

Candidate 2 evidence

Advanced Higher Biology Investigation

The effect of alcohol concentration on the activity of alpha amylase

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Abstract

Aim:

To investigate the effect of varying alcohol concentrations on the activity of alpha amylase.

Results:

As the concentration of ethanol increased starch hydrolysis continued at a similar rate throughout the varying concentrations and in the absence of ethanol. Therefore, ethanol was not inhibiting the activity of the enzyme alpha amylase.

1. Introduction

1.1 Aim:

To investigate the effect of varying alcohol concentrations on the activity of alpha amylase.

1.2 Hypothesis:

As the concentration of ethanol was increased, gradually the activity of alpha amylase would decrease due to ethanol inhibiting the action of amylase preventing starch hydrolysis.

1.3 Underlying Biology:

1.31 Amylase and starch

Amylase is an enzyme found predominantly in the saliva within the human body. It's a digestive enzyme that plays the role of breaking down carbohydrates such as starch, an insoluble compound, into maltose, a smaller soluble carbohydrate. Starch is a very complex carbohydrate made up of glucose monomers all joined by glycosidic bonds. Starch is produced in plants and acts as an energy store. For food to be useful to us it needs to be broken down to allow it to be absorbed into the bloodstream, its nutrients are then able to be transported around the body.

The process of breakdown proceeds by a hydrolysis reaction; this is the splitting of molecules by the addition of water. Maltose is composed of two glucose molecules, a disaccharide. Amylase begins the process of digestion in the mouth, breaking down large organic compounds into smaller micromolecules. There are two main types of amylase, alpha and beta. Alpha amylase is predominantly found in mammals, widespread in the human digestive system. Beta amylase is found mainly in plants, yeasts and bacteria but still carrying out a similar function. So for this investigation alpha amylase was chosen as it was looking at how alcohol would affect amylase in the human body.

Amylase is controlled by several inhibitors these controlling the conversion of starch into glucose. The six inhibitors associated with amylase are categorised by their tertiary structure. Competitive Inhibitors will bind to amylases active site and non-competitive inhibitors will bind to areas on the enzyme away from the active site but this then causes a change to the shape of the active site preventing the substrate from binding.



Figure 1.0 - Crystal structure of alpha amylase

1.32 Enzymes and Proteins

Enzymes are proteins that are used to catalyse reactions. Amino acids are the monomer molecules that join together by an elimination reaction known as a condensation reaction within a protein to form a long chain polymer, this being called a polypeptide. Each polypeptide is made up of many amino acids all synthesised in a specific order this determining the primary structure of the protein and inevitably its final structure. Hydrogen bonding exists between the different peptide bonds on the polypeptide backbone, the hydrogen bonds create regions of secondary structure within the backbone. Secondary structure comes in several forms; it can exist as alpha helices, parallel/antiparallel beta sheets or turns (Lloyd and Morgan, 2016). The R groups are left available to be involved in determination of tertiary structure or even in bonding of ligands. An alcohol is an organic compound with at least one hydroxyl (OH) group attached to a carbon. The alcohol found in alcoholic drinks is called ethanol (C₂H₅OH). Alcohols have a hydrogen attached to an oxygen so they can form hydrogen bonds and when alcohol hydrogen bonds with a protein the new hydrogen bonds disrupt hydrogen bonds in the secondary and tertiary structure. This changes the conformation of a protein and may even denature it.

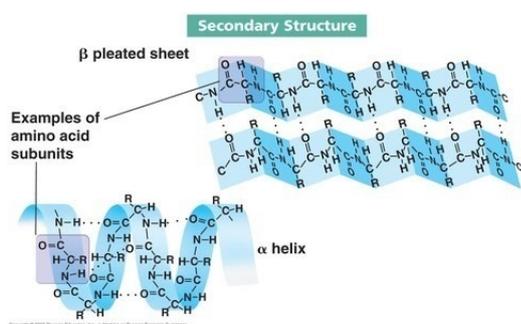


Figure 1.1- Secondary structure

Every enzyme is specific to a certain substrate this allowing it to catalyse one specific reaction, the active site of the enzyme is called the ligand binding site and is complementary to its specific substrate in shape and chemistry. When a substrate binds to an enzyme this can trigger a conformational change of the enzyme called an induced fit this helps to increase the binding action between the substrate and enzyme. The temporary change in conformation alters the function of the enzyme but also reduces the activation energy of the reaction since the bonds within the substrate are under tension and stressed due to the conformational change, thus the enzyme acting as a catalyst. Decreasing the activation energy of the reaction means less energy is required to reach the activated complex {a stable state midway in the reaction where the reacting molecules exist as neither reactant nor products}. The rate of enzymes catalysed reactions can be affected by certain variables such as concentration of the substrate, enzyme or end product.

