

Edexcel A Biology A-Level

Core Practical 4

Investigate the effect of enzyme and substrate concentrations on the initial rates of reactions.





The rate of reaction of an enzyme-controlled reaction is influenced by different factors: **the temperature, pH, concentration of the substrate, and the concentration of the enzyme**. The effect of each of these can be determined by **changing a single variable** and measuring its effect on the rate of reaction. It is important to **keep all other variables constant** so that they do not influence the results. Initial rate of reaction is measured because the **rate of an enzyme-controlled reaction is high**, this is because enzymes act as biological catalysts, so the concentration of reactants changes rapidly. The **initial rate** is the **only point** during the reaction when **concentration of reactants and products is known**.

Equipment

- Milk powder solution
- Trypsin solution (1%)
- Test tubes
- Test tube holder
- Stopclock
- 5cm³ pipettes
- Goggles
- Colorimeter
- Cuvettes
- Distilled water

Method

1. **Dilute** stock solution of trypsin with distilled water to produce solutions with concentrations of 0.2%, 0.4%, 0.6% and 0.8%.
1. Make a **control** by adding 2cm³ of trypsin solution and 2cm³ of distilled water. Use this to set the colorimeter absorbance to zero.
2. To another cuvette, add 2cm³ of milk suspension and 2cm³ of the stock trypsin solution. Mix, place in the colorimeter and **measure absorbance at 15 second intervals for 5 minutes**.
3. Rinse the cuvette with distilled water.

$$\text{Rate of reaction} = \frac{1}{\text{mean time}}$$

4. Repeat step 3 at **all trypsin concentrations**.





Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Broken glass	Cuts from sharp object	Take care when handling glass objects; keep away from edge of desk	Elevate cuts; apply pressure; do not remove glass from wound; seek medical assistance	Low
Hot liquids	Scalding	Handle with care; use tongs to remove boiling tubes from water bath; wear eye protection, keep away from edge of desk	Run burn under cold water; seek medical assistance	Low
Enzymes	Allergies	Avoid contact with skin/eyes; wear eye protection	Seek assistance	Low

Graph

- Plot a graph of **rate of reaction** against **temperature**.

Conclusion

- Milk contains a white protein called **casein** which, when broken down, causes the milk to turn **colourless**. **Trypsin** is a **protease enzyme** which **hydrolyses the casein**.
- As concentration of trypsin **increases**, the **number of enzyme-substrate complexes forming also increases** because enzymes and substrates are more likely to collide. This means that the **rate of reaction increases** up to the **optimum** enzyme concentration.



- The **rate plateaus** at the point where **all substrates** occupy an active site. Increasing the enzyme concentration won't increase rate as **substrate concentration** is limiting the rate.

Modification

The procedure can be modified to measure the effect of **substrate concentration** on initial rate of reaction by diluting the milk suspension to produce different concentrations and **controlling concentration of trypsin**.

