Personal Engagement: 2 points and 8% of score

Mark	Descriptor
0	☐ The student's report does not reach a standard described by the descriptors below
1	☐ **The evidence of personal engagement with the exploration is limited with little independent thinking, initiative or creativity.** ☐ The justification given for choosing the research question and/or the topic under investigation does not demonstrate **personal significance, interest or curiosity.** ☐ There is little evidence of **personal input and initiative** in the designing, implementation or presentation of the investigation.
2 2	☑ **The evidence of personal engagement with the exploration is clear with significant independent thinking, initiative or creativity.** ☑ The justification given for choosing the research question and/or the topic under investigation demonstrates **personal significance, interest or curiosity.** ☑ There is evidence of **personal input and initiative** in the designing, implementation or presentation of the investigation.

Communication: 4 points and 17% of score

Mark	Descriptor
0	☐ The student's report does not reach a standard described by the descriptors below
1-2	☐ **The presentation of the investigation is unclear, making it difficult to understand the focus, process and outcomes.** ☐ The report is not well structured and is unclear: the necessary information on focus, process and outcomes is missing or is presented in an incoherent or disorganized way. ☐ The understanding of the focus, process and outcomes of the investigation is obscured by the presence of inappropriate or irrelevant information. ☐ There are many errors in the use of subject specific terminology and conventions*.
3-4 4	☑ **The presentation of the investigation is clear. Any errors do not hamper understanding of the focus, process and outcomes.** ☑ The report is well structured and clear: the necessary information on focus, process and outcomes is present and presented in a coherent way. ☑ The report is relevant and concise thereby facilitating a ready understanding of the focus, process and outcomes of the investigation. ☑ The use of subject specific terminology and conventions is appropriate and correct. Any errors do not hamper understanding.

Exploration: 6 points and 25% of score

Mark	Descriptor
0	☐ The student's report does not reach a standard described by the descriptors below
1-2	☐ The topic of the investigation is identified and a research question of some relevance is **stated but it is not focused.** ☐ The background information provided for the investigation is **superficial** or of limited relevance and does not aid the understanding of the context of the investigation. ☐ The methodology of the investigation is only appropriate to address the research question to a very limited extent since it takes into consideration few of the significant factors that may influence the relevance, reliability and sufficiency of the collected data. ☐ The report shows evidence of limited awareness of the significant safety, ethical or environmental issues that are **relevant to the methodology of the investigation***.
3-4	☐ The topic of the investigation is identified and a relevant but not fully focused research question is described. ☐ The background information provided for the investigation is mainly appropriate and relevant and aids the understanding of the context of the investigation. ☐ The methodology of the investigation is mainly appropriate to address the research question but has limitations since it takes into consideration only some of the significant factors that may influence the relevance, reliability and sufficiency of the collected data. ☐ The report shows evidence of some awareness of the significant safety, ethical or environmental issues that are **relevant to the methodology of the investigation.***
5-6 6	☑ The topic of the investigation is identified and a relevant and fully focused research question is clearly described. ☑ The background information provided for the investigation is entirely appropriate and relevant and enhances the understanding of the context of the investigation. ☑ The methodology of the investigation is highly appropriate to address the research question because it takes into consideration all, or nearly all, of the significant factors that may influence the relevance, reliability and sufficiency of the collected data. ☑ The report shows evidence of full awareness of the significant safety, ethical or environmental issues that are **relevant to the methodology of the investigation.***

Analysis: 6 points and 25% of score

Mark	Descriptor
0	☐ The student's report does not reach a standard described by the descriptors below
1-2	☐ The report includes **insufficient relevant** raw data to support a valid conclusion to the research question. ☐ Some **basic** data processing is carried out but is either **too inaccurate or too insufficient to lead to a valid** conclusion. ☐ The report shows evidence of little consideration of the impact of measurement uncertainty on the analysis. ☐ The processed data is incorrectly or insufficiently interpreted so that the conclusion is invalid or very incomplete.
3-4	☐ The report includes relevant but incomplete quantitative and qualitative raw data that could support a simple or partially valid conclusion to the research question. ☐ Appropriate and sufficient data processing is carried out that could lead to a broadly valid conclusion but there are significant inaccuracies and inconsistencies in the processing. ☑ The report shows evidence of some consideration of the impact of measurement uncertainty on the analysis. ☐ The processed data is interpreted so that a broadly valid but incomplete or limited conclusion to the research question can be deduced
5-6 5	☑ The report includes sufficient relevant quantitative and qualitative raw data that could support a detailed and valid conclusion to the research question. ☑ Appropriate and sufficient data processing is carried out with **the accuracy** required to enable a conclusion to the research question to be drawn that is fully **consistent** with the experimental data. ☐ The report shows evidence of full and appropriate consideration of the impact of measurement uncertainty on the analysis. ☑ The processed data is correctly interpreted so that a completely valid and detailed conclusion to the research question can be deduced.

