Edexcel GCSE Biology

Your notes

Enzymes

Contents

Your notes

The Action of Enzymes

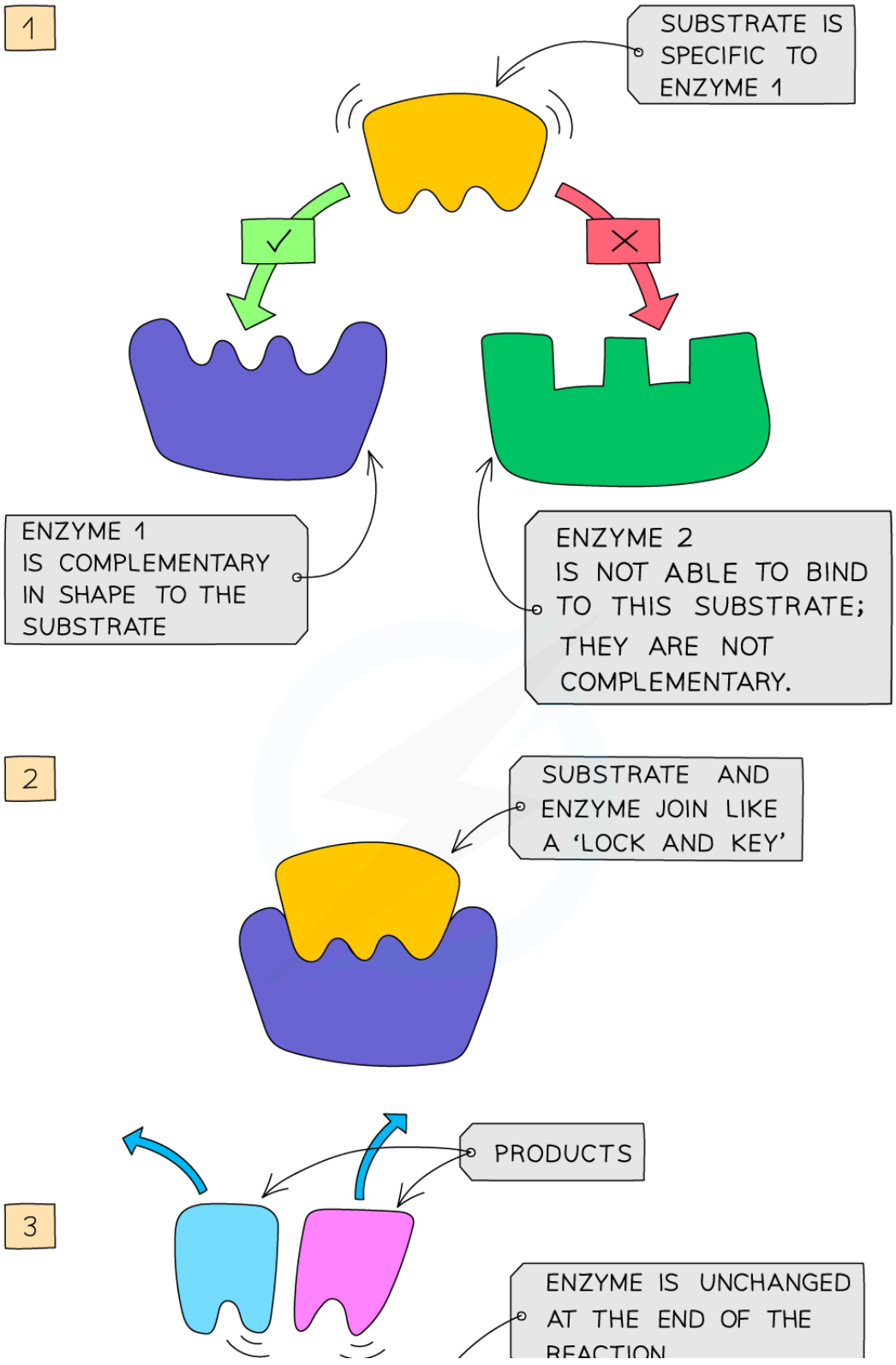
Enzymes

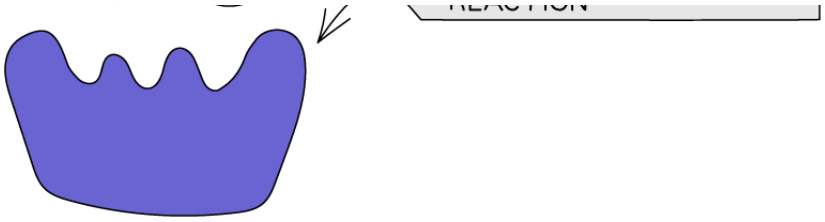
- Enzymes are proteins that act as **biological catalysts** to **speed up** the rate of a chemical reaction **without** being **changed** or **used up** in the reaction
- They are **biological** because they are made in **living cells**
- Enzymes are necessary to all living organisms as they allow all **metabolic reactions** to occur at a rate that can **sustain life**
  - For example, if we did not produce digestive enzymes, it would take around 2 - 3 weeks to digest one meal; with enzymes, it takes around 4 hours

The mechanism of enzyme action

- Enzymes are **specific** to one particular substrate(s) as the **active site** of the enzyme, where the substrate attaches, is a complementary shape to the substrate
- When the substrate moves into the enzyme's active site, the **enzyme-substrate complex** is formed
- After the reaction has occurred, the **products** leave the enzyme's active site, which is then free to take up another substrate
- The steps of an enzyme catalysed reaction are shown in the diagram below and can be summarised as follows:
  - **Step One:** Enzymes and substrates randomly move about in solution
  - **Step Two:** When an enzyme and its complementary substrate randomly collide, an enzyme-substrate complex forms and the reaction occurs
  - **Step Three:** A product (or products) forms (from the substrate) and is then released from the active site. The enzyme is unchanged and will go on to catalyse further reactions

Your notes





How enzymes work

Denaturation of enzymes

- Enzymes are **proteins** and have a **specific shape**, held in place by **bonds**
- This is extremely important around the **active site**, as the specific shape of this area of the enzyme is what ensures the **substrate will fit into the active site** and enable the reaction to proceed
- If the bonds that hold the enzyme together are **disrupted** or **broken** the active site it will **lose its shape** - this is known as **denaturation**
  - The enzyme is said to be **denatured**
  - Substrates cannot fit into denatured enzymes as the shape of their active site has been lost
  - Denaturation is **irreversible** – once enzymes are denatured they cannot regain their proper shape and **the reaction they are catalysing will stop**
  - Denaturation can occur due to **high temperatures** or **extremes of pH**