1.33 Membranes and Diffusion

Amylase in the human body is either found in the mouth as salivary amylase or in the small intestine as pancreatic amylase. When the salivary alpha amylase enters the stomach it is inactivated due to the low pH and acidity of the stomach. Further hydrolysis occurs later on, in the duodenum, pancreatic juices travel here, which consists of pancreatic alpha amylase. Alpha amylase is used in breaking down starch into smaller molecules allowing it to move through the membranes of cells.

Every cell has a phospholipid bilayer surrounding it called a membrane, this controls what enters and exits the cell. Ions and polar molecules found within the body can move across the membrane

through transmembrane proteins which are specific to certain molecules. For the uptake of glucose, glucose transport proteins are required. The glucose transporter protein binds to glucose, causing it to undergo a conformational change, allowing facilitated diffusion across the membrane of glucose. If in the body amylase was not present, starch wouldn't break down into maltose and furthermore glucose, meaning cells wouldn't get their required energy, as molecules like maltose are too large to diffuse across the membrane.

1.34 Previous related studies

After devising an aim and hypothesis for the study, research into similar investigations was done. From looking at a previous study that has undergone a similar investigation this allowed a brief hypothesis to be created. This study looked at how habitual alcohol drinking influenced the activity of alpha amylase. From looking at this study it was seen that people drinking alcohol frequently had a higher amylase activity and the controls (non-drinkers) had a lower amylase activity, suggesting that alcohol was promoting the activity of amylase rather than inhibiting (Onyesom and Erude, 2004). This was unexpected.

1.35 Biological significance

It can be seen throughout society that alcohol has a significant presence in many adults lives, whether it be drinking excessive amounts but only occasionally or small volumes frequently (Buddy, 2018). Excessive alcohol consumption is a forever worsening issue in modern society which can have severe effects on your body, specifically your liver. Enzymes ensure the breakdown of food/toxins however they are lesser spoke about when discussing the effects of alcohol in the body, but it is the inhibition of these enzymes that causes the problems within organs as toxins are left to accumulate which can cause destructive effects.

An enzyme that hasn't been widely explored when looking at effects of alcohol on enzymes is Amylase. Investigating this topic allows for a greater understanding of how ethanol affects enzymes, specifically amylase. Amylase is vital in allowing cells to gain energy from food, without it cells wouldn't acquire sufficient glucose to undergo respiration and therefore won't function properly.

2. Procedure

2.1 Pilot study:

All materials/solutions were prepared and assembled as described in the main study.

2.11 Experiment A

Aim: Determining the correct concentration and volume of amylase to be used in the main study.

1% starch solution was selected for this study, too low a concentration of starch would mean the reaction between the amylase and starch would happen quickly and be difficult to measure as the reaction time would be very short. Too high a concentration and the reaction mixture would have to be left for long periods to allow the amylase to break down the starch completely, which isn't time efficient. A lower absorbance reading was sought too as this allowed room for a large increase in the case of inhibition; given the correct range a noticeable change could be observed.

Twelve test tubes were labelled appropriately according to their contents (X-volume, Y-amylase concentration) and then placed in water bath set to 40 degrees Celsius. Three different concentrations of amylase were prepared: 0.5%, 1% and 3%. Each concentration of amylase was tested with four different volumes; 1cm³, 2 cm³, 3 cm³ and 4 cm³ thus to find the most suitable volume and corresponding concentration of amylase. 1 cm³ of pH7 buffer solution was pipetted into each of the 12 test tubes using a 1 cm³ syringe to increase precision.

Figure 2.0- The volumes of distilled water required for each amylase volume

Amylase volume (cm ³)	Distilled water volume (cm ³)
1	5
2	4
3	3
4	2

Once the buffer and distilled water were added 5 cm³ of 1% starch solution was drawn into a pipette, an X volume/concentration (depending on the repeat) of amylase was drawn into a separate pipette, the two separate pipettes were added into the same test tube simultaneously and a timer was started. This was repeated for each volume and corresponding concentration of amylase until all combinations were completed. After ten minutes the test tube was removed from the bath and two drops of iodine were added, this was left to settle for 1 minute before testing and obtaining an absorbance reading. The absorbance reading was recorded again 1 minute later. It was found the reaction was continuing as the absorbance reading had decreased. To prevent this, an ice bath was introduced; this was used in the main study as it helped to stop the reaction.

Figure 2.1 shows the results from measuring the absorbance after a reaction time of 10 minutes in the 40°C water bath plus one minute in the ice bath.

10-minute reaction time

Amylase concentration (%)	Absorbance reading per volume of amylase (cm ³)			
	1(cm ³)	2(cm ³)	3(cm ³)	4(cm ³)
0.5%	1.81	1.53	1.42	1.20
1%	1.43	1.29	0.84	0.55
3%	0.48	0.42	0.31	0.14

Figure 2.1

This investigation was repeated with the same technique as before except the reaction time was reduced to five minutes to see how this altered the results, these absorbance readings were recorded in *figure 2.2*.