Evaluation: 6 points and 25% of score

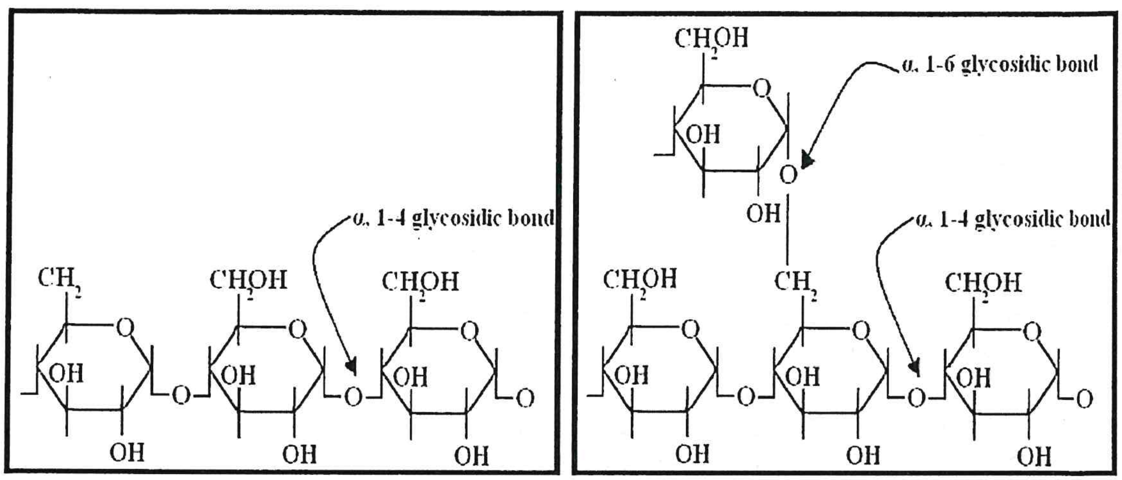
Mark	Descriptor
0	☐ The student's report does not reach a standard described by the descriptors below
1-2	☐ A conclusion is **outlined** which is not relevant to the research question or is not supported by the data presented. ☐ The conclusion makes superficial comparison to the accepted scientific context. ☐ Strengths and weaknesses of the investigation, such as limitations of the data and sources of error, are **outlined** but are restricted to an **account of the practical or procedural issues** faced. ☐ The student has **outlined** very few realistic and relevant suggestions for the improvement and extension of the investigation.
3-4	☐ A conclusion is **described** which is relevant to the research question and supported by the data presented. ☐ A conclusion is described which makes some relevant comparison to the accepted scientific context. ☐ Strengths and weaknesses of the investigation, such as limitations of the data and sources of error, are **described** and provide evidence of **some awareness of the methodological issues*** involved in establishing the conclusion. ☑ The student has **described** some realistic and relevant suggestions for the improvement and extension of the investigation.
5-6 5	☑ A detailed conclusion is **described and justified** which is entirely relevant to the research question and fully supported by the data presented. ☑ A conclusion is correctly **described and justified** through relevant comparison to the accepted scientific context. ☑ Strengths and weaknesses of the investigation, such as limitations of the data and sources of error, are discussed and provide evidence of a **clear understanding of the methodological issues*** involved in establishing the conclusion. ☐ The student has **discussed** realistic and relevant suggestions for the improvement and extension of the investigation.