Your notes

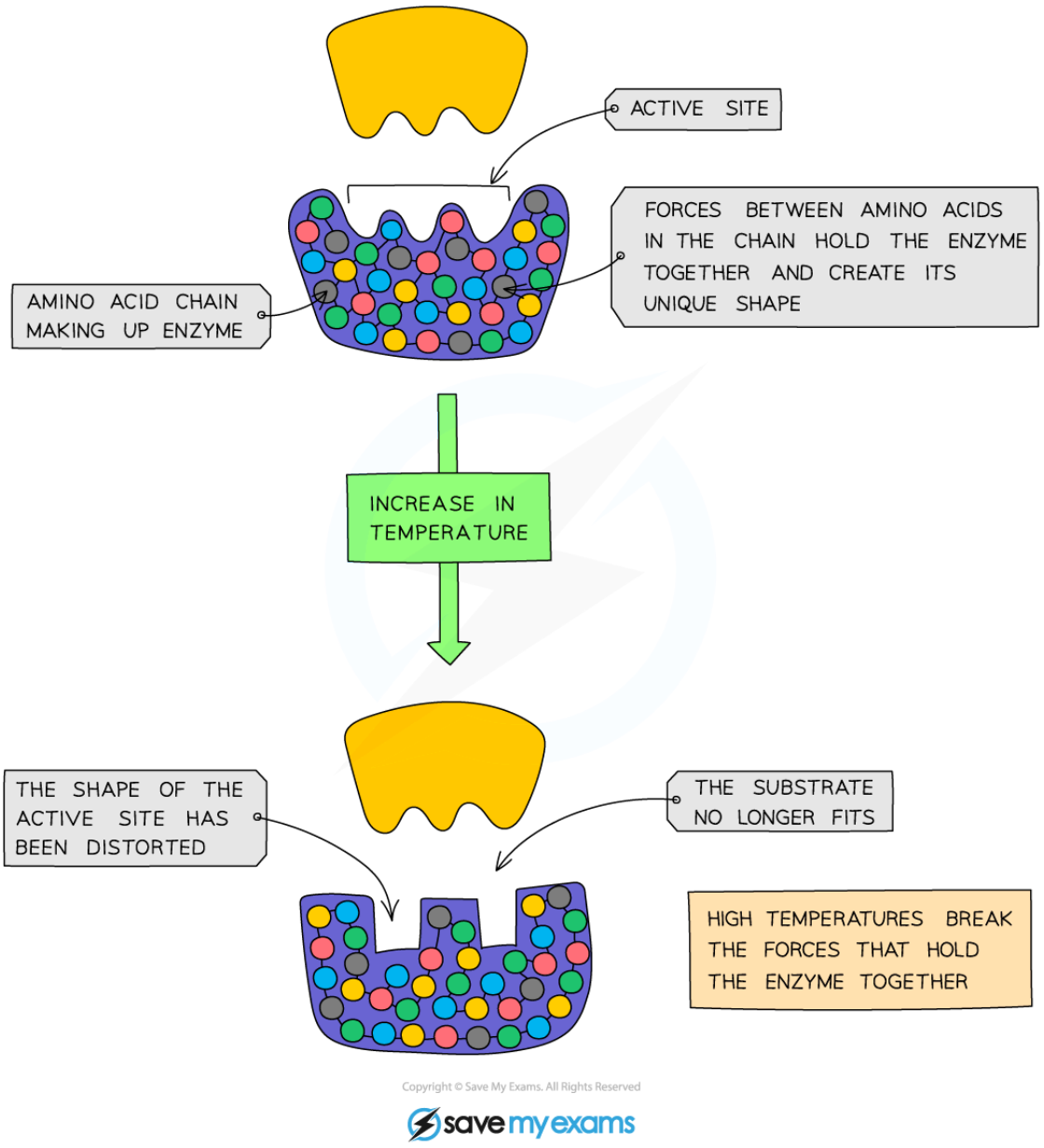
Factors Affecting Enzymes


Your notes

Factors Affecting Enzyme Action: Temperature

- Enzymes work fastest at their '**optimum temperature**'
  - In the human body, this optimum temperature is about **37°C**
- Heating to high temperatures (**beyond the optimum**) will **break the bonds** that hold the enzyme together and the active site will **lose its shape**
  - The enzyme has been **denatured**

Your notes

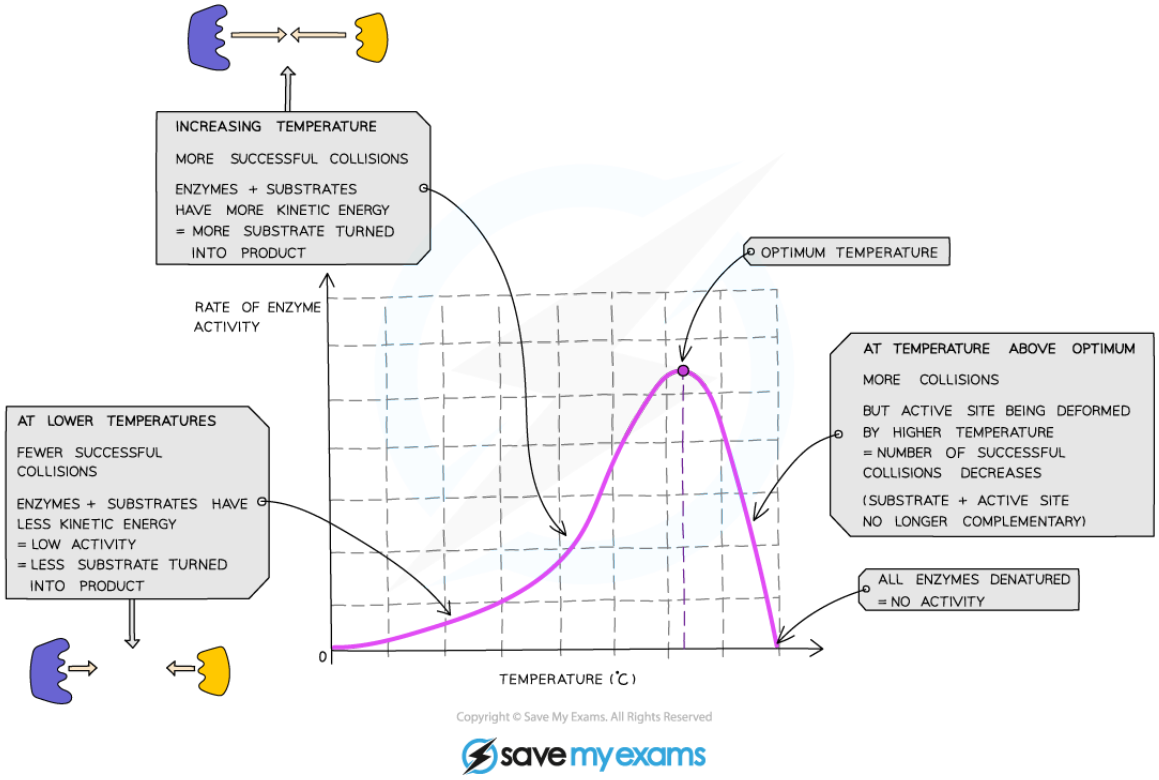


The effect of temperature on enzyme activity

- As **temperature increases** (towards the optimum) **the activity** of enzymes **increases**
  - This is because the molecules have more **kinetic energy, move faster** and have more **successful collisions** with the substrate molecules. This leads to a **faster rate of reaction**

Your notes

- This means that **low temperatures do not denature enzymes**, they just make them work **more slowly** due to a **lack of kinetic energy**



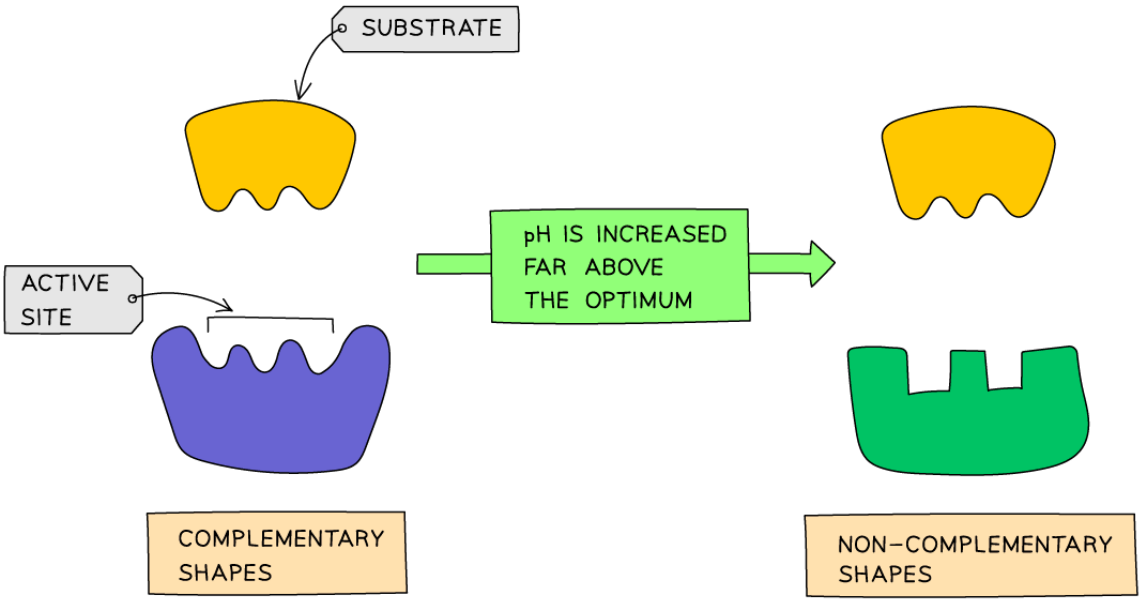
Graph showing the effect of temperature on the rate of enzyme activity

Factors Affecting Enzyme Action: pH

- The **optimum pH** for most human enzymes is **pH 7**
  - Some enzymes that are produced in **acidic conditions**, such as the **stomach**, have a **lower optimum pH (pH 2)**
  - Some that are produced in **alkaline conditions**, such as the **duodenum**, have a **higher optimum pH (pH 8 or 9)**
- If the pH is **too far above** or **too far below** the **optimum**, the bonds that hold the amino acid chain together to make up the protein can be **disrupted** or **broken**
- This will **change the shape of the active site**, so the **substrate can no longer fit** into it, **reducing the rate of activity**