5-minute reaction time

Amylase concentration (%)	Absorbance reading per volume of amylase (cm ³)			
	1(cm ³)	2(cm ³)	3(cm ³)	4(cm ³)
0.5	1.89	1.69	1.58	1.51
1	1.62	1.51	1.31	1.12
3	0.64	0.56	0.51	0.39

Figure 2.2

The control in this study used a test tube containing 1 cm³ of the buffer, 5 cm³ of starch solution and 4 cm³ of distilled water, this was left for five minutes before adding the iodine. This was to see whether it was solely the amylase causing the breakdown of starch.

From pilot study A it was concluded that 1 cm³ of 3% of alpha amylase when left for five minutes was best suited. This combination yielded a reading low enough to allow for an increase when the inhibitor was added but also used minimal volumes of amylase and was left for a shorter time. Using 4 cm³ of 1% when left for 10 minutes would also be appropriate as this yielded useful results and uses a small supply of amylase however it wasn't as time efficient. The main study therefore used 5cm³ of 1% starch solution and 1 cm³ of 3% amylase solution and had an overall reaction time of five minutes.

2.12 Experiment B

Aim: Determining the sample size required for the main study.

Each test tube was set up containing 1 cm³ of buffer solution and 3 cm³ of distilled water. The investigation was carried out with the concentrations of starch and amylase determined in pilot study A. 1 cm³ of 3% alpha amylase solution was collected in a pipette and so too was 5 cm³ of 1% starch solution, the contents of these two pipettes were then pipetted into the test tube simultaneously and left for 5 minutes before being transferred to an ice bath where two drops of iodine were added to the test tube. A minute later an absorbance reading was taken. The experiment was then repeated 4 more times until an appropriate sample size was determined.

Sample size	Absorbance reading					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	average
1	0.4	-	-	-	-	0.40
2	0.4	0.44	-	-	-	0.42
3	0.4	0.44	0.39	-	-	0.41
4	0.4	0.44	0.39	0.38	-	0.40
5	0.4	0.44	0.39	0.38	0.4	0.40

Figure 2.3

Figure 2.3 shows that the results obtained using 3% amylase solution and 1% starch solution were on average consistent. A sample size of four was chosen to minimise the effects of any experimental error due to fluctuations.

2.2 Main study

2.21 Preparation of solutions:

All solutions were transferred into boiling tubes, labelled accordingly to their contents and placed in a 40°C water bath. This allowed their contents to heat up to the temperature the reaction was going to take place at, thus controlling confounding variables.

Alpha amylase solution:

3% alpha amylase solution was prepared by mixing 0.750 g of the amylase powder with a minimum amount of distilled water until it all completely dissolved. With rinsings this was transferred to a 250 cm³ volumetric flask and distilled water was added up to the graduation mark.

Starch solution:

1% starch solution was prepared by weighing out 1.000 g of starch powder with a minimum volume of boiling distilled water was added. The starch solution was stirred until all the starch had completely dissolved. The solution was transferred to 100 cm³ standard flask with rinsings and distilled water was added up to the graduation mark.

Iodine solution

0.01M iodine solution was used to ensure colorimeter readings were achievable.

Diluted Ethanol solutions:

All five ethanol concentrations 10%, 20%, 30%, 40%, 50% were prepared using the same method but with varying ethanol volumes. For the 10% ethanol solution 10 cm³ of ethanol was pipetted into a 100 cm³ volumetric flask and then 90 cm³ of water was added.

pH7 buffer

The pH7 buffer solution was prepared by dissolving a pH7 buffer tablet in 100 cm³ of distilled water.

2.22 Method:

List of apparatus:

12 Boiling tubes
12 Test tubes
1 Balance
2 Water baths
1 Ice bath
1 Timer
1 Colorimeter
2 Thermometers
12 Beakers
7 Standard flasks
8 Pipettes
2 Syringes
1 Glass Stirring rod

Procedure for test tube 1:

This method is also applicable for the experimentation of test tubes 2 through to 9; these experiments were carried out with the exact same method except for including different volumes and concentrations of specific solutions or in the case of controls not including certain solutions and this being replaced with distilled water as shown respectively in *figure 2.4*.

Prior to adding contents all test tubes were placed in the water bath. Using different pipettes for each solution avoiding cross contamination 5 cm³ of distilled water, 1 cm³ of the buffer pH7 solution and 2 cm³ of 10% ethanol was added to test tube 1. Different pipettes were used for each concentration of ethanol despite it being the same substance, this helped avoid affecting their standard concentrations. 5 cm³ of the 1% starch solution was drawn into a 5 cm³ syringe and 1 cm³ of 3% amylase was drawn into separate 1 cm³ syringe. Syringes were used at this point instead of pipettes as it was proven easier to hold the liquids in syringes for a prolonged time. Both the amylase and starch solution were pipetted into the test tube simultaneously, this making sure the amylase and starch began reacting at the same time, and a timer was started.

After 5 minutes the test tube was placed in an ice bath where it was left for 1 minute to cool before the iodine was added. This was tested in a colorimeter against a blank containing buffer and iodine solution. The colorimeter was set at a wavelength of 420nm. This method was repeated for each test tube four times as this was the sample size for the investigation.

