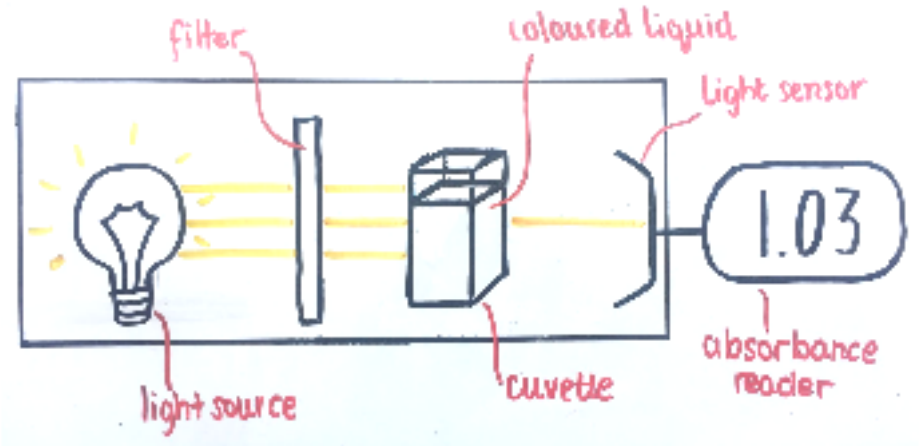
Edexcel IAL Biology A Level

Core Practical 4

Investigate the effect of temperature, pH, enzyme concentration and substrate concentration on the initial rate of enzyme-catalysed reactions.

This experiment investigates the effects of different factors on the rate of the digestion of milk by the enzyme **trypsin**. As the milk protein called casein is digested the **white, opaque milk colour** becomes **more pale and translucent**, eventually turning **colourless. More light** passes through the **transparent and lighter solutions** so a colorimeter can be used to measure the absorbance of the solution which in turn indicates the rate of reaction of the experiment. The faster the rate of the reaction, the **lower** the initial absorbance reading will be - **as paler solutions absorb less light.**



Equipment list

- Colorimeter
- Cuvettes
- Water baths at the following temperatures: 20°C, 30°C, 40°C, 50°C and 60°C
- pH buffers at the following pHs: 5, 6, 7, 8 and 9
- Distilled water
- Stopwatch
- Test tubes
- Test tube rack
- Protease enzyme solution - 1% trypsin enzyme solution
- 2% semi-skimmed milk
- Pipettes
- Tongs

Method

Factor 1 - the effect of temperature

1. Prepare the 5 water baths and **monitor their temperatures** using thermometers.
2. Use a pipette to add 2 cm^3 of trypsin solution to 5 test tubes and place one in each water bath.
3. Then use a pipette to add 2 cm^3 of milk to 5 test tubes and again place one in each water bath.
4. Start the stopwatch and time for 5 minutes, **allowing the contents of each test tube to reach the temperature of the water bath.**

5. Pipette 2 cm^3 of trypsin and 2 cm^3 of distilled water into a cuvette and take a colorimetry reading to set the reference absorbance of the colorimeter to **zero.**
6. Now remove the milk test tube and the trypsin test tube from the 20°C water bath and add the contents of each to a single cuvette, shaking it and then immediately placing it in the colorimeter.
7. Take an initial reading and then record the absorbance reading at 15 second intervals for 5 minutes or until there is only a **slight change** in absorbance between readings.
8. Repeat steps 6-7 for the remaining 4 temperatures, being sure to **rinse the cuvettes** between each temperature and setting the **absorbance to zero with the water and trypsin cuvette each time.**

Factor 2 - pH

1. Prepare the reference cuvette by adding 1 cm^3 of buffer solution, 1 cm^3 of trypsin and 2 cm^3 of water to it and setting the colorimeter absorbance of this to **zero.**
2. Prepare 5 cuvettes each containing 1 cm^3 of a **different pH buffer solution** and 1 cm^3 of trypsin solution. Prepare a further 5 cuvettes all containing 2 cm^3 of milk solution.
3. Add the contents of 1 of the milk cuvettes to the cuvette containing the pH 5 buffer solution then **immediately** place it in the colorimeter.
4. Take an initial reading and then record the absorbance reading at **15 second intervals** for 5 minutes or until there is only a **slight change** in absorbance between readings.
5. Repeat steps 3 and 4 with the remaining 4 pH buffer solutions.

Factor 3 - Enzyme concentration

1. Prepare these 10 cm^3 solutions of different enzyme concentrations using the following quantities of distilled water and 1% trypsin solution:

Solution concentration (%)	Volume of trypsin (cm^3)	Volume of distilled water (cm^3)
1	10	0
0.8	8	2
0.6	6	4
0.4	4	6
0.2	2	8

2. Pipette 2 cm^3 of trypsin and 2 cm^3 of distilled water into a cuvette and take a colorimetry reading to set the reference absorbance of the colorimeter to **zero.**
3. Add 2 cm^3 of milk to a cuvette, then add 2 cm^3 of the 1% enzyme solution to the cuvette. Mix quickly then place in the colorimeter.

4. Take an initial reading and then record the absorbance reading at **15 second intervals** for 5 minutes or until there is only a **slight change** in absorbance between readings.
5. Repeat steps 3 and 4 with the remaining 4 enzyme solutions.

Factor 4 - substrate concentration

1. Prepare these 10 cm^3 solutions of different substrate concentrations using the following quantities of distilled water and 2% milk solution:
    -

Solution concentration (%)	Volume of milk (cm^3)	Volume of distilled water (cm^3)
1	5	5
0.8	4	6
0.6	3	7
0.4	2	8
0.2	1	9

2. Pipette 2 cm^3 of trypsin and 2 cm^3 of distilled water into a cuvette and take a colorimetry reading to set the reference absorbance of the colorimeter to zero.
3. Add 2 cm^3 of milk to a cuvette, then add 2 cm^3 of the 1% milk solution to the cuvette. **Mix quickly** then place in the colorimeter.
4. Take an initial reading and then record the absorbance reading at **15 second intervals** for 5 minutes or until there is only a slight change in absorbance between readings.
5. Repeat steps 3 and 4 with the remaining 4 milk substrate solutions.

Risk assessment

Risk	Hazard	Precaution
Liquids	Spillage that could cause surfaces to be slippery leading to an accident	Wipe up any liquid spillages as soon as they occur Put lids on bottles and put them away once used Keep away from edge of desk
Hot water bath	Scalding	Take care in removing and replacing the water bath lid Have a first aid kit nearby Remove test tubes from the water bath with tongs Keep away from edge of desk
Glassware	Cuts from sharp objects	Take care when handling glass objects Keep away from edge of desk
Trypsin	May cause an allergic reaction or respiratory problems if inhaled	Wear gloves when handling to avoid skin contact, if it gets on skin rinse thoroughly with cold water

Results table

Time (seconds)	Absorbance reading
15	
30	
45	
60	
...	