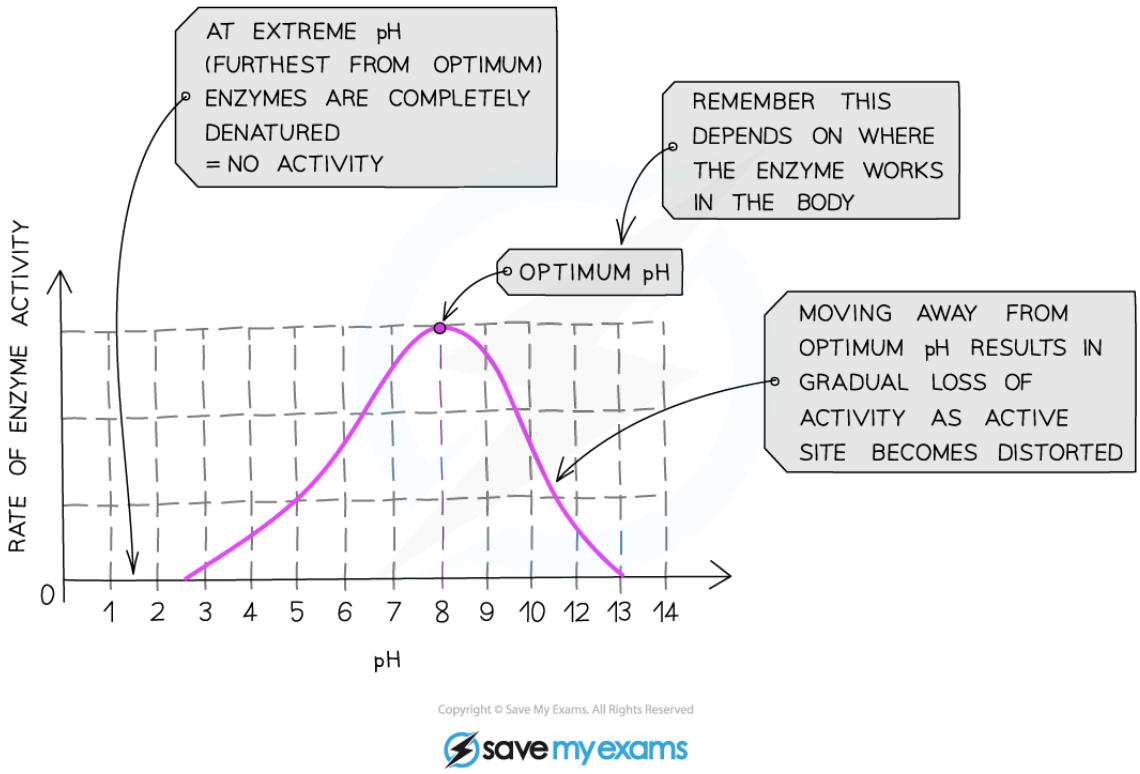
- Moving too far away from the optimum pH will cause the enzyme to **denature** and the reaction it is catalysing will **stop**

Your notes



Effect of pH on enzyme activity

Your notes



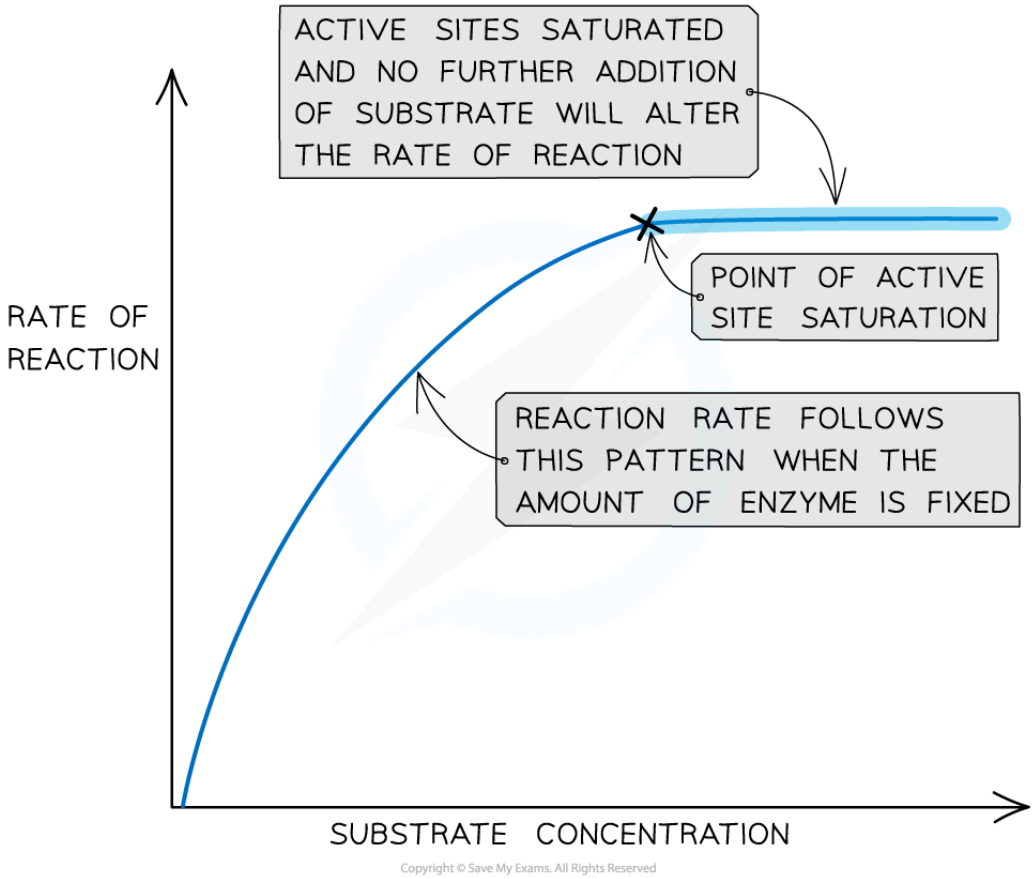
Graph showing the effect of pH on the rate of activity for an enzyme from the duodenum

Factors Affecting Enzyme Action: Substrate Concentration

- The **greater** the **substrate concentration**, the **greater** the **enzyme activity** and the **higher** the **rate of reaction:**
  - As the number of substrate molecules increases, the likelihood of enzyme-substrate complex formation increases
  - If the enzyme concentration remains **fixed** but the amount of substrate is increased past a certain point, however, all available active sites eventually become **saturated** and any further increase in substrate concentration will **not increase** the reaction rate
  - When the **active sites** of the enzymes are **all full**, any substrate molecules that are added have **nowhere to bind** in order to form an **enzyme-substrate complex**
- For this reason, in the graph below there is a **linear increase** in reaction rate as substrate is added, which then **plateaus** when all active sites become occupied

- At this point (known as the **saturation point**), the substrate molecules are effectively 'queuing up' for an active site to become available

Your notes



The effect of substrate concentration on the rate of an enzyme-catalysed reaction

Examiner Tips and Tricks

Remember the terminology when writing about enzymes is very important. Make sure you refer to an enzyme becoming 'denatured' not 'dying'.Being able to describe AND explain the effect of each environmental condition on enzyme action is key.Practise describing and explaining using the graphs and then check your descriptions against your notes.

Your notes

Practical: Enzymes & pH

- **Amylase** is an enzyme that digests **starch** (a polysaccharide of glucose) **into maltose** (a disaccharide of glucose)
- The effect of different pH levels on the activity of amylase can be investigated

Apparatus

- Spotting tile
- Measuring cylinder
- Test Tube
- Syringe
- Pipette
- Stopwatch
- Buffer solutions
- Iodine
- Starch solution
- Amylase solution