The entire experiment was then repeated on a further date to obtain a second set of results. This second repeat was completely independent of the first replicate. All materials prepared for second replicate were made using fresh materials to ensure complete independence. The exact same equipment and method was used to control all other variables.

Figure 2.4 shows the volumes of solutions added to each varying test tube.

Solution	Volumes of solutions (cm ³)								
	Test tube								
	1 (10%)	2 (20%)	3 (30%)	4 (40%)	5 (50%)	6 (0%)	7 (control)	8 (control)	9 (control)
Ethanol	2	2	2	2	2	-	-	-	-
pH7 buffer	1	1	1	1	1	1	-	1	1
1% starch solution	5	5	5	5	5	5	5	-	5
3% amylase	1	1	1	1	1	1	1	1	-
Distilled water	5	5	5	5	5	7	8	12	8

Figure 2.4

2.3 Control:

Figure 2.4 shows the controls respectively. Test tube 9, being a negative control, containing no amylase allowed for certainty that it was the amylase causing the breakdown of starch and not another constituent. The starch wasn't broken down shown by an absorbance reading of 2.0 which is off the scale, certifying that amylase is the primary reason for the breakdown of starch. Test tube 8 with the absence of starch showed it was starch causing the indicator change. In test tube 7 no buffer was added to see if the buffer was having an adverse effect on the rate of reaction or causing other disruptions. Each control was carried out with each repeat to ensure the starch and amylase were always working and the starch was reacting with the iodine. The total volume for each test tube for the controls was 14 cm³.

3. Results

3.1 Replicate 1

Figure 3.0 shows the averages and standard deviations for the absorbance readings of the solution as the ethanol concentration increased.

Ethanol concentration (%)	Absorbance Reading	
	Average	Standard Deviation
0	0.52	0.05
10	0.60	0.03
20	0.57	0.02
30	0.62	0.01
40	0.55	0.06
50	0.54	0.02

Figure 3.0

As the ethanol concentration increased from 0 - 50% the absorbance readings fluctuate with no significant trend. There is no visible correlation between ethanol concentration and amylase activity.

Average standard deviation value: 0.0334

Calculation :

Sample calculation for standard deviations is found in the appendix.

Figure 3.1 shows how the absorbance values vary as the ethanol concentration was increased throughout replicate 1.

