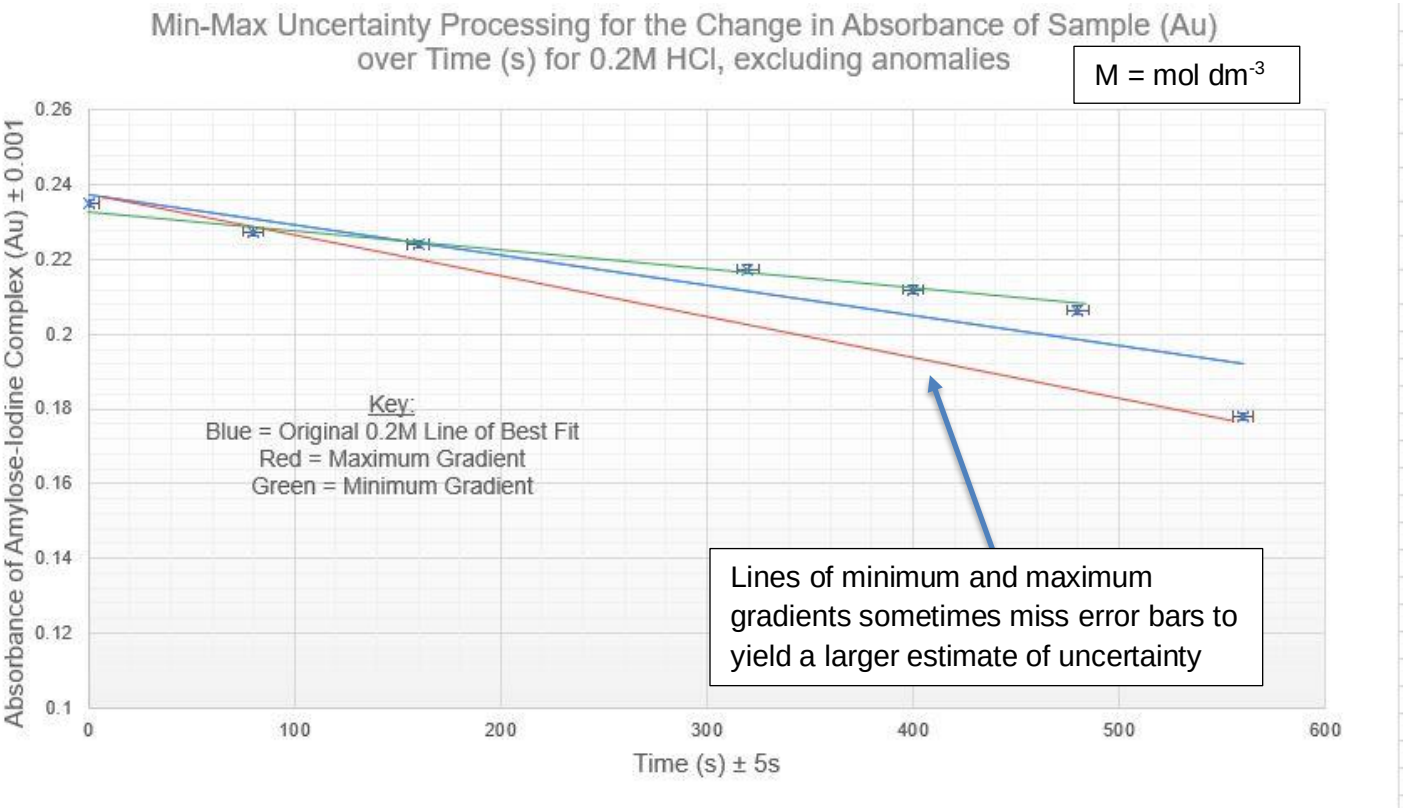


Figure 12 – Calculating Average Gradient/Rate and Uncertainties for Each HCl Concentration

Hydrochloric acid Concentration/ mol dm^{-3}	Maximum Gradient (x 10^{-5}) (3sf in order to calculate a more precise average gradient)	Minimum Gradient (x 10^{-5}) (3sf)	Average Gradient (Maximum + Minimum Gradient/2) (x 10^{-5}) *To nearest integer to match with uncertainty*	Slope Uncertainty (Maximum - Minimum Gradient/2) (x 10^{-5})
0.200 ± 0.006	-10.8	-3.61	-7.19 ≈ -7	-3.5801 = -4 (1sf)
0.400 ± 0.004	-14.6	-8.67	-11.7 ≈ -12 [1.7 times -7]	-3 (1sf)
0.800 ± 0.009	-21.3	-8.00	-14.7 ≈ -15 [2.1 times -7]	-6.6...= -7 (1sf)

