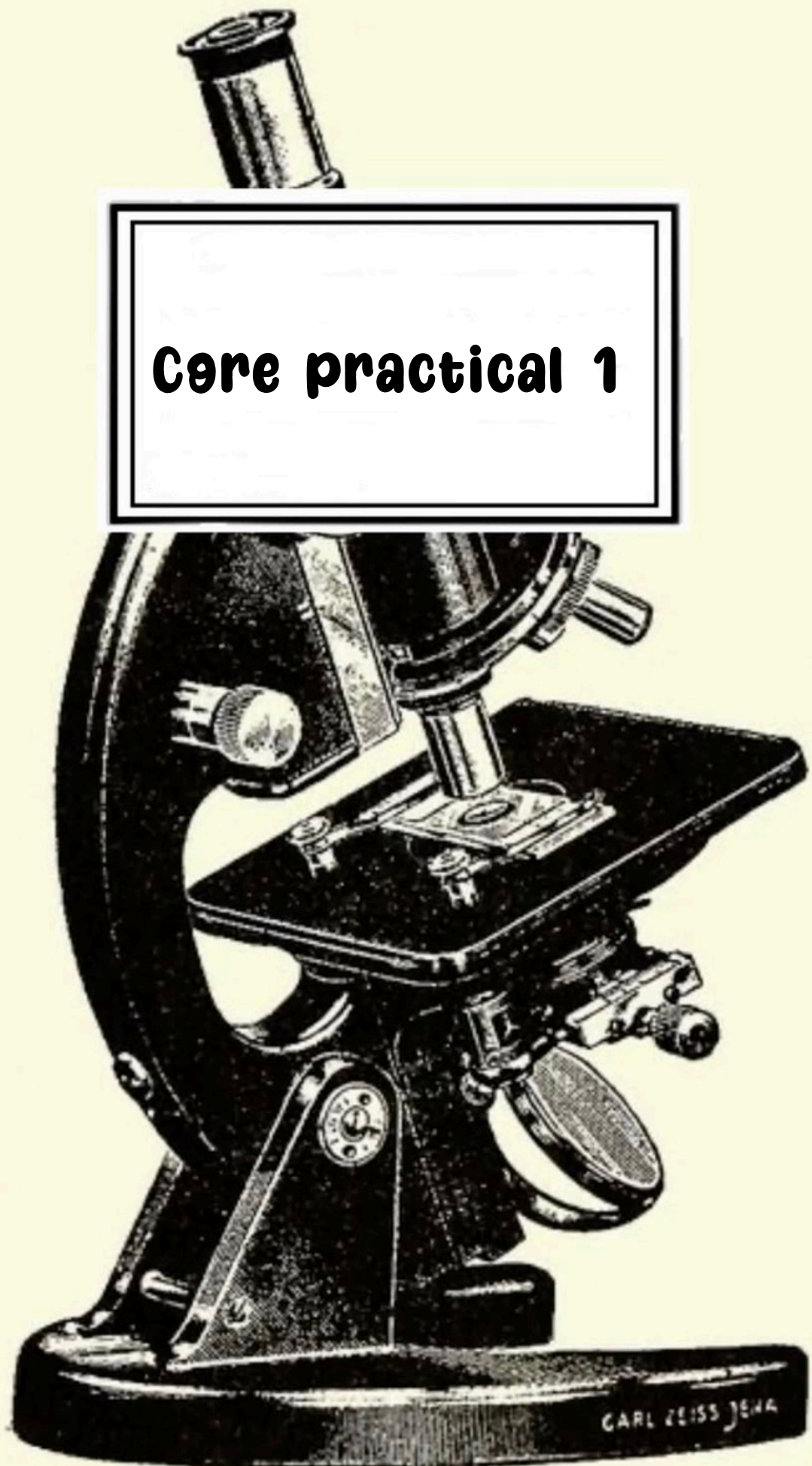


Core practical 1



key concepts

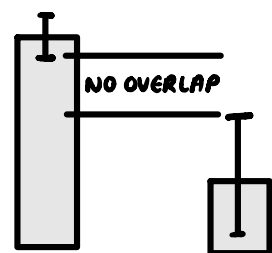
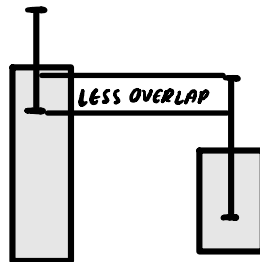
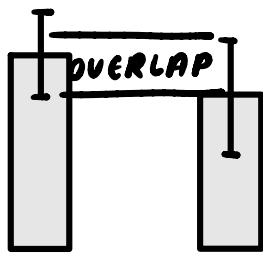
accuracy → The difference between actual values and measure values. The higher the difference the lower the accuracy. Accuracy is increased by changing the measuring method and using a more sensitive measuring apparatus