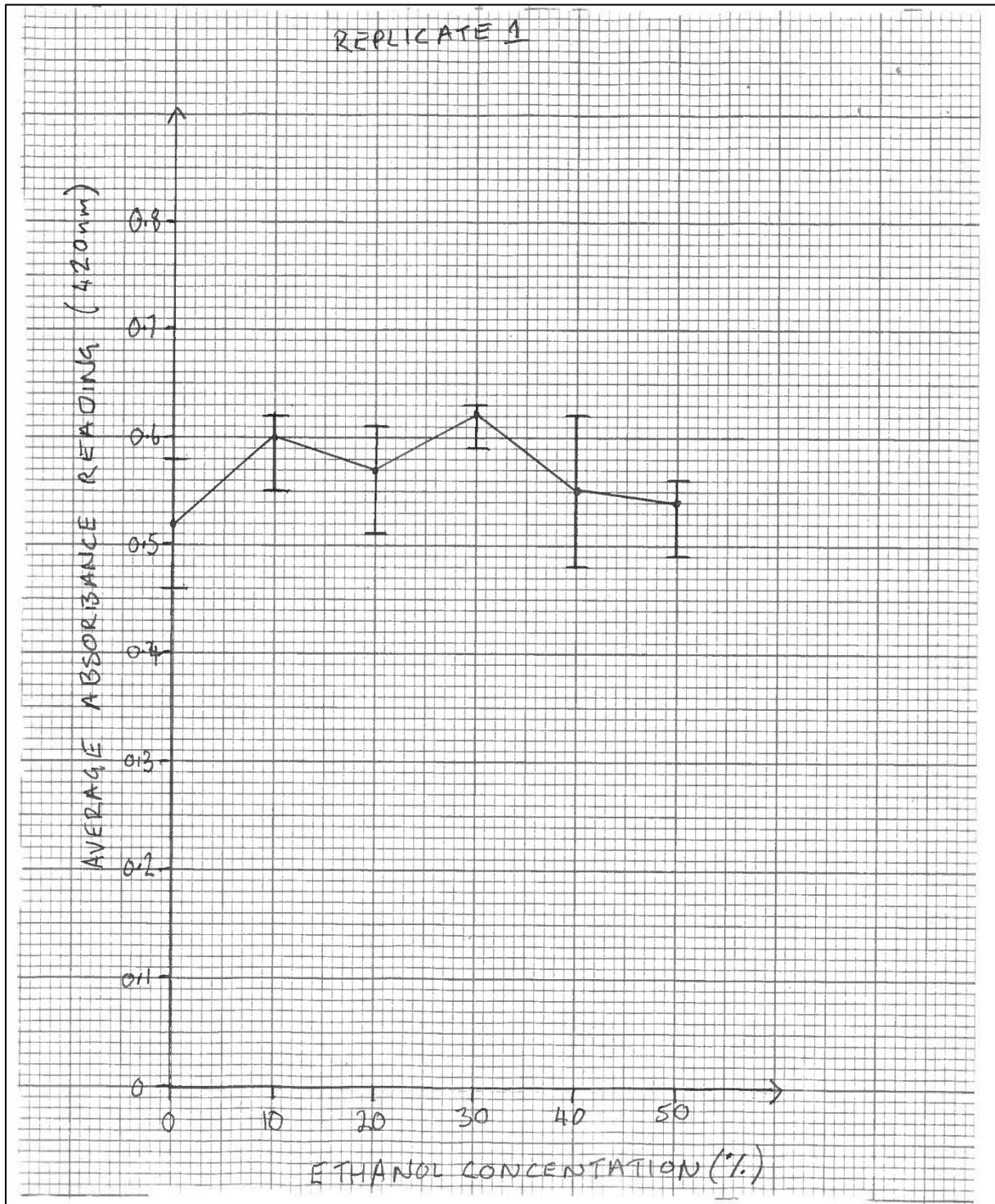


Figure 3.1

3.2 Replicate 2

Figure 3.2 shows how increasing the alcohol concentration affects the absorbance of the solution

Ethanol concentration (%)	Absorbance Readings	
	Average	Standard Deviation
0	0.65	0.07
10	0.63	0.04
20	0.64	0.07
30	0.59	0.04
40	0.57	0.05
50	0.61	0.05

Figure 3.2

Again as the ethanol concentration increased from 0 - 50% the absorbance readings fluctuated throughout representing no correlation between ethanol concentration and absorbance readings.

Average standard deviation: 0.0512

Calculation:

See sample calculation carried out for replicate 1.

Figure 3.3 shows graphically how the absorbance values vary as the ethanol concentration is increased in replicate 2.