7 = 22/24

Investigation of the effect of sodium chloride concentration on the activity of amylase in the hydrolysis of starch

Background Information:

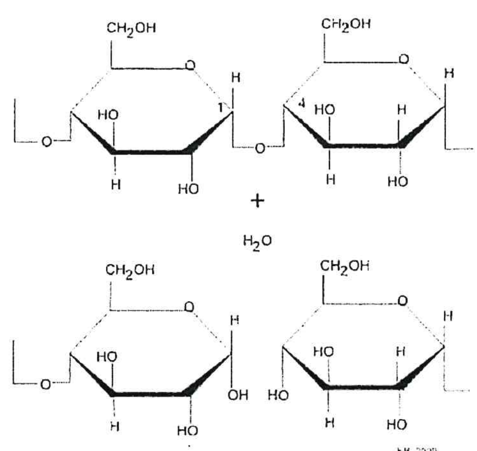
Starch, an energy storage polysaccharide found in plants, is made up of two types of molecules; amylose and amylopectin. Both forms are composed of α-D-glucose units joined together by glycosidic bonds. Amylose is an unbranched molecule with glucose units joined by α-1,4-glycosidic bonds, whereas amylopectin is branched with additional α-1,6-glycosidic bonds at the branch points. **("a-Amylase.")**



(a) amylose (b) amylopectin

("Action of Alpha- Amylase on Starch.")

During hydrolysis, the glycosidic bonds are split by water to produce a mixture of simpler carbohydrates consisting of maltose and glucose. In humans, the hydrolysis of starch is catalysed by α-amylase in saliva and the pancreas. **("Starch.")**



Hydrolysis of 1:4α Linkage

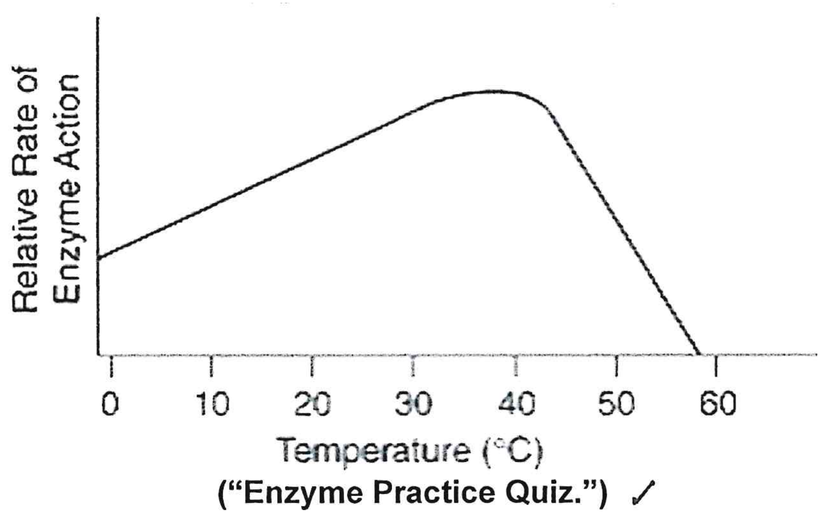
("Mechanisms of Chemical Digestion - Boundless Open Textbook.")

Amylase is an enzyme, which catalyses the hydrolysis of starch by providing an alternate pathway with a lower activation energy (E_a). When a starch molecule binds to the active site of amylase, the glycosidic bond is weakened, resulting in cleavage. ✓

There are several factors that affect enzyme activity, including enzyme and substrate concentrations, pH, temperature, and the presence of inhibitors and activators. ✓

Enzymes have an optimum pH, where the activity of the enzyme is at its maximum. The optimum pH of amylase (saliva and pancreas) varies from 6.7 to 7. At extremes of pH, the enzyme becomes denatured. At low or high pH, the subsequent change in hydrogen ion concentration results in the loss of some ionic bonds that stabilise the tertiary structure of the enzyme, causing a change in shape of the active site and hence a decrease in enzyme activity. **("Introduction to Enzymes.")** ✓

Likewise, amylase has an optimum temperature of 37°C. According to the collision theory, as temperature is increased, more amylase molecules have kinetic energy greater than the activation energy, resulting in more frequent successful collisions between amylase and starch molecules and an increase in enzyme activity. However, above the optimum temperature, the enzyme activity falls considerably with temperature. Heat energy disrupts hydrogen bonds that stabilise the tertiary structure, causing the enzyme to denature, preventing the starch molecule from binding to the active site of amylase. **("Kinetic Inhibition of Human Salivary Alpha-Amylase by a Novel Cellobiose-Containing Tetrasaccharide.")** ✓



("Enzyme Practice Quiz.") ✓

Activators and inhibitors also affect enzyme activity. Activators bind to enzymes and increase their activity. Examples are cofactors, such as Fe in haemoglobin, and coenzymes. Inhibitors reduce enzyme activity and can either compete with the substrate to bind at the active site (competitive inhibitor), or bind to a site other than the active site, known as the allosteric site (non competitive inhibitor).