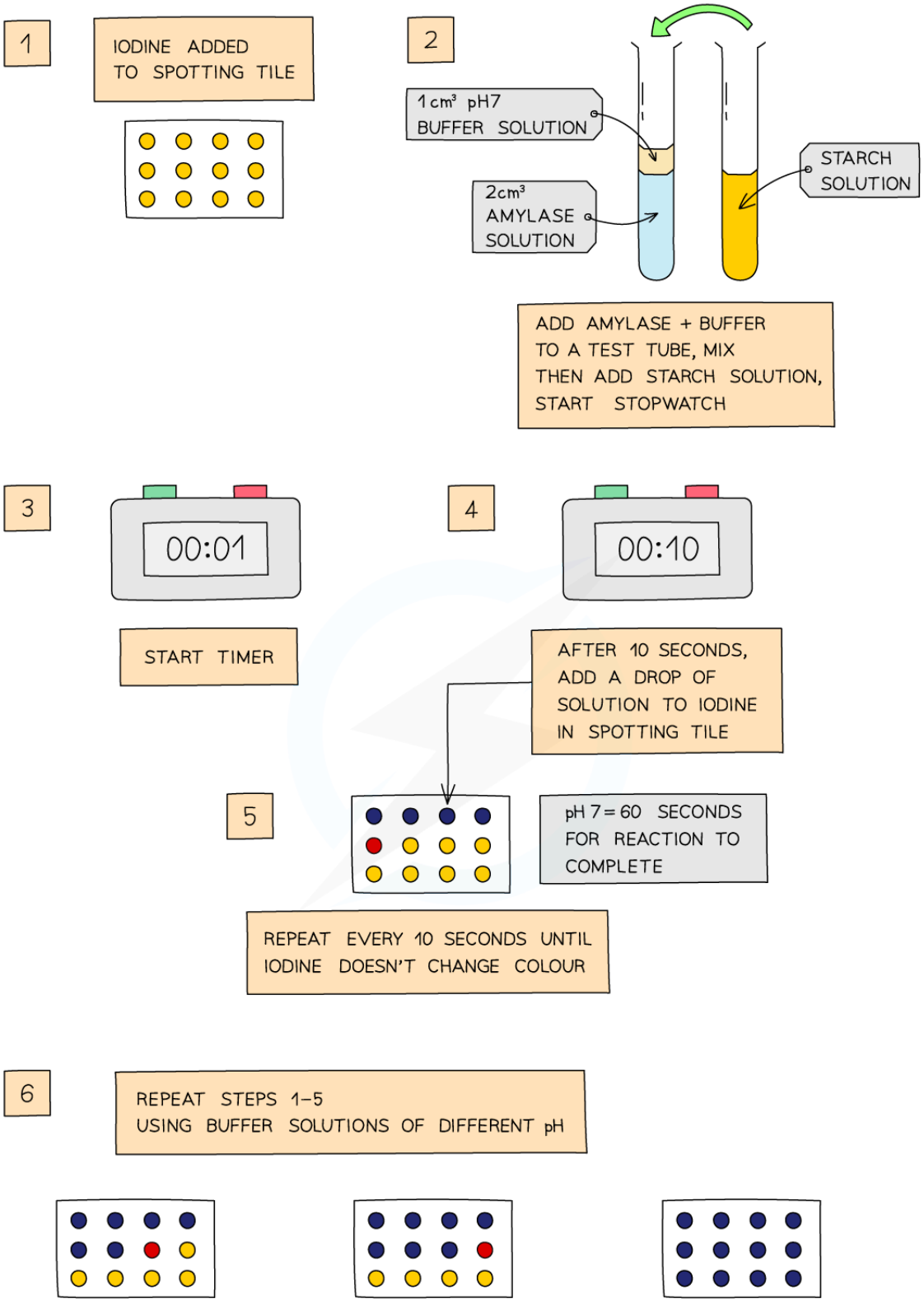
Method

- Add a drop of **iodine** to each of the wells of a spotting tile
- Use a syringe to place 2 cm^3 of **amylase** into a test tube
- Add 1cm^3 of **buffer solution** (at pH 2) to the test tube using a syringe
- Use another test tube to add 2 cm^3 of **starch solution** to the amylase and buffer solution, start the stopwatch whilst mixing using a pipette
- Every 10 seconds, transfer a droplet of the solution to a new well of iodine solution (which should turn blue-black)
- Repeat this transfer process every 10 seconds until the iodine solution **stops turning blue-black** (this means the amylase has broken down all the starch)
- **Record the time** taken for the reaction to be completed

- Repeat the investigation with buffers at different pH values (ranging from pH 3.0 to pH 7.0)


Your notes

Your notes



Investigating the effect of pH on enzyme activity

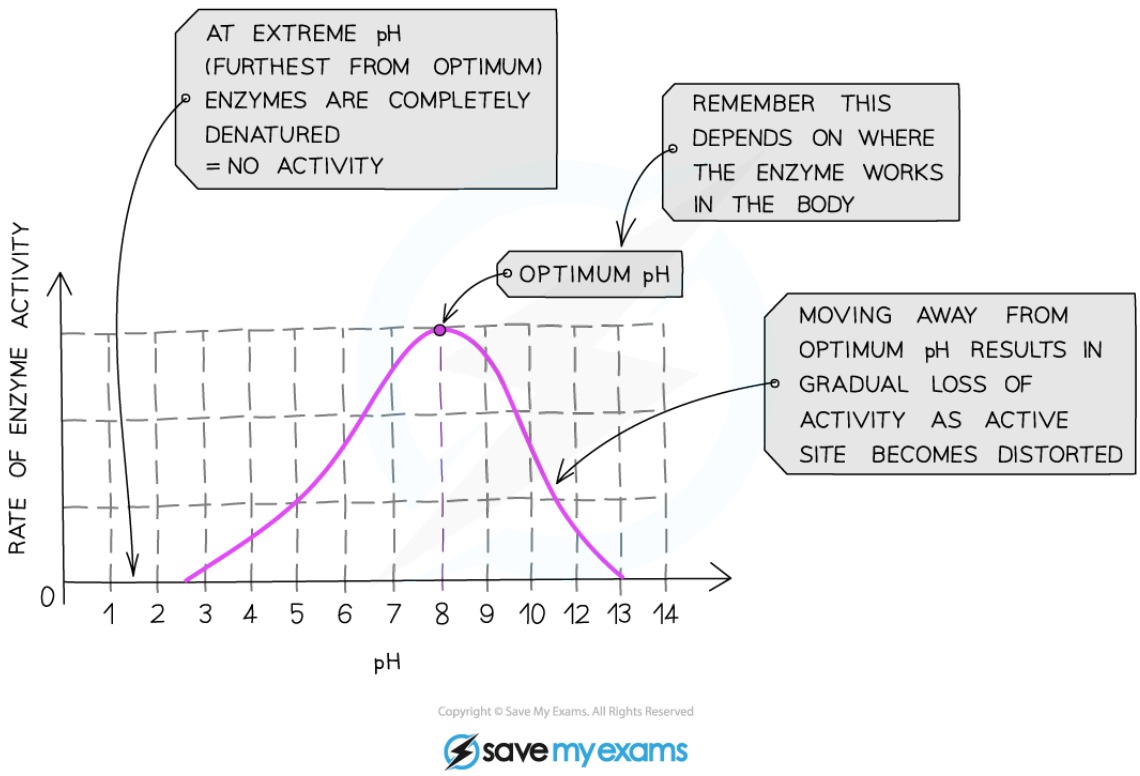
Your notes

Results and Analysis

- Amylase is an enzyme which **breaks down starch**
- When the iodine solution remains orange-brown, all the starch has been digested
- This investigation shows:
  - At the **optimum pH**, the iodine stopped turning blue-black and remained orange-brown within the shortest amount of time
    - This is because the enzyme is working at its fastest rate and has digested all the starch
- At **higher or lower pH's** (above or below the optimum) the iodine took a longer time to stop turning blue-black or continued to turn blue-black for the entire investigation
  - This is because on either side of the optimum pH, the enzymes are starting to become denatured and as a result are unable to bind with the starch or break it down

Limitations

- The starch and amylase solutions that need to be used should be placed in a water bath at optimum temperature before being used
- A **colorimeter** can be used to measure the progress of the reaction more accurately by measuring the absorbance/transmission of light through the coloured solution
  - A control of iodine solution would be used for comparison

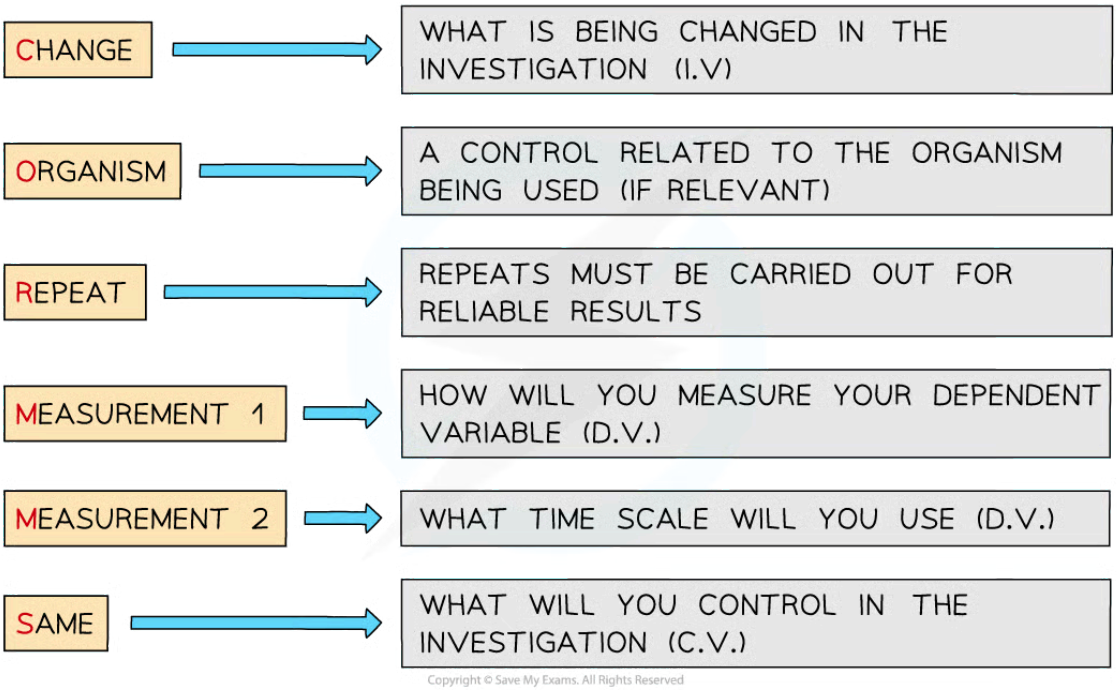


A graph showing the optimum pH for an enzyme from a region of the small intestine

Applying CORMS to practical work

- When working with practical investigations, remember to consider your CORMS evaluation

Your notes



Your notes

CORMS Evaluation

- In this investigation, your evaluation should look something like this:
  - **C** - We are changing the pH of the environment
  - **O** - This is not relevant to this investigation as we aren't using an organism
  - **R** - We will repeat the investigation several times to ensure reliability
  - **M1** - We will measure the time taken for
  - **M2** - the iodine to stop turning black
  - **S** - We will control the concentration and volume of the amylase, iodine and starch solution used in the investigation

Examiner Tips and Tricks

When describing the effect of pH on enzyme activity, it is important to remember that any pH outside of the optimum can lead to the enzyme becoming permanently denatured.