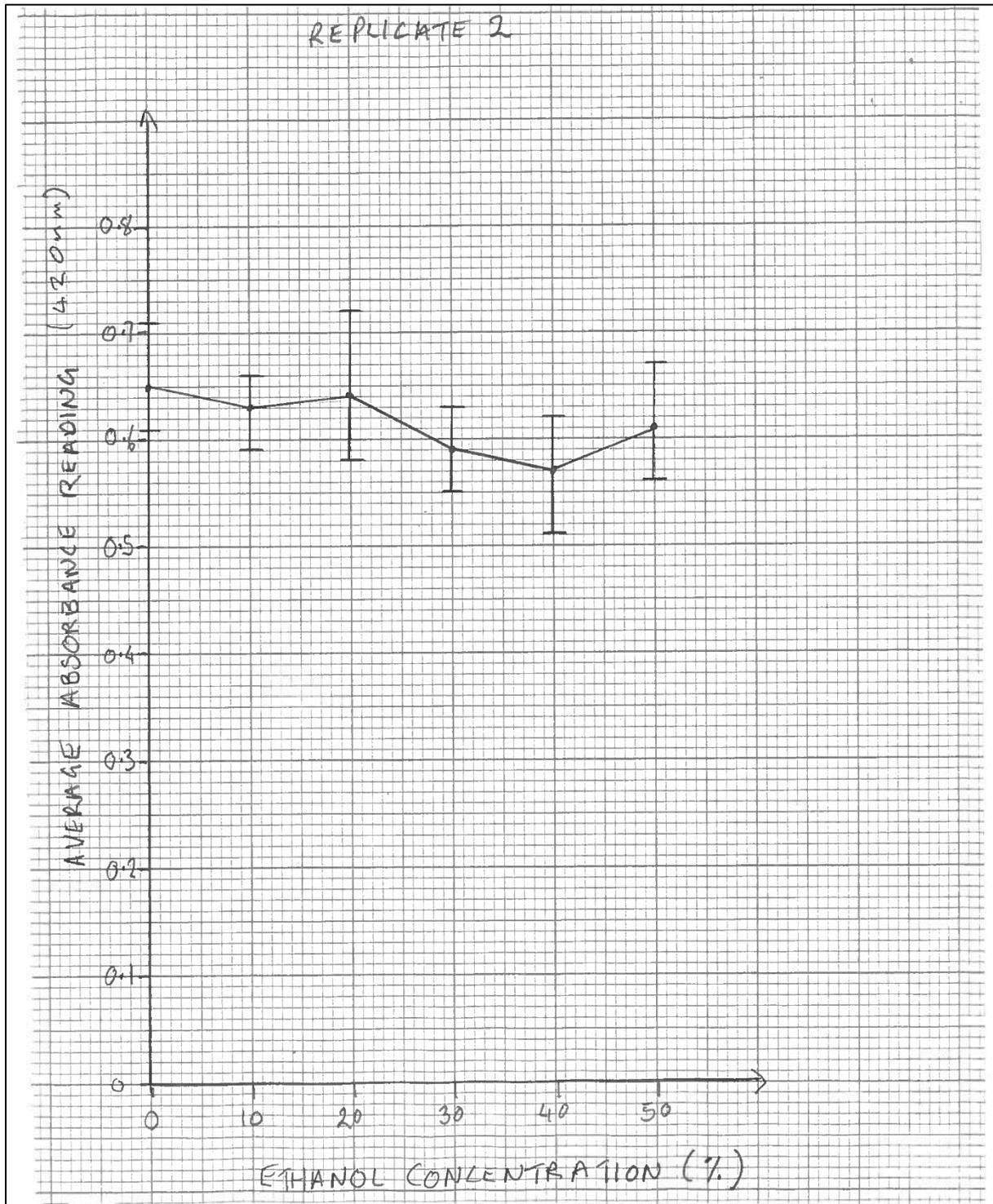


Figure 3.3

3.3 Average across replicate 1 and 2

Figure 3.4 combines the results from replicate 1 and 2 creating overall absorbance readings across both replicates

Concentration of ethanol (%)	Average absorbance across replicate 1 and 2	
	Overall average	Standard deviation
0	0.59	0.04
10	0.62	0.03
20	0.61	0.03
30	0.61	0.01
40	0.56	0.04
50	0.58	0.02

Figure 3.4

Figure 3.4 shows no visible trend corresponding between the absorbance values and concentration of ethanol used.

Figure 3.5 shows the average absorbance readings graphically from figure 3.4.