Salt concentration is another factor that can affect enzyme activity. A change in concentration of dissolved salts results in the addition or removal of positive or negative ions, which disrupt attractions between charged amino acid side chains, causing a change in secondary and tertiary structures and subsequent denaturation of the enzyme. ✓

I am interested in learning about the absorption of oral drugs in the body, since my aim is to follow a career in pharmacy. Besides its active ingredients, oral drugs contain inactive excipients, such as binders. Binders provide cohesion between the powders and hold them together during transport, yet allow the tablet to disintegrate so that the active ingredients are released into the body for absorption. Starch is a common binding agent used in the manufacture of tablets. In order to release the active ingredients in the tablet for absorption in the body, the starch binding agent has to be hydrolysed. This requires administering the tablet into the body through a pathway where the starch binding agent hydrolyses the fastest. **("Tablet Binders.")** ✓

Acini, the glands that produce saliva, produce an ion-rich solution containing potassium (K^+), hydrogencarbonate (HCO_3^-), sodium (Na^+) and chloride (Cl^-) ions. Hence, the naturally produced amylase that is present in saliva, catalyses the hydrolysis of starch in the presence of sodium chloride (NaCl). **("Saliva Secretion | Process of Salivary Flow, Functions, Control.")** ✓

This experiment will determine the enzyme activity of amylase in the hydrolysis of starch at different concentrations of sodium chloride (NaCl). The reaction will monitor the disappearance of starch using iodine solution (iodine in potassium iodide) as an indicator. ✓

Appropriate, relevant background information.

Research Question:

What is the optimum concentration of sodium chloride (NaCl) for the hydrolysis of starch by amylase at its optimum temperature (37 °C) and pH (7), using sodium chloride concentrations of 0 %, 10 %, 20 %, 30 %, 40 %?

Fully focused research question.

Variables:

Independent Variable: concentration of NaCl solution. Five different concentrations of sodium chloride solution will be used (0 %, 10 %, 20 %, 30 %, 40 %). ✓

Dependent Variable: activity of amylase (rate of hydrolysis of starch reaction), measured by taking samples of the starch-amylase reaction mixture at 10 second intervals and testing for the presence of starch using iodine solution. ✓

Controlled Variables:

Variable	Reason for control	How it will be controlled
pH	Enzyme becomes denatured at extremes of pH. The optimum pH for amylase is 6.7-7 (Using 7.63).	Use 1 cm^3 of pH 7.63 buffer solution for each experiment. Measure the pH of the buffer solution using a pH meter at the start of each experiment.
Quantity of starch solution	The enzyme activity increases with starch concentration (until it becomes saturated) because the frequency of successful collisions between enzyme and substrate molecules increases.	Use a graduated pipette to measure 4 cm^3 of 1 % starch solution, for each experiment.
Quantity of amylase solution	The enzyme activity increases with enzyme concentration because there are more available active sites, which increases the frequency of successful collisions between enzyme and substrate molecules.	Use a graduated pipette to measure 4 cm^3 of 1 % amylase solution, for each experiment.
Quantity of iodine solution	The colour intensity of the starch/iodine complex depends on the amount of starch/iodine.	Use a dropping pipette to transfer 1 drop of iodine solution to each dimple of the dimple tray for each experiment.
Sampling time	The amount of starch in the reaction mixture decreases with time as it is hydrolysed into glucose, and depends on the rate of reaction at time t.	Use a stopwatch to take samples of the reaction mixture every 10 s.
Temperature	The enzyme becomes denatured above the optimum temperature. As temperature decreases below the optimum temperature, enzyme activity decreases because the proportion of enzyme molecules with kinetic energy greater than the activation energy decreases. The optimum temperature of amylase is 37 °C.	Use a thermostat-controlled water bath to set the temperature of each experiment at 37°C. Measure the temperature of the water bath using a thermometer at the start of each experiment.

