

Unit 3 Advanced Subsidiary

T1 Exemplar Coursework

Investigation 4

Effect of copper sulphate on a solution of amylase and starch using iodine

EFFECT OF COPPER SULPHATE ON A SOLUTION OF AMYLASE AND STARCH USING IODINE.

What was my experiment about ?

My experiment was to find out the effect of changing the copper sulphate concentration on the rate of the hydrolysis of starch to maltose using the enzyme amylase. Copper sulphate was used because it acted as an inhibitor.

Hypothesis,

I predicted that the more copper sulphate added to the solution the longer the reaction would take to complete. This was due to the fact that copper sulphate would interfere with the starch for the active sites on the amylase molecules because it is a inhibitor.

Biological Knowledge,

Amylase acts as a catalyst and starch is a complex carbohydrate.

The amylase was used to hydrolyse the alternate glycosidic bonds in starch. By doing this it meant that the starch, a polysaccharide was broken down into maltose, a disaccharide. However, copper sulphate would interfere with starch for the active sites and so prevent the formation of enzyme-substrate complexes. Fewer complexes formed means that the time taken to hydrolyse the starch would be greater and the rate of the reaction would be slower.

My test was to find out what effect copper sulphate had on a solution of amylase and starch using iodine.

Outline Method,

- select different amounts of copper sulphate, 0.02, 0.04, 0.06, 0.08 and 0.1 g.
- dissolve the copper sulphate in 10 ml of water
- control 10 ml of water with no copper sulphate
- set up boiling tubes containing 20ml amylase
- set up boiling tubes containing 20ml starch,
- set up water bath at controlled temperature (40 C), insert all tubes for 10 mins to get them all to the same temperature
- mix tubes of starch and amylase and add copper sulphate
- place boiling tubes retained in a boiling tube rack into water bath,
- take a reading at a controlled time interval (every minute) by placing a drop of stirred mixture with a drop of iodine in a spotting tray

Controlled variables,

I decided to control the following variables:-

1. same volume and concentration of amylase
2. same volume and concentration of starch
3. same volume and concentration of iodine
4. same temperature by using a water bath
5. same amount of stirring, three complete turns for each test with the glass rod.

Equipment,

2% Amylase,
2% Starch,
Copper Sulphate,
3 10ml syringe,
6 Glass rods,
18 Boiling tubes,
Spoon,
3 Test tube racks,
Water bath - set at 40 °C,
Scales,
Iodine,
Spotter trays,
Timer

Safety

I had to take the usual the usual care by using safety glasses and to clear up any spillage, but I was not dealing with anything particularly hazardous.

Outline of my Results.

All the tests started of black, this indicated the presence of starch. At different times each different solution went a green colour, then they went a dark brown, before finally going light brown, this indicated the lack of starch due to it having been broken down to maltose.

Conclusion.

If we look at the graph showing time taken we see a trend, this being that the more copper sulphate added to the solution the longer the reaction would take to be completed, we can say that the graph shows that this relationship is directly proportional. This is due to the interference of the copper sulphate and how easily the starch can link with the active sites on the enzyme. The inhibition of the enzyme and the formation of enzyme-substrate complexes has increased the time taken for all the starch to be hydrolysed.

Whereas the graph showing the rate of the reaction contradicts that of the graph showing the time taken, I say this because the less copper sulphate present the higher the rate is. We can say that the graph is inversely proportional. This means that the rate at which the reaction took is faster with a low concentration of copper sulphate compared to that of a high concentration of copper sulphate. This is the same as the time taken graph, again this is due to the starch being broken down faster with lower concentrations of copper sulphate.

My only anomalous result seems to be at 0 g. of copper sulphate which seems to work faster than if a straight line had been drawn through all the points of the graph.

