An Experiment to show the affect of Copper Sulphate Solution on Catalase.

Introduction.

Reactions of chemicals are an important part of life. Enzymes are biological catalysts. A catalyst is a substance which increases the rate of a reaction by attracting the reactants, this makes them more likely to collide and react. There are other conditions which can effect the rate of a reaction (these are mention in the prediction).

Aim.

The aim of this experiment is to discover the effect that copper sulphate has of the enzyme catalase. This will be achieved by using hydrogen peroxide, catalase/potato (rich in catalase) and a buffer. The effect will be the amount of oxygen gas that is released which is measured using a graduated gas syringe.

Hypotheses.

Hypothesis: Copper Sulphate solution will inhibit (slow down) the rate of the reaction between Hydrogen peroxide and catalase. The concentration of the Copper sulphate solution is inversely proportional to the rate of the reaction.

Null Hypothesis: Copper Sulphate solution has no effect of the rate of reaction between Hydrogen Peroxide and catalase. As the Concentration of the copper sulphate solution is altered the rate of reaction will remain constant.

To prove one of these hypotheses a fair test experiment will have to be strictly obeyed to ensure a true representation.

Prediction.

An increase in the concentration of the copper sulphate solution is inversely proportional to the rate of the reaction. High Concentration = Slow Rate of reaction etc.

I predict this because copper sulphate solution contains the heavy metal ion Copper. Heavy metal ions are good inhibitors. They break down the structural disulphide bonds which deform the enzyme.

Temperature: Biological enzymes work most efficiently at a temperature slightly above that of the body. If the temperature is lower than this then the particles in the reaction have less kinetic energy so they move slower. Slow moving particles do not collide as often as particles at an optimum temperature of approximately 40°C.

If the temperature increases to over 60°C, the enzymes become denatured. This means that they are unable to react. The increased vibration of the molecules causes hydrogen bonds to be broken. Hydrogen bonds are the structural frame the atom which gives the enzyme it's three-dimensional shape. The active site is deformed so the substrate cannot enter. The reaction cannot take place with the aid of a catalyst. Denaturation means that the enzyme cannot fulfil it's role as a catalyst.

The temperature that the reaction takes place at can be effected by body temperature. It the conical flask is being held it will heat up. An increase in temperature will lead to the expansion of the oxygen gas. The reading on the gas syringe will be inaccurate due to this excess oxygen gas which has been forced out of the conical flask as the result of the increase in body temperature. Not only this but also the temperature of that the reactants are at will be altered. A constant temperature would be impossible to maintain in such conditions.

pH: Most enzymes have an optimum pH of around 7 (neutral). This is the condition that they work most efficiently at.

H+ ions are present in large quantities in acids (low pH numbers). Fewer are present in alkaline substances. Hydrogen ions are capable of breaking down hydrogen bonds which give the enzyme's three-dimensional structure. If too many H+ ions are present, the enzymes become denatured and do not catalyse the reaction. (pepsin is an enzyme which does not have an optimum pH of neutral. Pepsin is found in very acidic conditions in the stomach. To survive and be able to catalyse reactions in the stomach, it has to be adapted to have an optimum temperature of around pH 2.)

Graphs to show the effect of temperature/pH on the enzyme catalase.

Concentration can affect the rate of a reaction. An increase in the concentration of the substrate means there is more of the substance for the other reactant to react with. Therefore, the rate of reaction is increased.

If the concentration of the enzyme, catalase, is increased, it is capable of acting on more particles of the reactants. The enzyme brings more of the reacting particles together so the rate of the reaction is increased. A decrease in either of these concentrations would result in a slower reaction.

An inhibitor is a substrate which competes with the catalyst. It aims to deactivate the enzyme's active site. It achieves this by either deforming the structure of the enzyme (a non-competitive inhibitor) or by occupying the active site (a competitive inhibitor).

The presence of an inhibitor would slow down the rate of the reaction to it's normal rate (how it would be without the catalyst).

Copper in CuSO4 is a heavy metal ion. The nature of these ions makes them very good inhibitors. They cause the structural disulphide bonds of the enzyme to break; therefore the three-dimensional shape is lost. The deformed enzyme destroys the active site so the reactants are unable to react under the influence of the catalyst. The reaction occurs at its 'normal' speed.

A Graph To Show The Effect of Rate of the Reaction Against the Concentration of Copper Sulphate.

Diagrams to Show the process of inhibition

Variables.

- Presence of an inhibitor – If the copper sulphate solution contains an inhibitor, the rate of the reaction would be slow. This can only be proven if the variable are kept constant (apart from the concentration of the

copper sulphate solution). The indication of the copper sulphate solution being an inhibitor will be confirmed by the comparison of the results with the results of the 'standard test'. If a sign of inhibition is also shown in the standard test this will prove that it is not the copper sulphate solution which inhibits the reaction of hydrogen peroxide and catalase. Another variable would have to be altered to discover the inhibitor of the reaction.