Materials:

Equipment for hydrolysis of starch:

- Sodium chloride solution (concentration: 0 %, 10 %, 20 %, 30 %, 40 %) 100 cm^3
- Starch solution, 1 % concentration, 100 cm^3
- Amylase solution, 1 % concentration, 100 cm^3
- Iodine solution, 50 cm^3
- Buffer solution pH 7.63, 50 cm^3
- Transparent dimple (15) trays x6 (2 per trial)
- Dropping pipette x15 (x3 per experiment)
- Graduated dropping pipette x20 (x4 per trial) ($\pm$ 0.02 ml for 2 ml and $\pm$ 0.05 ml for 5 ml)
- Thermometer -10 – 110 °C ($\pm$0.5 °C)
- Thermostat-controlled water bath with test tube rack
- Stop clock ($\pm$ 0.01 s)
- Marker pen (for labelling test tubes)
- Safety goggles
- Electronic mass balance ($\pm$ 0.001 g) Equipment uncertainties stated.

Risk Assessment:

Chemical	Hazard	Precaution
1 % starch solution	Low hazard.	Do not consume food/drink in the lab.
1 % amylase solution	Low hazard.	Wear eye protection. If contact is made with the eye, flood the eye with gently running tap water for 10 minutes.
Amylase powder	May be a sensitiser and may produce allergic reactions. Can cause asthma and irritate the eyes, nose and skin.	Use a fume cupboard when handling amylase powder.
Iodine solution (less than 1 mol dm^{-3})	Low hazard.	Wear eye protection. If contact is made with the eye, flood the eye with gently running tap water for 10 minutes. If swallowed, rinse the mouth with water, do not induce vomiting. See a doctor. If spilled on the skin, remove contaminated clothing and drench the skin with plenty of water.
Buffer solution of pH 7.63	Low hazard.	Wear eye protection. If contact is made with the eye, flood the eye with gently running tap water for 10 minutes.

Wear safety goggles throughout the experiment and take care when handling glassware. Broken glass can cause cuts. **("STUDENT SAFETY SHEETS.")**

Awareness of safety issues.

Method:
1. Using a thermometer to monitor the temperature, warm the water in the water bath to 37 °C.
2. Make the following solutions: 1 % starch solution (1 g starch powder dissolved in 100 ml of distilled water), 1% amylase solution (1 g of amylase powder dissolved in 100 ml of distilled water).
3. Label five test tubes T1, T2, T3, T4, and T5. Using a graduated pipette, transfer 2 cm^3 of 1 % amylase solution to each of the three test tubes.
4. Using two separate graduated pipettes, add 2 cm^3 of 0 % salt solution and 2 cm^3 of pH 7.63 buffer to the amylase in each test tube. Put a stopper on each test tube, gently shake each mixture, and place the test tubes in the water bath.
5. Using a graduated pipette, transfer 4 cm^3 of 1 % starch solution to five separate test tubes and place them in the water bath.
6. Allow the solutions in the test tubes in the water bath to incubate to 37 °C.
7. Place two transparent dimple trays on a white surface and add one drop of iodine solution to each dimple, using a dropping pipette.
8. Once the solutions in the test tubes have reached 37 °C, add 4 cm^3 of starch solution from one of the test tubes to the amylase/salt/buffer mixture in test tube T1. Gently shake the mixture in the test tube, return it to the water bath, and immediately start the stop clock.
9. After 10 seconds, using a clean dropping pipette, transfer one drop of the reaction mixture to the first dimple of the dimple tray containing iodine solution. The iodine solution should turn blue-black if starch is present.
10. Repeat step 8 every 10 seconds until the iodine-reaction mixture in the dimple tray remains orange.
11. Repeat steps 1 to 10 two more times (for test tubes T2, T3, T4, and T5).
12. Repeat steps 1 to 11 for different concentrations of sodium chloride solution (10 %, 20 %, 30 %, 40 %). Very clear detailed method.