Validity → how correct is your experimental procedure. Validity is increased by keeping all other factors except the one that is being investigated constant to ensure a fair test. These factors are known as controlled variables.

Reliability → how similar your results are after repeating the experiment numerous times.

Two ways of presenting the data in a reliability of data

- Calculating a mean → avoids anomalous results
- Using SD/ error bars → Size of error bars indicates to the degree of spread out of data from mean value. the larger the error bar the more the deviation / spread out of data from the mean value so less reliable. Overlapping of error bars if error bars overlap so no significant difference (less reliable). No over lapping there is a significant difference between data



Planning an investigation

1. Independent variable (change) → use 5 different values for example use 5 different temperatures
2. Dependent variable (organism) → keep the species, age, gender, size of organism the same for example when testing effect of mineral ions on plants, ensure to keep the age, height, type of species of the plant the same.
3. Mention 2 measurements for example after one week (time period is a common measurement you can use in every answer of yours) measure the increase in height using a ruler of the plant after placing it in nitrate solution (2nd measurement).
4. Control variables - environmental factor that will affect the outcome of the experiment for example in the case of plants, carbon dioxide concentration, temperature, light intensity
5. Repeat the experiment to increase reliability and identify and discarb anomalous results and calculate a mean
6. Safety precautions relevant to the experiment

core practical 01: Test for reducing sugars, proteins and starch

Independent variable: different food samples

Dependent variable: the concentration of reducing sugars in a food sample

KEYWORDS

A quantitative test tells you whether something is present or absent

A quantitative test enables you to determine exactly how much of the substance is present

A semi-quantitative test enables you to estimate roughly how much of the substance is present

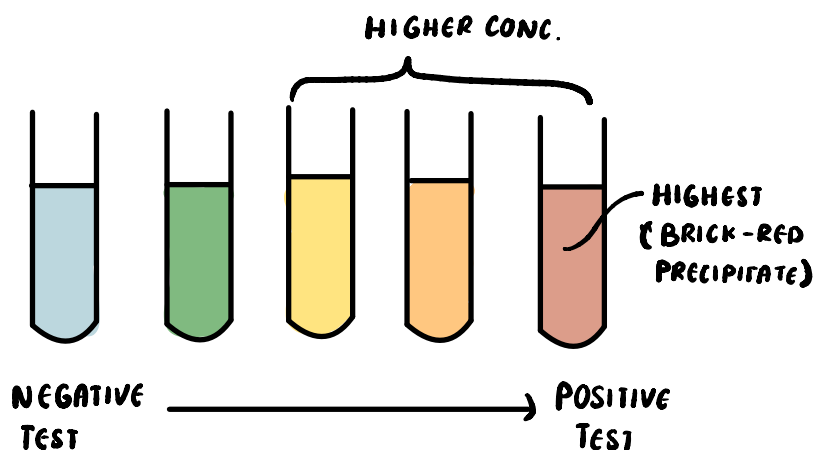


safety precautions

- wear eye protection
- avoid skin contact with Benedict's reagent, iodine and hot solutions,
- Handle the test tubes with tongs to avoid burns
- Do not taste the sample solutions
- Wear lab coat and gloves