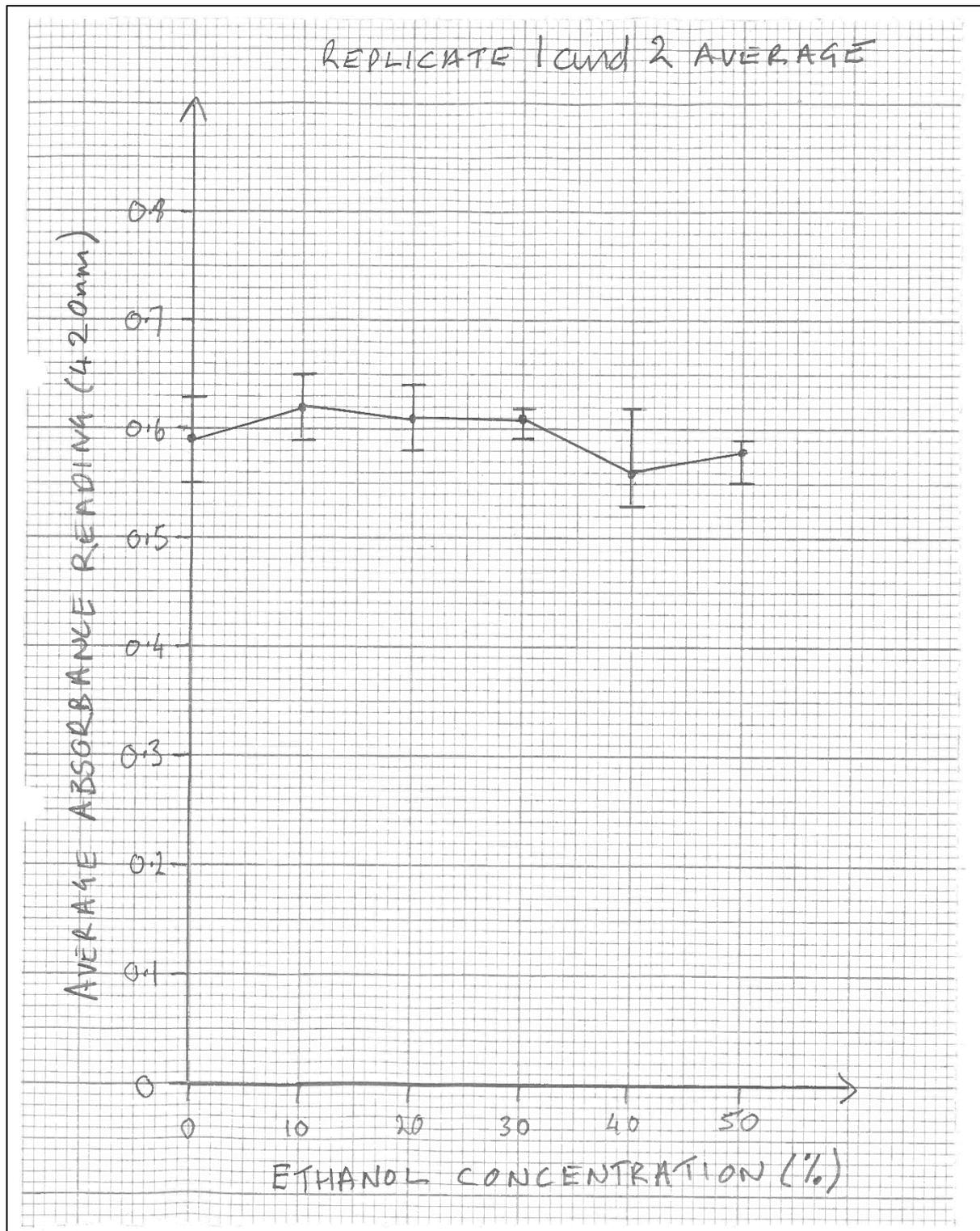


Figure 3.5

4. Discussion

4.1 Conclusion

Varying the alcohol concentrations had no effect on the absorbance of the solution. Looking at the averages across replicate 1 and 2 as the ethanol concentration increased from 0-10% the average absorbance reading increased from 0.59-0.63, from 10-20% it decreased from 0.63-0.61, 20-30%, it remained constant at 0.61, from 30-40% it decreased from 0.61-0.56 and from 40-50% increased from 0.56-0.58. Fluctuations in absorbance readings occur throughout and lack any significant trend. From a concentration of 0-50% ethanol the activity of the alpha amylase varied very little indicating that ethanol doesn't inhibit the activity of alpha amylase as it continued to break starch down at a similar rate throughout varying concentrations and without ethanol.

4.2 Evaluation of procedure

Each replicate was conducted using freshly made solutions. This increased reliability of results as if any experimental error occurred when preparing the solutions this could be observed if the results didn't correspond. Using the same solutions for each replicate would mean that if the solutions were faulty there would be experimental error within both sets of results unknowingly, both replicates would correspond because the final interpretation would consist of two incorrect sets of results.

The pilot study was carried out to determine the correct timings for the reaction, the most suitable concentrations and volumes of starch/amylase to be used and the most suited sample size, it also allowed for practice of experimental technique. It was found that when using cold water to prepare the starch solution the starch didn't completely dissolve and it sank to the bottom of the test tube affecting the overall concentration, the bottom being a lot more concentrated than the top. Inverting the test tube couldn't account for this as the starch would sink too quickly. Boiling water was used in the main study, this helping the starch to fully dissolve.

A test tube was prepared without ethanol for each repeat, this allowed a comparison to see whether ethanol was the only inhibiting factor of amylase.

The reason for the ethanol having no effect on the activity of alpha amylase could be due to the low concentrations of ethanol used. A second investigation could be carried out using higher concentrations of ethanol such as 50%, 60%, 70%, 80%, 90% and 100% this would allow us to see if it was the concentration of the ethanol was the inhibiting factor of the procedure.

The investigation was performed to see how alcohol consumption could affect the process of digestion in humans. Temperature is a very important factor when discussing enzymes, too high a temperature could cause denaturation of the enzyme. The temperature of a healthy human body is 37°C, so the ideal temperature for this procedure would be at 37°C this increasing the validity of the experiment. However, the water bath only had a scale using multiples of ten so using 37°C would be an estimate. 40°C was used instead as this was a temperature that could be accurately stated. Because of this, the experiment was not exactly replicating how amylase and ethanol interact in the body, finding a water bath with a smaller scale would increase accuracy of the procedure.

All solutions in boiling tubes were placed in the water bath set to 40°C allowing the solutions to heat up to the investigation temperature, thus maintaining a constant temperature throughout every repeat and increasing validity.

4.3 Evaluation of results

Comparing the absorbance values of the varying concentrations of ethanol against using no ethanol produced similar results suggesting ethanol wasn't affecting the activity of amylase.

Looking across both replicates combined, as the ethanol concentration increased from 0-10% the average absorbance reading increased, from 10-20% it decreased, from 20-30% it stayed the same, from 30-40% it decreased and from 40-50% it increased, indicating no significant trend. It is clear from these results that altering the concentration of ethanol was not affecting the rate of starch hydrolysis therefore ethanol was not inhibiting alpha amylases activity.

Overall looking at both replicates, replicate 1 on average produced lower absorbance values than replicate 2, but not to any significant amount that the results were disagreeing or showing controversy. The difference in variation and overall average absorbance values could be due to the use of different solutions between replicates as all materials were prepared fresh. All the solutions were prepared with the exact same technique however it cannot be said with exact certainty that the solutions were of the exact same concentrations between replicates, as uncertainties associated with the three figure balance are present and transfer losses are associated.

Replicate 2's results were more precise with smaller fluctuations between results as shown by the average standard deviations. The precision of results varied between all replicates. There was no specific concentration that yielded the most consistent results. Replicate 1, 30% ethanol on average showed the lowest standard deviation suggesting its results were most precise. Replicate 2, 30% and 10% ethanol shared the lowest standard deviation value. On average, 30% ethanol produced the lowest standard deviation suggesting the most consistent results. The experiment overall has high precision indicated by low standard deviation values, however still not providing a meaningful trend as expected.