Your notes

Your notes

Rate Calculations for Enzyme Activity

- Rate calculations are important in determining how **fast** an **enzyme** is **working** (i.e. the **rate of reaction**)
- To perform a rate calculation, use the following formula:

rate = change ÷ time

- **Change** = the change in the substance being measured
  - E.g. the **amount of substrate used up** in the reaction or the **amount of product formed**
- **Time** = the **time taken** for that **change** to **occur**

Worked Example

Amylase catalyses the breakdown of starch into maltose. 15 grams of starch were added to a solution containing amylase. It took 2 hours for all the starch to be broken down.

Calculate the rate of reaction.

Answer:

Step 1: write out the equation for calculating the rate of enzyme activity

rate = change ÷ time

In this case: rate = amount of substrate used ÷ time

Step 2: substitute in the known values and calculate the rate

rate = 15 g ÷ 2 hours

rate = 7.5 g / hr **or** 7.5 g hr^{-1}

Worked Example

The enzyme catalase catalyses the breakdown of hydrogen peroxide into water and oxygen. In one experiment, a student found that 45 cm^3 of oxygen was released in 5 minutes.

Your notes

Calculate the rate of reaction.

Answer:

Step 1: write out the equation for calculating the rate of enzyme activity

rate = change ÷ time

In this case: rate = amount of product formed ÷ time

Step 2: substitute in the known values and calculate the rate

rate = 45 cm^3 ÷ 5 minutes

rate = 9 cm^3 / min **or** 9 cm^3 min^{-1}

- In some situations you **may not be told** how much something has **changed** during a reaction; instead, you may only be told the **time taken** for the **reaction to occur**
- In this case you can still calculate the rate of reaction by using the following (slightly different) formula:

rate = 1 ÷ time

Worked Example

A student adds a set volume of starch solution to a set volume of amylase solution at a range of different pH values. At each pH, the student times how long it takes for the amylase to break down all of the starch. At pH 6 the time taken for amylase to break down all of the starch was 50 seconds.

Calculate the rate of reaction at pH 6.

Answer:

Step 1: write out the equation for calculating the rate of enzyme activity

rate = 1 ÷ time

Step 2: substitute in the known values and calculate the rate

rate = 1 ÷ 50 seconds

rate = 0.02 s^{-1}

The units for the calculation above are in **s^{-1}** because rate is given **per unit time**.

Examiner Tips and Tricks

In an exam you could be asked to plot the reaction rates (from an enzyme catalysed reaction) on a graph. However, using the equation 'rate = 1 ÷ time' often gives small numbers that are difficult to plot on a graph. In these cases, you can also use the equation:

rate = 1000 ÷ time

This equation give you bigger numbers that are easier to plot on a graph. So, for the calculation in the worked example above, you would get:

rate = 1000 ÷ 50 seconds

rate = **20** s^{-1}


Your notes

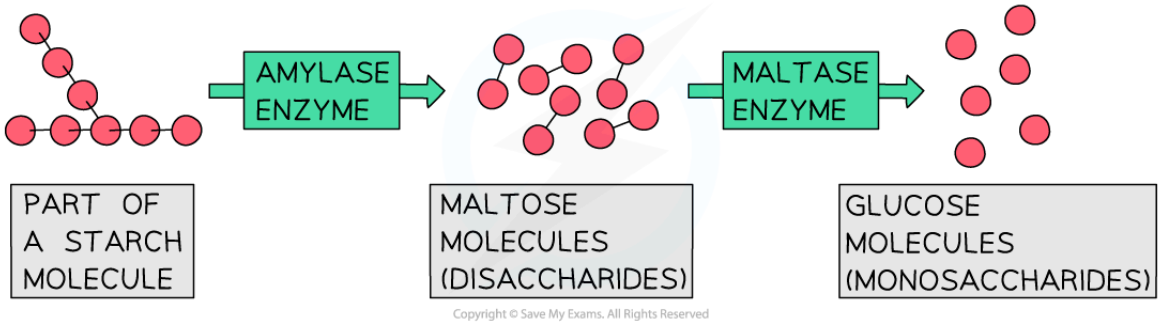
Your notes

Enzymes as Biological Catalysts

- The purpose of digestion is to **break down large, insoluble molecules** into **smaller, soluble molecules** that can be **absorbed** into the **bloodstream**
- Food is partially digested **mechanically** (by chewing, churning and emulsification) in order to break large pieces of food into smaller pieces of food
  - This **increases** the **surface area** for enzymes to work on
- Digestion mainly takes place **chemically**, where **bonds** holding the **large molecules together** are **broken** to make smaller and smaller molecules
- Chemical digestion is **controlled** by **enzymes** that are produced in different areas of the digestive system
- Enzymes are **biological catalysts** – they speed up chemical reactions without themselves being used up or changed in the reaction
- There are three main types of **digestive enzymes**: **carbohydrases, proteases and lipases**

Carbohydrases

- Carbohydrases are enzymes that break down **carbohydrates** into **simple sugars** such as **glucose**
  - **Amylase** is a carbohydrase that is made in the salivary glands, the pancreas and the small intestine
  - **Amylase** breaks down **starch** into **maltose**
  - **Maltase** then breaks down **maltose** into **glucose**

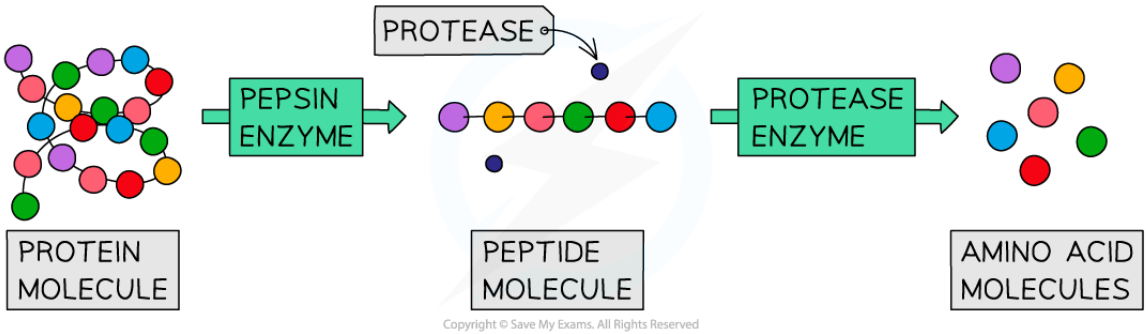


Starch is broken down into glucose using two enzymes: amylase and maltase

Proteases

Your notes

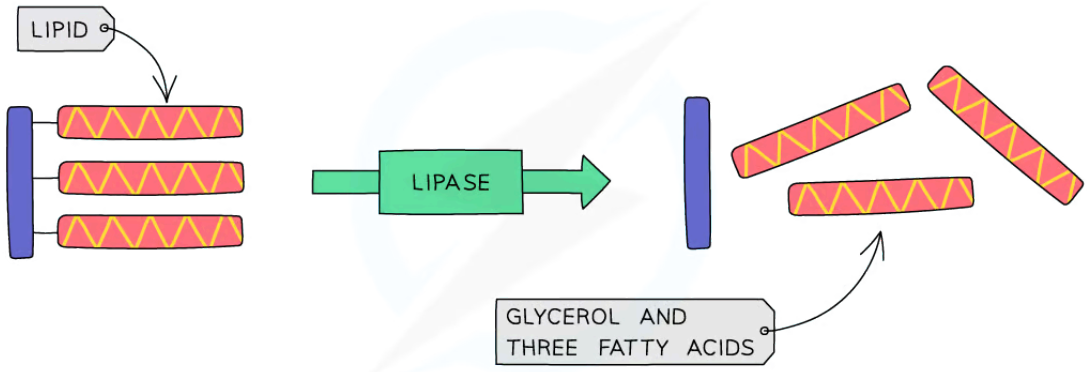
- Proteases are a group of enzymes that break down **proteins** into **amino acids**
  - **Pepsin** is an enzyme made in the **stomach** that breaks down proteins into smaller **polypeptide chains**
  - **Proteases** made in the **pancreas** and **small intestine** break the polypeptides into **amino acids**



Proteins are broken down using pepsin and other proteases

Lipases

- Lipases are enzymes that break down **lipids** (fats) to **glycerol** and **fatty acids**
  - Lipase enzymes are produced in the **pancreas** and secreted into the **small intestine**



Lipids are broken down by lipase enzymes

Synthesis of carbohydrates, proteins and lipids


Your notes

- Enzymes are not just important in **breaking down** larger molecules into smaller ones
- They are also required for the **synthesis** of larger molecules (building small molecules back up into bigger ones)
- Enzymes are required by organisms to **synthesise carbohydrates, proteins and lipids**
  - **Carbohydrates** are synthesised by **joining simple sugars** together
    - For example, **glycogen synthase** is an enzyme that joins together many **chains of glucose molecules** to form **glycogen** (an energy-storage molecule in animals)
  - **Proteins** are synthesised by **joining amino acids** together
    - Again, enzymes catalyse the reactions required to do this
  - **Many enzymes** are involved in the **synthesis of lipids** from fatty acids and glycerol

Examiner Tips and Tricks

The pancreas is an **accessory organ** in the digestive system. Food does not pass **directly through it**, but it has a key role in **producing digestive enzymes**, as well as the hormones that regulate blood sugar (insulin and glucagon).