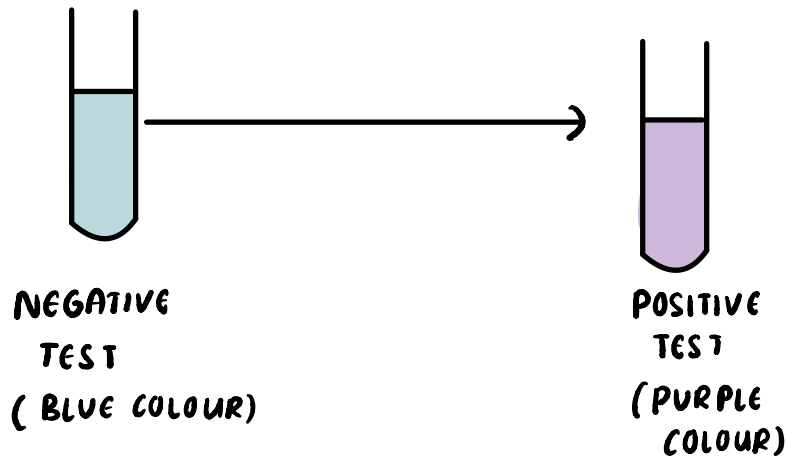
Test for reducing sugars

1. Take 5cm³ of milk each from different brands in different test tubes of same diameter and add 1cm³ of Benedict's reagent to each of this.
2. Place these test tubes in a water bath kept at 70°C and start the stopwatch.
3. After 2 minutes take the test out from the water bath and observe the colour change from blue to green to red.
4. Use a standard colour chart to identify concentration of reducing sugar in each milk product
5. Repeat at least three times for each milk brand and find the mean concentration.
6. Throughout the experiment, the variables such as temperature, using temperature- controlled water bath and volume of Benedict's reagent used using graduated pipette



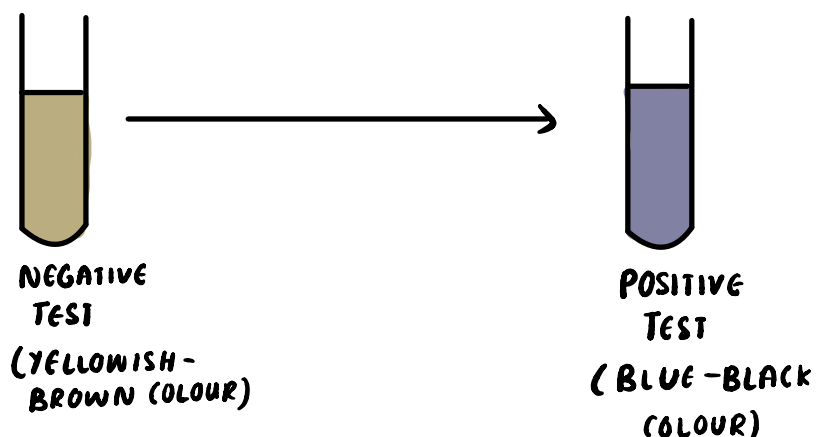
test for Proteins

1. Take 5cm^3 of milk each from different brands in different test tubes of same diameter and add 1cm^3 of Biuret's reagent to each of this.
2. . After 30 seconds, compare the purple color in the test tube.
3. . Use a standard colour chart to identify concentration of protein in each milk product
4. Repeat at least three times for each milk brand and find the mean concentration.
5. . Throughout the experiment, control the variables such as temperature, using temperature - controlled water bath and volume of milk and biuret's reagent used using a graduated pipette.



Test for starch

1. Use same extraction method, same mass of potato, same volume of water added to crushed potato
2. Measure equal volumes of potato solution using a graduated pipette in same diameter
3. Add equal volumes of iodine solution to each test tube.
4. Use a standard colour chart to identify concentration of starch concentration in each potato extract
5. Repeat at least three times for each potato species and find the mean concentration.
6. Throughout the experiment control the variables such as temperature, using temperature-controlled water bath and volume of potato extract using a graduated pipette