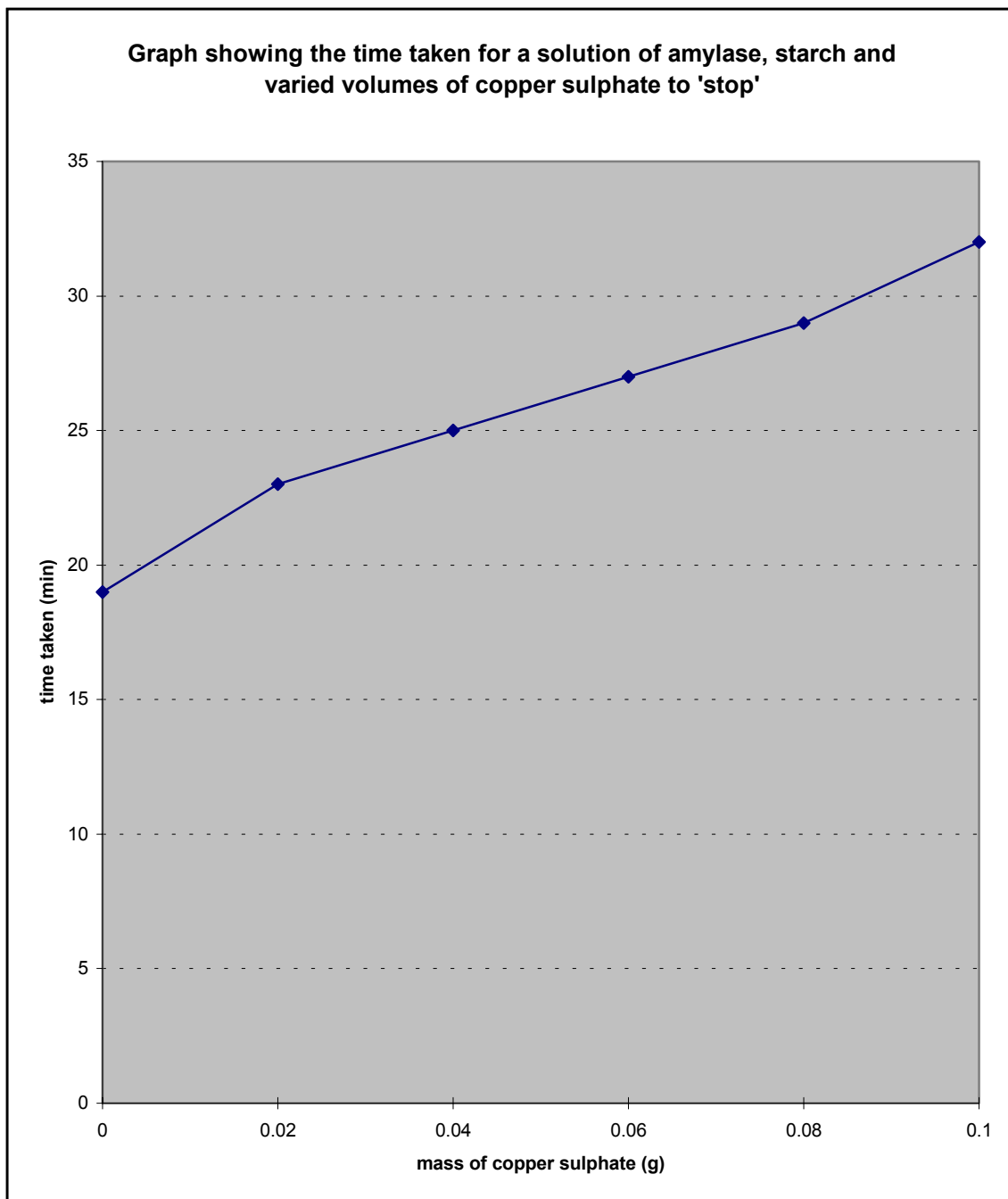
Evaluation.