Your notes

Practical: Food Tests

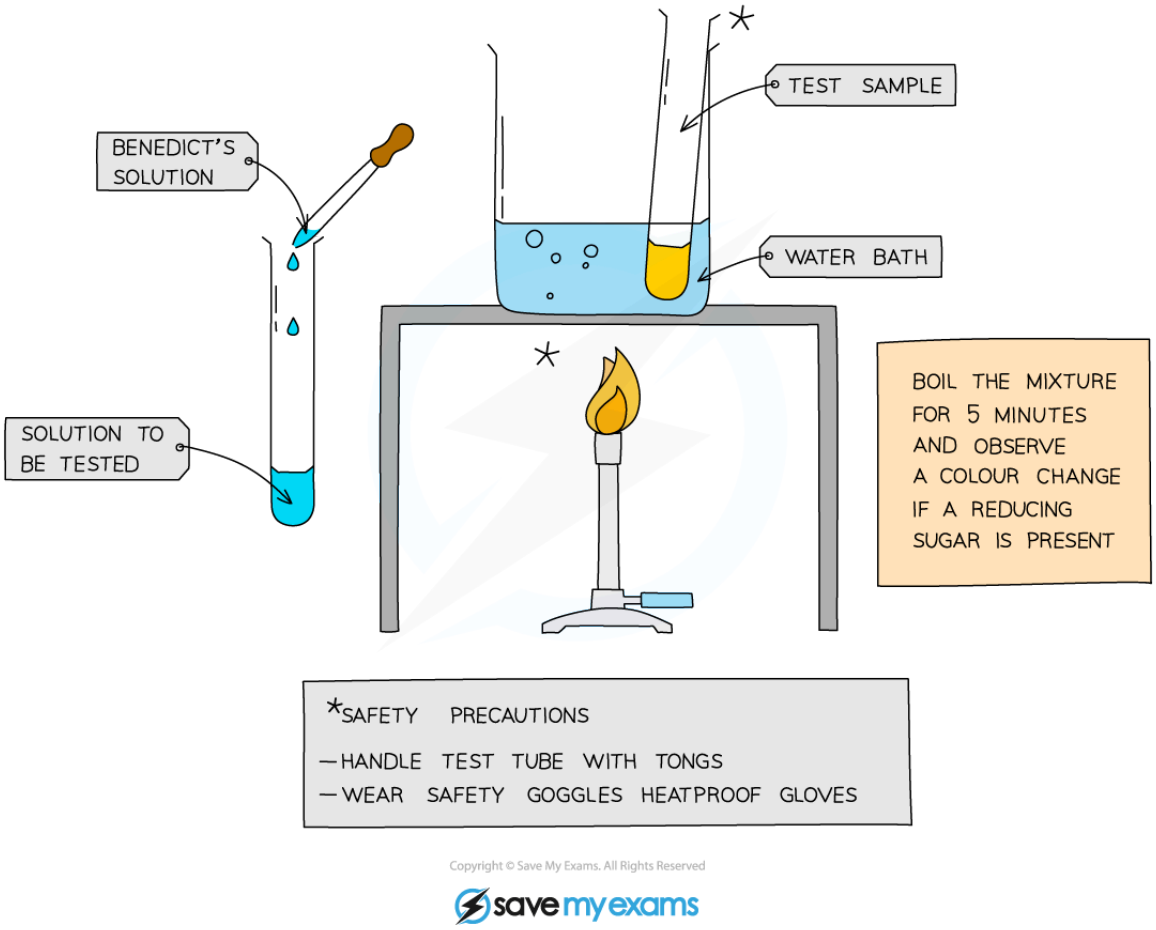
Preparing a sample

- Before you can carry out any of the food tests described below, you may need to prepare a food sample first (especially for solid foods to be tested)
- To do this:
  - Break up the food using a pestle and mortar
  - Transfer to a test tube and add distilled water
  - Mix the food with the water by stirring with a glass rod
  - Filter the mixture using a funnel and filter paper, collecting the solution
  - Proceed with the food tests

Test for glucose (a reducing sugar)

- Add **Benedict's solution** to the sample solution in a test tube
- **Heat** in a boiling water bath for **5 minutes**
- Take the test tube out of the water bath and observe the colour
- A positive test will show a colour change from **blue to orange / brick red**

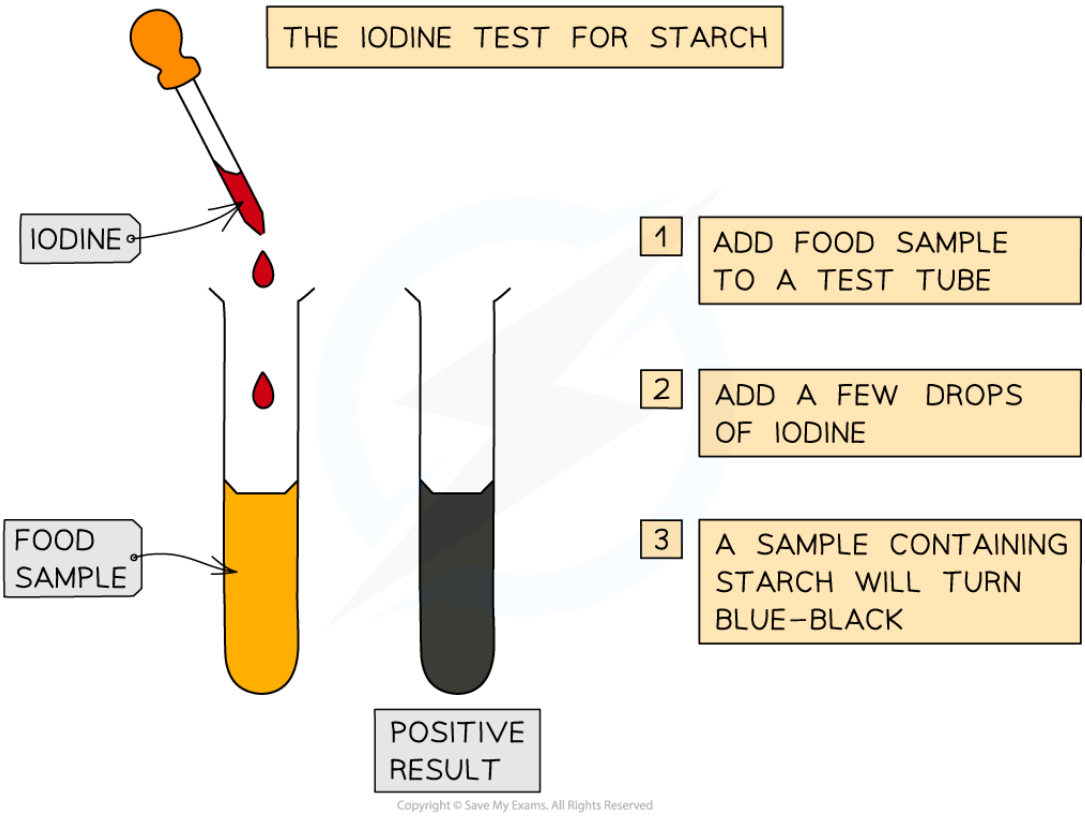
Your notes



The Benedict's test for glucose

Test for starch using iodine

- We can use iodine to test for the presence or absence of starch in a food sample
- Add drops of **iodine solution** to the food sample
- A positive test will show a colour change from **orange-brown to blue-black**