Previous experiments showed that as alcohol consumption increased gradually the activity of the alpha amylase increased, suggesting that ethanol was promoting the activity of alpha amylase rather than inhibiting. However, the results obtained in this investigation showed that as the concentration of ethanol increased the activity of alpha amylase remained unaffected and it continued to break down starch at a similar rate, with slight fluctuations throughout, thus tacking a significant trend. The reasons could be due experimental error associated with uncertainties throughout the procedure involving the apparatus and chemicals.

The graphs of figures 3.1, 3.3 and 3.5 show overlapping of almost every error bar suggesting the results show no statistical significance as the difference between the means is not of such a value that it can be confidently stated the results weren't just by chance. Comparing the graphs separately from replicate 1 and 2 shows this experiment has not proven statistical correlation between replicates as they don't share a common trend.

5. References

Buddy T (2018) "Alcohol Metabolism Could Be Key to Alcohol's Dangers" [online] accessed 02/19 at <https://www.verywellmind.com/alcohol-metabolism-key-to-alcohols-dangers-66524>

Campbell D, Smith B (2015), "SCHOLAR Study Guide CfE Advanced Higher Biology Unit 1: Cells and Proteins" [online] accessed 01/19 at [http://www.standrewspaisley.com/uploads/6/0/2/3/60230905/ah_cbio_unit1\[1\].pdf](http://www.standrewspaisley.com/uploads/6/0/2/3/60230905/ah_cbio_unit1[1].pdf)

Erude H (2004), "Amylase activity in the saliva and plasma of habitual alcohol drinkers" BIOKEMISTRI 16(1):11-14 [online] accessed 02/19 at <http://www.bioline.org.br/pdf?bk04002>

John A (n.d.) "The Effects of Alcohol on Enzymes" (online) accessed 10/18 at <https://www.livestrong.com/article/426731-the-effects-of-alcohol-on-enzymes/>

Lloyd D, Morgan G (2016), "BrightRED Study Guide, CfE Advanced Higher Biology" BrightRed Publishing, Edinburgh

SQA (2015), "Advanced Higher Biology Course/Unit Support Notes" [online] accessed 11/18 at https://www.sga.org.uk/files_ccc/AHCUSNBiology.pdf

Wang NS (n.d.), "STARCH HYDROLYSIS BY AMYLASE" [online] accessed 01/19 at <https://user.eng.umd.edu/~nsw/ench485/lab5.htm>

Wikipedia Contributors (2019), "Protein secondary structure" [online] accessed 01/19 at https://en.wikipedia.org/wiki/Protein_secondary_structure

Wikipedia Contributors (2018) "Alpha Amylase" [online] accessed 11/18 at <https://en.wikipedia.org/wiki/Alpha-amylase>

6. Appendix

6.1 Replicate 1

Ethanol concentration (%)	Absorbance Reading					
	Repeat				Average	Standard deviation
	1	2	3	4		
0%	0.49	0.46	0.54	0.58	0.52	0.05
10%	0.59	0.62	0.62	0.55	0.60	0.03
20%	0.57	0.59	0.51	0.61	0.57	0.02
30%	0.59	0.62	0.63	0.63	0.62	0.01
40%	0.62	0.51	0.48	0.58	0.55	0.06
50%	0.54	0.55	0.49	0.56	0.54	0.02

Figure 6.0

6.2 Replicate 2

Ethanol concentration (%)	Absorbance Readings					
	Repeat				Average	Standard deviation
	1	2	3	4		
0	0.61	0.65	0.71	0.62	0.65	0.066
10	0.59	0.67	0.65	0.61	0.63	0.037
20	0.58	0.59	0.72	0.66	0.64	0.066
30	0.63	0.62	0.55	0.56	0.59	0.041
40	0.62	0.57	0.58	0.51	0.57	0.050
so	0.56	0.62	0.67	0.59	0.61	0.047

Figure 6.1

6.3 Standard deviation calculation:

Using results for ethanol concentration 10%

Average:

$$\frac{(0.59 + 0.62 + 0.62 + 0.55)}{4}$$
$$= 0.60$$

Standard deviation:

SD= standard deviation

= sum of

X = sample value

b = mean value = 0.60

n = number of values

Mean(x) = 24

Repeat 1: $(x - b) = (0.59 - 0.6) = 0.01$

$$(x - b)^2 = 0.01^2 = 0.0001$$

Repeat 2: $(x - b) = (0.62 - 0.6) = 0.02$

$$(x - b)^2 = 0.02^2 = 0.0004$$

Repeat 3: $(x - b) = 0.62 - 0.6 = 0.02$

$$(x - b)^2 = 0.02^2 = 0.0004$$

Repeat 4: $(x - b) = 0.55 - 0.6 = 0.05$

$$(x - b)^2 = 0.05^2 = 0.0025$$

Sum of $(x - x) = 0.0001 + 0.0004 + 0.0004 + 0.0025 = 0.0034$

$$SD = (0.0034/4 - 1)^{1/2} = 0.03366501646 = 0.034$$

All standard deviations calculated were rounded to two decimal places.