I had one anomalous result, the value for the control, did not sit exactly on a straight line of best fit. This straight line suggests that I can be reasonably confident that my results are reliable and that the rate of reaction is proportional to the concentration of inhibitor. During my experiment there may have been some limitations, such as the copper sulphate concentrations, by this I mean that I could have made up several more different concentrations of copper sulphate so that I was able to obtain a wider range of results. I never found that the copper sulphate completely prevented the reaction from happening, at higher concentrations of copper sulphate I might find that this happens. I only measured the colour every minute so that my results were not very precise, trying to record the colour every 10 or 15 seconds would have been better. I did not repeat any of my results, which I should really have done so that I could be sure my results were reliable. The size of the drops on the glass rod and in the spotting tray may have varied so that I cannot be sure of all my results. It was difficult to see colour change so my method could have been unreliable. I could have used a colorimeter, which measures the intensity of the colours formed.

My results show that the copper sulphate has inhibited the hydrolysis of starch, however I have not found out if the copper sulphate competes with the starch for the active sites or not. I could investigate this by also altering the starch substrate concentration, because if there was competition then the inhibitor would have a reduced effect if there was more starch.

Results

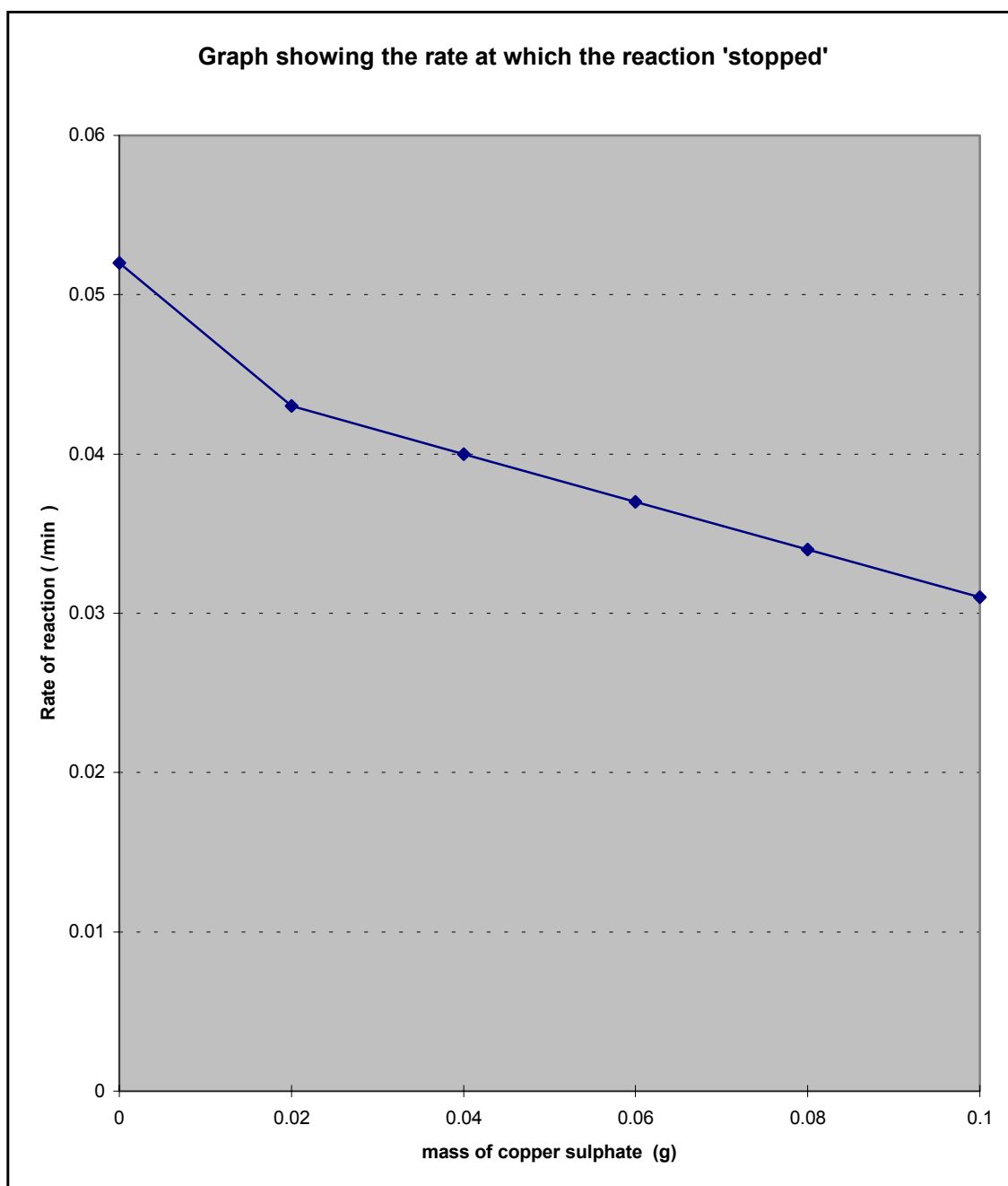
CuSO (g)	Time Taken (min)
0	19
0.02	23
0.04	25
0.06	27
0.08	29
0.1	32