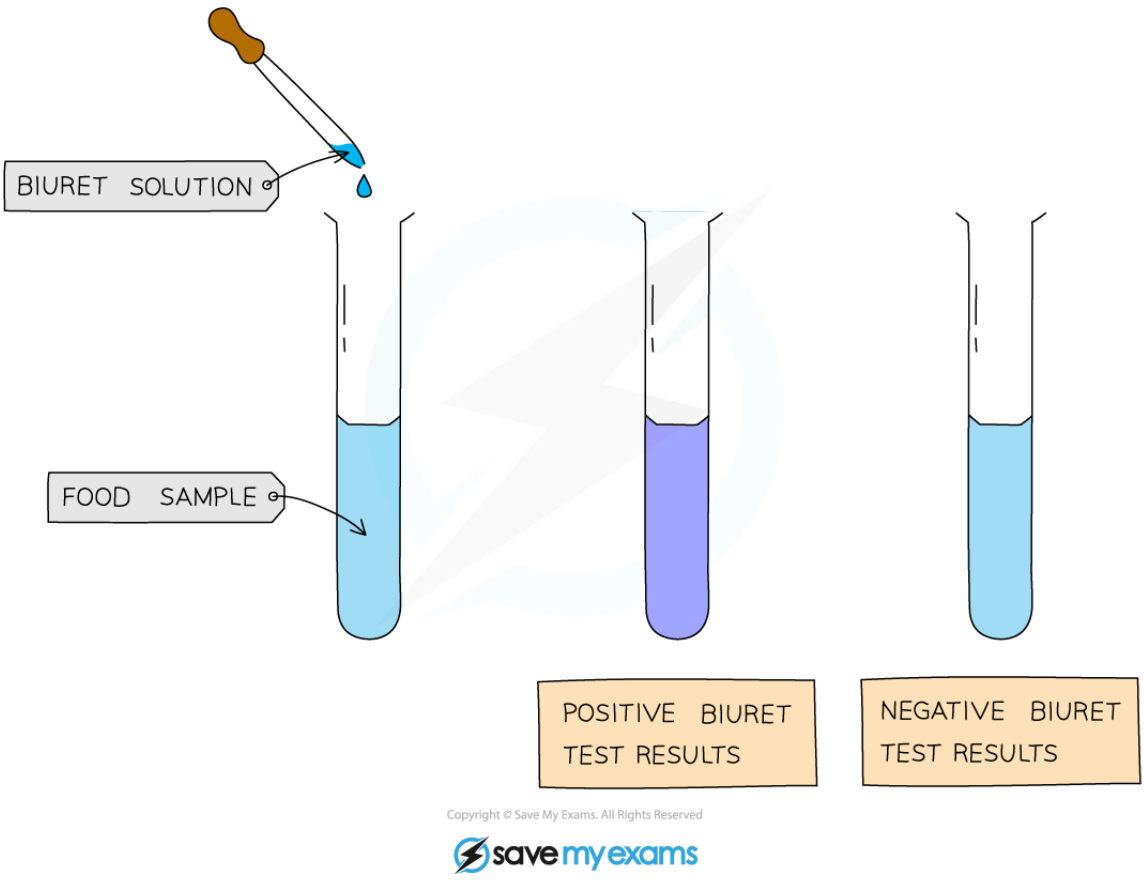
In the presence of starch, iodine will turn from brown to blue-black

Test for protein

- Add drops of **Biuret solution** to the food sample
- A positive test will show a colour change from **blue to violet / purple**

Your notes

Your notes

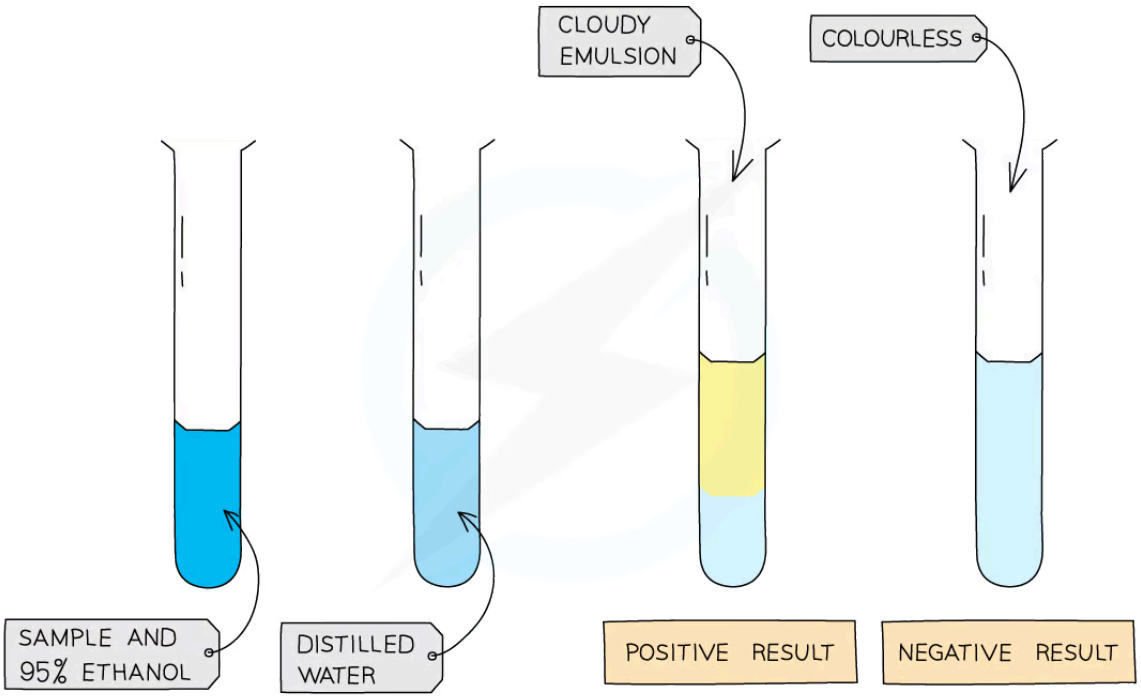


The Biuret test for protein

Test for lipids

- Mix the food sample with **4cm^3 of ethanol** and shake
- Allow time for the sample to dissolve in the ethanol
- Strain the ethanol solution into another test tube
- Add the ethanol solution to an equal volume of **cold distilled water (4cm^3)**
- A positive test will show a **cloudy emulsion** forming

Your notes



The ethanol test for lipids

Food Test Results Table

Your notes

Food Test	Colour of reagent	Positive test result	Negative test result
Iodine for starch	orange–brown	blue–black	orange–brown (no change)
Benedict's for sugar	light blue	green to brick–red	light blue (no change)
Ethanol for lipid	colourless	cloudy emulsion	colourless (no change)
Biuret for protein	blue	lilac–purple	blue (no change)

Important hazards

- Whilst carrying out this practical you should try to identify the main hazards and be thinking of ways to reduce harm
- Biuret solution contains **copper (II) sulfate** which is dangerous particularly if it gets in the eyes, so always wear **goggles**
- Iodine is also an irritant to the eyes
- **Sodium hydroxide** in biuret solution is **corrosive**, if any chemicals get onto your skin wash your hands immediately
- **Ethanol** is highly **flammable;** keep it away from any Bunsen burner
- The Bunsen burner itself is a hazard due to the open flame

Worked Example

Food tests: analysis

Your notes

Name of food tested	Colour produced with Benedict's solution	Colour produced with iodine solution	Cloudy layer produced with ethanol	Colour produced with Biuret solution
Potato	Blue	Black	✗	Blue
Olive oil	Blue	Orange	✓	Blue
Egg yolk	Blue	Orange	✓	Purple
Apple	Orange	Dark blue	✗	Blue
Tofu	Blue	Orange	✗	Purple
Biscuit	Yellow	Orange	✓	Blue

Write a conclusion to state which food groups are present one of the food samples you tested and an explanation of how you know this.

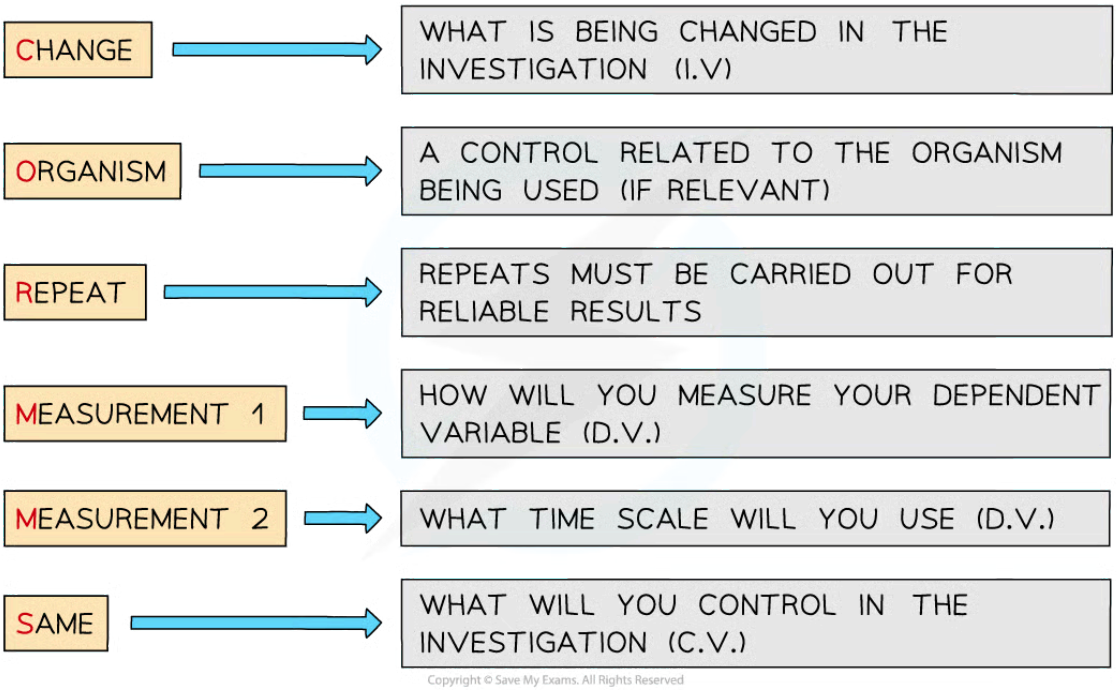
Conclusion:

The apple contained both starch and sugar as it tested positive for both the iodine test (orange → blue - black) and the benedict's test (blue → orange).