Exploration = 6/6

Qualitative Data:

Concentration (% of sodium chloride)	Observations
0	First drop of solution shown as to be dark orange. Solution colour after the first drop was much lighter (bright yellow).
10	First drops of swimming pool water had a similar colour to the solution with 0 % sodium chloride, but were slightly darker. Solution colour after the first drop was much lighter (bright yellow).
20	Solution droplet looks dark brown with a slight tint of purple. Second droplet was brown. Colour of droplets after the previous solutions were yellow.
30	First and second droplets look very dark brown/purple. Colour of solution after the first and second droplets were brown/orange.
40	Solution turned to a very dark purple/black colour and took at least two pipettes for solution to show a change in colour to orange/yellow.

Raw Quantitative Data:

Table 1: Reaction time (to the nearest 10 seconds) at different concentrations of sodium chloride.

Concentration /% of sodium chloride	Reaction time /s ($\pm$ 10 s) ✓				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0	10	10	10	20	10
10	20	20	20	20	10
20	30	20	20	20	20
30	30	20	30	30	40
40	40	30	50	40	40

✓

Processed Data: Sufficient quantitative and qualitative raw data.

Table 2: Average reaction time and average rate of reaction for different concentrations of sodium chloride.

Concentration /% of sodium chloride	Average reaction time /s	Average rate of reaction /s^{-1} ✓
0	12	0.083
10	18	0.056
20	22	0.046
30	30	0.033
40	40	0.025

✓

The average reaction time for each concentration of sodium chloride was calculated using the formula:

Average reaction time = (trial 1 + trial 2 + trial 3 + trial 4 + trial 5)/5

For example, experiment 1:

Average reaction time = (10 + 10 + 10 + 10 + 10)/5 = 10 s

The rate of reaction for each concentration of sodium chloride was calculated using the formula:

Average rate of reaction = 1/average reaction time

For example, experiment 1:

Average rate of reaction = 1/10 = 0.1 s^{-1} ✓

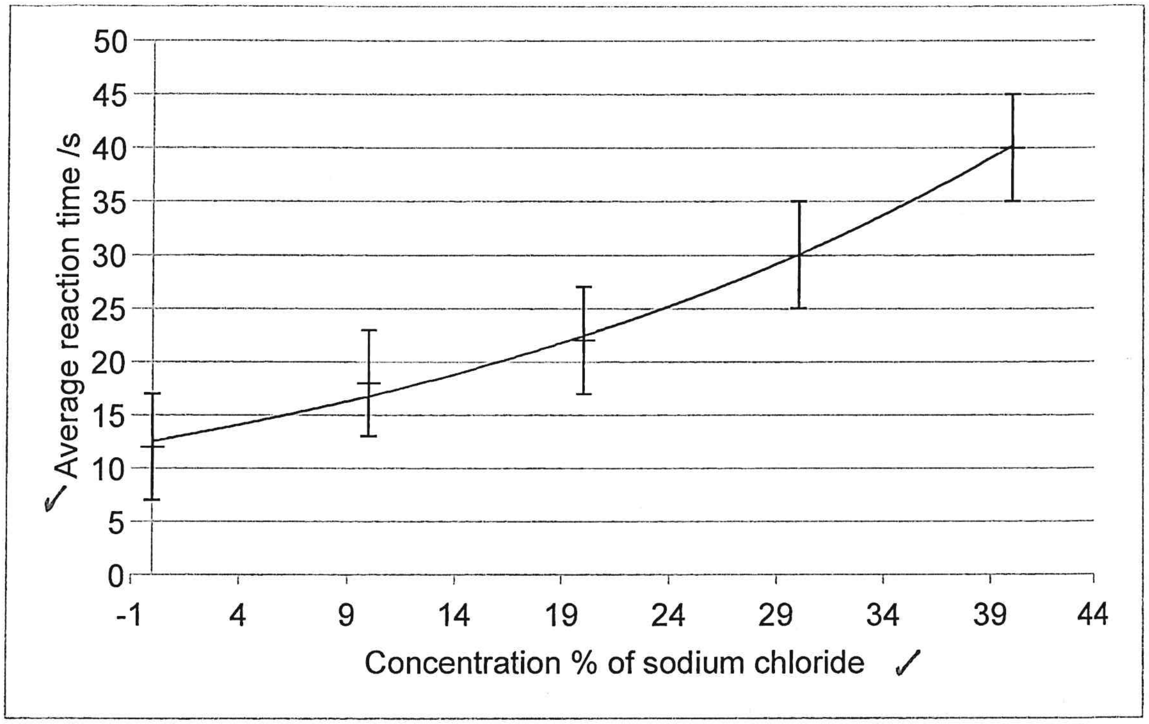


Figure 1 – The average reaction time /s of complete change in colour of amylase/starch solution at different concentrations % of sodium chloride