Results

CuSO (g)	Rate (/min)
0	0.052
0.02	0.043
0.04	0.04
0.06	0.037
0.08	0.034
0.1	0.031

$$\text{Rate} = \frac{1}{\text{time}}$$



Commentary

Planning

- (d) A testable hypothesis hypothesis is formulated independently and is stated very concisely. Biological knowledge is used to explain the prediction. The quality is better than 4 and towards 6 marks. The nature of inhibition is not defined at all. 5 marks is probably appropriate.
- (e) The important variables to be controlled are described. The apparatus is listed but not all justified. A range of concentrations is identified but there is no indication why this particular range was chosen and no suggestions for repeating the collection of data are included. Again this is better than 4 but not up to 6.
- (f) The attempts to assess safety are superficial and merit little more than 4 marks.

Overall there are aspects to merit marks of between 4 and 6; so the mark to be awarded will be in the third tier as all the criteria for 4 are met, 5 is most appropriate.

Implementing

- (a) Simple apparatus was used competently. The range of manipulative techniques was narrow but used safely. 4 marks.
- (b) The work was carried out independently and in an organised and safe manner. 4+ marks.
- (c) Mass measurements were made to 0.01 g, but time only to minutes, which are not SI units; candidates often misuse minutes and seconds as decimals. Precision was thus only reasonable. Data were recorded in suitably headed tables. 4 marks

Overall relatively simple techniques and low degree of precision, but the investigation was carried out independently. 4 marks.

Analysing

- (a) Summary tables are presented and the choice of graphs was selective and illustrated rate of reaction as well as time. The use of ICT included correctly plotted points, not a smoothed curve. Appropriate format was used. 6 marks.
- (b) Trends and patterns in the data were clearly recognised and commented on. The anomaly of the control was indicated. 6 marks.
- (c) The explanations were sound and related to fairly basic biological knowledge. 5 marks.

Overall, this is working Summary tables are presented and the choice of graphs was towards 6 marks but is not really worth 6 marks, particularly as the explanations are simple. 5 marks.

Evaluating

- (a) The evaluation repeats the anomaly of the control but this is not further explored. The candidate is aware that the results may not be entirely reliable, but suggests that the straight line graphs support the conclusion drawn. 5 marks.
- (b) The candidate is aware of the limitations of the colour change and the poor precision over timing and proposes sensible extensions of the inquiry by investigating changes in substrate concentration to explore whether the inhibition is competitive. 6 marks

Overall the candidate is reasonably aware of the lack of precision and makes some sensible suggestions for improvement and extension. 5 marks.

Planning	-	5
Implementing	-	4
Analysing	-	5
Evaluating	-	5
Total	-	19/32