Comparing the size of the trend to the magnitude of errors and uncertainties - large uncertainties in the slope gradient (rate) indicate significant sources of uncertainty. They are so large that it is difficult to work out order wrt HCl. However, if I factor in the plausible range from uncertainties, it is theoretically possible the true rate of reaction (gradient) may have doubled from –7 to -14 when acid concentration was doubled from 0.2 to 0.4 mol dm^{-3} or that it may have almost doubled from -12 to -22 when acid concentration was doubled from 0.4 to 0.8 mol dm^{-3}. Either observation alone is compatible with the literature suggestions of a first-order reaction (though percentage differences can't be calculated as those experiments didn't use the same methodology). Coming back to the original aim, I have confirmed increasing HCl concentration (by releasing more protons) would indeed increase the rate of starch breakdown.

Evaluation and Future Possibilities:

There were some strengths to the experiment. Firstly, the increments in time and acid concentration were great enough so the error bars didn't overlap, and significant results were produced. High R^2 values are signs of a good correlation once the anomalies are removed from consideration. Secondly, I was able to maintain the control variables quite well. Making standard solutions for Iodine and 1% starch solution ensured they were effectively controlled. I also regulated temperature by adding hot water if it decreased below 60°C. In the final experiment, it never dipped below ~57°C so the rate constant, k, did not change throughout the experiment. This could be improved even further if the beaker was insulated more and draft excluders were introduced.

Accuracy and precision could be improved in many areas to draw more definitive conclusions. There were human and random errors, such as adding too much iodine solution accidentally to some of the test tubes (which probably caused the anomalies), but there were also systematic errors that couldn't have been avoided with the experimental methodology as it stood:

Figure 13 – Errors and Uncertainties

	Manifestation in this Experiment	**Improvements for Future**
Systematic	The **largest** uncertainty (more so than random) was systematic and was the time of reaction - the time taken to transfer the solution could have been up to 5s by estimation. The results always deviated in one direction as the "time" (length of reaction) graphed was always **earlier** than reality. This measurement uncertainty is **significant** as when calculating rate, I didn't truly know the difference in time between readings. However, a strength was that increments between readings were sufficiently large such that each absorbance reading was different from the previous sampling time.	Create an experimental setup such that the reactant solution could be transferred more quickly to the $NaHCO_3$: conduct the experiment in a water bath (at a constant temperature) with a valve and every 80s turn the valve to release solution into a pre-prepared test tube of $NaHCO_3$. The advantage would be reducing the time taken to transfer the solution after the reaction was supposed to be complete, but it would be harder to add the same volumes to the test tube at each time interval. This method or using Benedict's quantitative reagent (see Introduction) could be used as comparison against my method or provide further evidence for my conclusions.
Random	The same cuvette was not used, possibly altering absorbance values despite no change in the concentration of the amylose-triiodide complex.	Use the same cuvettes to keep optical path length constant.

The major factors affecting how strongly my conclusion could be asserted were:

1) The range of the independent variable - much more than 3 acid concentrations are needed to sufficiently draw a conclusion of what order this reaction is with respect to hydrochloric acid. In the future, this experiment could be expanded to accommodate the ranges of 1.6 and 2.4 mol dm^{-3} HCl and so on (keeping starch solution in excess) to do this.

2) The small number of repeats (two) for each acid concentration was an even greater problem than usual because for each repeat the absorbance (even for the same acid concentration) varied considerably (i.e. they were not concordant) so if there was an anomaly in one of the repeats and the value was used for the other data point in isolation instead it could yield an unexpectedly high or low result when graphing the averages of each time of reaction reading. Adding more repeats would increase the reliability, as well as reduce the role of random errors as discussed above.

In the future, the breakdown of other polysaccharides by acid could be explored, such as glycogen by hydrochloric acid to indicate other possible orders of reaction. Otherwise, it could be worth comparing how effective acid hydrolysis of starch is alone compared to with the combined or isolated effects of amylases or other compounds present in human digestion.