- pH – This is controlled by the volume of the buffer and it's pH. The pH of the solution will be tested before each experiment using a pH meter. The pH should measure roughly 7, as this is the optimum pH of catalase (and the majority of enzymes). This variable must be kept constant in order to abide by the fair test criteria.

- Temperature – A water bath can be used to maintain a constant temperature throughout the reaction. The reactant are put in the water bath for a minute to achieve equilibrium. The Conical flask should not be hand held. This would increase the temperature inside the conical flask which would cause the oxygen gas to expand and move at a quicker rate into the gas syringe. The results would then be inaccurate.
  Using an optimum temperature of 30°C means that the reaction is at it's most efficient temperature, therefore larger volumes of gas will be produced (if it is not inhibited). Large volumes of gas are easily measured accurately. It will be easier to differentiate between the inhibited reaction and the uninhibited reaction.
  This variable must be kept constant in order to abide by the fair test criteria.

- Concentration of enzyme – A powder form of catalase can be used and mixed with water to form a catalase solution. The solution must be stirred so a constant concentration is maintained throughout the solution. The amount of catalase will be weighed, using a top pan balance, to the value of 1.00g (to the nearest 1/100 of a gram).
  The Rate of the reaction is directly proportional to the number of collisions, which is increased by the use of more of the enzyme.
  This variable must be kept constant in order to abide by the fair test criteria.

- Concentration of copper sulphate solution – This can be adjusted by diluting the copper sulphate with distilled water.

- Concentration of reactants/surface area of the reactants – This can be controlled by weighing the catalase powder, and using a pipette for the distilled water-to obtain accurate volumes.

This variable must be kept constant in order to abide by the fair test criteria.

- Time length for which results are recorded – The results will be recorded for a short period of time approximately 10 seconds. This time limit will be confirmed by the trial experiment.
  The rate of a reaction slows down after about 20 seconds. The result would not show a figure true to a steady release of gas is the time limit was above 20 seconds.
  This variable must be kept constant in order to abide by the fair test criteria.

- Constant concentration of Substrate (hydrogen peroxide) – This can be achieved by using the same equipment (the volume of a gas syringe may vary slightly from another). To measure out the solutions a pipette could be used for a good degree of accuracy.
  This variable must be kept constant in order to abide by the fair test criteria.

- The Volume of the equipment – The same equipment should be used. Apparatus often varies slightly in volume and performance rate. For example, the same graduated gas syringe should be used because the ease at which gas can enter the syringe varies (performance rate), and also the graduations may vary slightly.
  This variable must be kept constant in order to abide by the fair test criteria.

- The reactant should be mixed thoroughly as soon as the reaction starts. This ensures that a constant concentration will be maintained throughout the reacting mixture.

Apparatus: Conical flask with side arm, delivery tube with bungs, graduated gas syringe 30cm^3, pipette with filler, stopwatch, buffer solution (pH7), hydrogen peroxide, copper sulphate solution, catalase powder, & distilled water.

Method.

The Standard.

This is done by setting up a conical flask as show in the diagram above but without adding the Copper sulphate solution. If my prediction is correct the rate of the reaction should be faster in this conical flask compared to conical flask when the copper sulphate was present.

The control will give the experiment a marker. If the results of the experiments with the presence of he copper sulphate solution are shown to have a decrease in the speed of the reaction, it will be clear that copper sulphate inhibits a catalase reaction. If the further results show a similar result of the control, then it will show that copper sulphate solution has no effect on a catalase reaction.

Control Experiment.

Put $5cm^3$ of the buffer (pH 7) in a conical flask with $2cm^3$ of Hydrogen peroxide and water. The control is set up to test if the rate of the reaction is dependent on the concentration of the copper sulphate solution. In the copper sulphate solution inhibits the

reaction this control should have a faster rate of reaction. This will prove whether the presence of copper sulphate solution is the inhibitor or if it is another substance within the reaction i.e. Hydrogen Peroxide.

Method with Copper Sulphate Solution.

The apparatus was set up as shown in the diagram above. They were placed in a water bath of temperature 30°C and left to equilibrilate.

$5cm^3$ of the buffer (pH 7) was placed in a conical flask with $2cm^3$ of hydrogen peroxide. 1g of catalase powder/potato sample was added to the conical flask. The volume of gas produced by the reaction was measured using a graduated gas syringe (which was connect to the conical flask using a delivery tube). The volume of gas was recorded in a table (shown in the results section).

NOTE: The hydrogen peroxide was placed in the syringe and added to the mixture when all bungs had been placed on. This allows all gas from the start of the reaction to be collected. The conical flask is then gentle swirled to mix the reactants.

The procedure was repeated but with adding copper sulphate solution of amounts: $2cm^3$, $4cm^3$, $6cm^3$, $8cm^3$,& $10cm^3$ in different test tubes.

All results were recorded using a table which can be found in the results section.