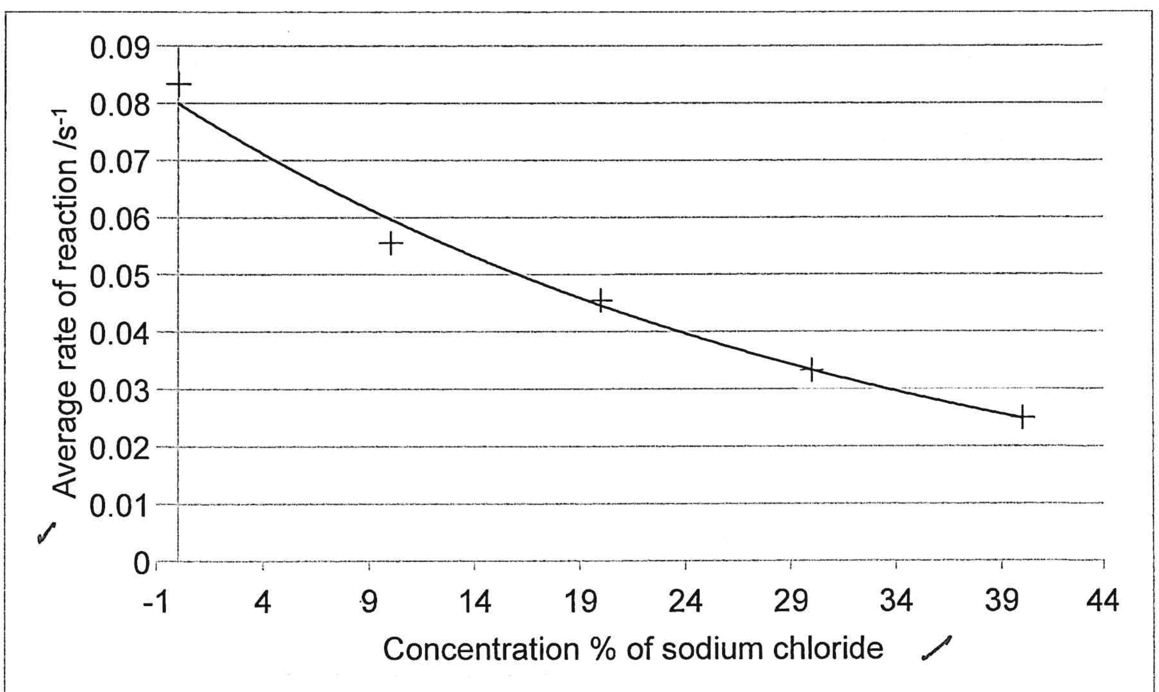


Figure 2 – The average rate of reaction /s^{-1} of complete change in colour of amylase/starch solution at different concentrations % of sodium chloride

Appropriate, sufficient, accurate data processing.

Analysis = 5/6

Conclusion:

Table 2 and Figure 2 show that the rate of reaction for the hydrolysis of starch at 37 °C and pH 7.65 decreases as the concentration of sodium chloride solution increases for values of 0, 10, 20, 30 and 40 %. As the concentration of sodium chloride is increased from 0 % to 40 %, the average rate of reaction decreases from 0.0833 to 0.0250 s^{-1}. This supports the theory that a change in sodium chloride concentration causes denaturation of amylase by disrupting ionic interactions between amino acid R groups, and changing the secondary and tertiary structures of the enzyme. ✓

Figure 2 shows that the average rate of reaction is at its minimum value of 0.0250 s^{-1} at a sodium chloride concentration of 40 % under the conditions of 37 °C and pH 7.65. However, the shape of the graph between all of the respective sodium chloride concentration values cannot be deduced, as it is unclear whether the graph is a straight line or a curve within this region. Hence the exact range of values that give the optimum sodium chloride concentration, under the conditions of 37 °C and pH 7.65, cannot be determined. ✓

A detailed, focused conclusion with correct reference to rate.