The apple did not contain protein or lipid (fat) as the biuret and emulsion tests were both negative.

Applying CORMS to practical work

- When working with practical investigations, remember to consider your CORMS evaluation.



Your notes

CORMS evaluation

- In this investigation, your evaluation should look something like this:
  - **C** - We are changing the type of food in the sample
  - **O** - This is not relevant to this investigation as we aren't using an organism
  - **R** - We will repeat the investigation several times for each food sample to ensure a reliable result
  - **M1** - The presence of the specific biological molecule in each food type by noting the colour change
  - **M2 –** ....after testing with each specific testing agent
  - **S** - We will control the volume of each testing agent used, the quantity of the food sample, the concentration of the testing agents, the temperature of the water bath for the Benedicts test. There may be other examples that you can think of

Examiner Tips and Tricks

When describing food tests in exam answers, make sure you give the **starting colour** of the solution and **the colour it changes to** for a positive result.


Your notes

Your notes

Practical: Energy Content of a Food Sample

We can investigate the energy content of food in a simple calorimetry experiment

Apparatus

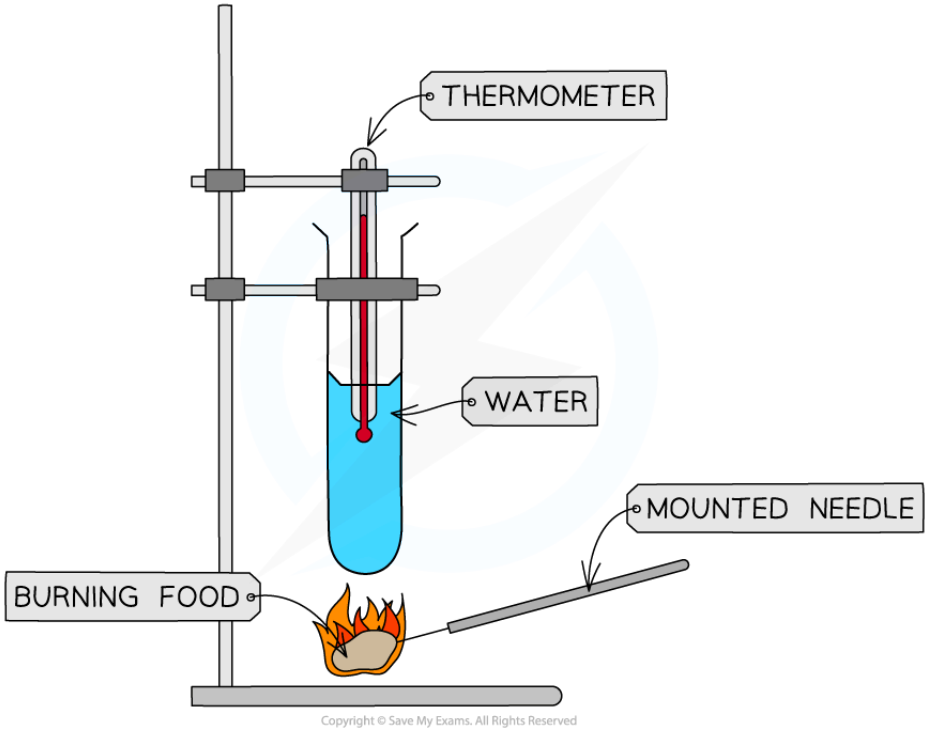
- Boiling tube
- Boiling tube holder
- Bunsen burner
- Mounted needle
- Measuring cylinder
- Balance/scales
- Thermometer
- Water
- Food samples

Method

- Use the measuring cylinder to measure out 25 cm^3 of **water** and pour it into the boiling tube
- Record the **starting temperature** of the water using the thermometer
- Record the **mass** of the food sample
- Set fire to the sample of food using the bunsen burner and hold the sample 2 cm from the boiling tube until it has **completely burned**
- Record the **final temperature** of the water
- **Repeat** the process with different food samples
    - E.g. popcorn, nuts, crisps

Investigating the energy content of food samples diagram

Your notes



Different food samples can be burned in a simple calorimetry experiment to compare the energy contents of the samples

Results

- The **larger the increase in water temperature**, the **more energy** is stored in the sample
- We can calculate the energy in each food sample using the following equation:

$$\text{energy transferred per gram of food (J)} = \frac{\text{mass of water (g)} \times \text{temperature increase (°C)} \times 4.2}{\text{mass of food sample (g)}}$$

- 4.2 kJ is the specific heat capacity of water, meaning that it is the energy required to raise 1 kg of water by 1 °C
- 1 cm^3 of water has a mass of 1 g

The energy content of food samples table

Food sample	Mass of water / g	Mass of food / g	Initial water temperature / °C	Final water temperature / °C	Change in water temperature / °C	Energy transferred per gram of food (J)
Popcorn	25	8.5	20.5	31.2	10.7	132.2
Walnut	25	8.1	20.4	34.1	13.7	177.6

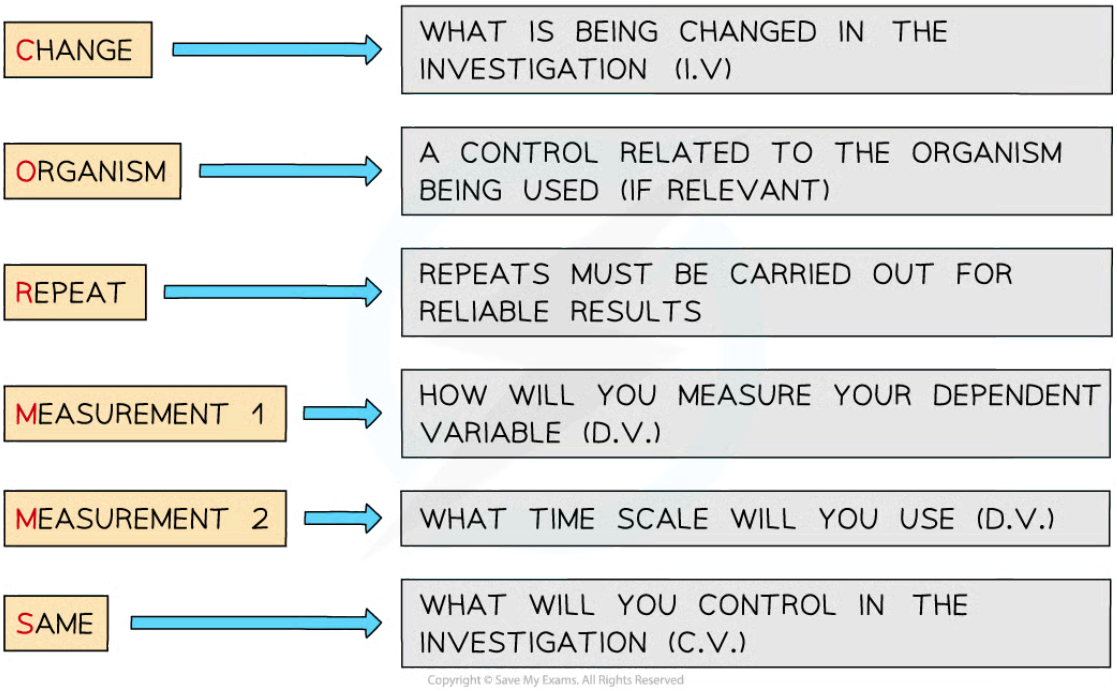

Your notes

Limitations

- **Incomplete burning** of the food sample
  - Solution: Relight the food sample until it no longer lights up
- **Heat energy is lost** to the surroundings
  - Solution: Whilst heat lost means that the energy calculation is not very accurate, so long as the procedure is carried out in exactly the same way each time (with the same distance between food sample and boiling tube), we can still compare the results

Applying CORMS evaluation to practical work

- When writing about practical investigations the CORMS evaluation can be used:



Your notes

CORMS provides a framework for writing about practical investigations

- In this investigation CORMS can be applied as follows:
  - **Change**
    - We are changing the **type of food** in the sample
  - **Organisms**
    - This is not relevant to this investigation as we aren't using an organism
  - **Repeat**
    - We will **repeat** the investigation several times for each food sample
  - **Measurement 1**
    - We will measure the **change in temperature** of the water
  - **Measurement 2**
    - The **mass of the food** will be measured after the food sample has burned out
  - **Same**

- We will control the **volume of water** used and the **distance** between the food sample and the boiling tube during burning
- The food will also be **relit** every time it goes out until it no longer relights


Your notes