Evaluation:

Table 2 and Figure 2 show that there is some correlation between the average rate of reaction and sodium chloride concentration, for salt concentrations of 0, 10, 20, 30 and 40 %. However, it is unclear exactly what the relationship is between the average rate of reaction and the sodium chloride concentration. In order to determine this relationship, more experiments at different concentration values between 20 % and 40 % would be needed. Since the greatest decrease in rate of reaction is between sodium chloride concentration values of 20-30 % and 30-40 %, more experiments within this range would be required. ✓

Table 1 shows that the rate of reaction varied across the trials for sodium chloride concentration values of 0, 10, 20, 30 and 40 %. More trials would be needed for each value of sodium chloride concentration in order to determine the reliability of the data and identify anomalies more easily. ✓

There are a number of ways in which the experiment could be improved to give more reliable data and so allow a more accurate conclusion to be drawn:

Error	**How could it affect the results?**	**How could this be improved?**
Number of different values for sodium chloride concentration	Limited data (five values for sodium chloride concentration) collected to establish the relationship between average reaction time and sodium chloride concentration. More values of sodium chloride concentration within the range 10-40 % would allow a more accurate determination for the optimum sodium chloride concentration.	Carry out more experiments within the range of 0-40% sodium chloride concentration. Experiments with the following sodium chloride concentrations could be used: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 %.✓
Sampling time	Samples of the reaction mixture were taken every 10 s, making reaction time accurate to the nearest 10 s (± 10 s). Difficult to determine anomalous results. Data used to calculate average reaction time and rate of reaction maybe inaccurate, leading to inaccurate interpretation and conclusion.	Decrease the sampling time to reduce the uncertainty of reaction time. Take samples of the reaction mixture every 5 s.✓
Uncertainty in judging colour change	Uncertainty in judging the point when the iodine solution remained orange. Affects the measured reaction time, since samples of the reaction mixture were taken every 10 s.	Use a colorimeter to determine the point when all the starch is hydrolysed. Colorimeter removes the uncertainty due to judging colour change. Also reduces uncertainty in sampling time. Gives more accurate interpretation and conclusion. ✓
Uncertainties in measurement	Graduated pipette 5 ml: ± 0.05 ml Graduated pipette 2 ml: ± 0.02 ml Volumetric flask 100 ml: ± 0.20 ml Thermometer -10 to +110 °C: ± 0.5 °C Electronic balance: ± 0.001 g Smartphone stopwatch: ± 0.01 s	Small uncertainties in measurement can be reduced further if larger measurements are used. Doubling the volumes of the reaction mixture will decrease the error. ✓
Optimum pH 6.7-7	The pH of the buffer used in the experiment was 7.63. This means that the amylase was not at its optimum pH, therefore the reduction in enzyme activity was due to salt concentration and pH.	Use a pH buffer that is within the range of the optimum pH 6.7-7. ✓

Detailed improvements

Possible extension of the investigation:

The composition of electrolytes in saliva is not constant. The table shown below gives the range of concentrations for ions present in saliva:

Ion	mmol/l
Sodium (Na^+)	2-21
Potassium (K^+)	10-36
Calcium (Ca^{2+})	1.2-2.8
Magnesium (Mg^{2+})	0.08-0.5
Chloride (Cl^-)	5-40
Hydrogencarbonate (HCO_3^-)	25
Phosphate (PO_4^{3-})	1.4-39

Sodium, potassium, magnesium, and calcium ions have different charge densities due to their different charge and ionic radii. Magnesium ions have the greatest charge density out of these four cations, since magnesium ions have the greatest charge (2+) and the smallest ionic radii. Hence, the magnesium ion is able to form the strongest ionic bond with an anion, such as the chloride ion. A higher concentration of magnesium ions in saliva may disrupt attractions between charged amino acid side chains more readily than sodium ions, causing a greater degree of denaturation of the enzyme.

An extension of this investigation could involve determining the optimum concentration of magnesium chloride for the hydrolysis of starch by amylase at its optimum temperature (37 °C) and pH (7). **("Saliva Composition and Functions.", "Saliva.")**

The suggestion for extending the investigation is described.

Evaluation = 5/6

Personal Engagement = 2/2

The student demonstrated initiative and interest throughout the investigation, and carried out the method with attention to detail.

Communication = 4/4

The report is well structured and uses correct scientific terminology throughout.

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