serial dilution

a stepwise dilution of a solution

At each step, the concentration decreases by the same dilution factor

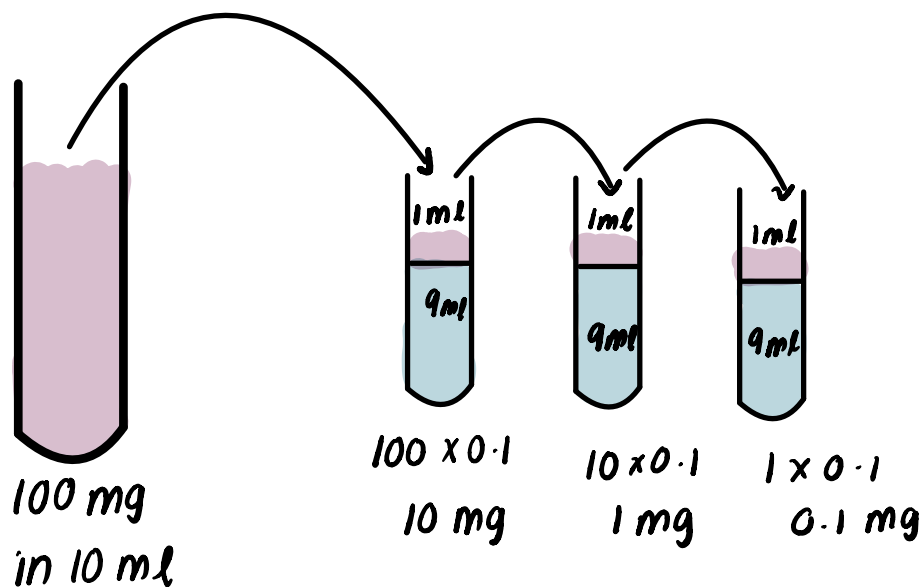
For example: 1:10 dilution means the concentration becomes 10 times smaller each step

1:2 dilution means the concentration becomes half at each step

$$\text{DILUTION FACTOR} = \frac{\text{VOLUME OF SAMPLE}}{\text{VOLUME OF SAMPLE} + \text{VOLUME OF DILUENT}}$$

sample = original solution

diluent = distilled water



$$\text{dilution factor} = \frac{1}{1 + 9} = 0.1$$

- 3 Many food crops, such as potatoes and bananas, contain starch. The starch content can affect the taste and cooking properties of the food.

Potatoes can be boiled or baked. Potatoes with a high starch content are good for baking and those with a lower starch content are good for boiling.

It is suggested that King Edward and Russet potatoes are better for baking and Nicola and Purple Congo potatoes are better for boiling.

A student decided to test these suggestions.

A standard set of starch solutions was prepared and tested.

The table shows the results.

Starch concentration (%)	Colour of solution after addition of iodine solution
1.0	black
0.1	dark blue
0.01	pale blue
0.001	very pale blue
0.0001	pale brown

- (a) Describe how the data in the table could have been obtained using a 1% starch solution.

(4)

.....
carry out a serial dilution using the 1% available stock solution

.....
To 1 ml of 1% stock solution and 9ml distilled water to obtain the 0.1% solution

.....
transfer 1 ml of 0.1% solution to 9 ml of distilled water to obtain 0.01% solution.

.....
Continue this dilution trend two more times to obtain 0.0001% solution

.....
To each test tube of starch solution add iodine of same concentration and volume.

.....
Observe the color change of the resultant solution in a test tube similar in diameter

.....
Note down the observed color change of the solution

.....

.....

.....

.....

.....

- 2 An investigation was carried out to determine the relative concentration of reducing sugars in a range of foods.

In each case, some food was ground up with distilled water. The resulting liquid was filtered to give an extract.

This extract was then tested for the presence of reducing sugars using a reagent.

- (a) (i) Describe how a test for reducing sugars is carried out.

(2)

Add Benedict's reagent to the sample solution

Heat the test tube in a water bath

- (ii) Explain how this investigation should be carried out to give a semi-quantitative estimate of the concentration of the reducing sugars by comparing the colour of the precipitate with a colour chart.

(3)

Use sugar solutions of known concentrations to produce a color chart

Use the same mass or volume of each food sample

Mix with a fixed volume of distilled water

Add a fixed volume of Benedict's reagent to the sample solution

Place the test tube containing the sample solution in a thermostatically controlled water bath

Compare the results to the color chart

(iii) It was assumed that the extract contained all the reducing sugars from the food sample.

Suggest how this assumption could be tested.

(2)

Test the food residue in the filter paper for reducing sugars with Benedict's reagent

.....

.....

.....

.....

(b) The table shows the results of the investigation.

Food	Colour of precipitate
apple	brick red
bread	green
cashew nut	no precipitate
potato	yellow
sweet potato	orange

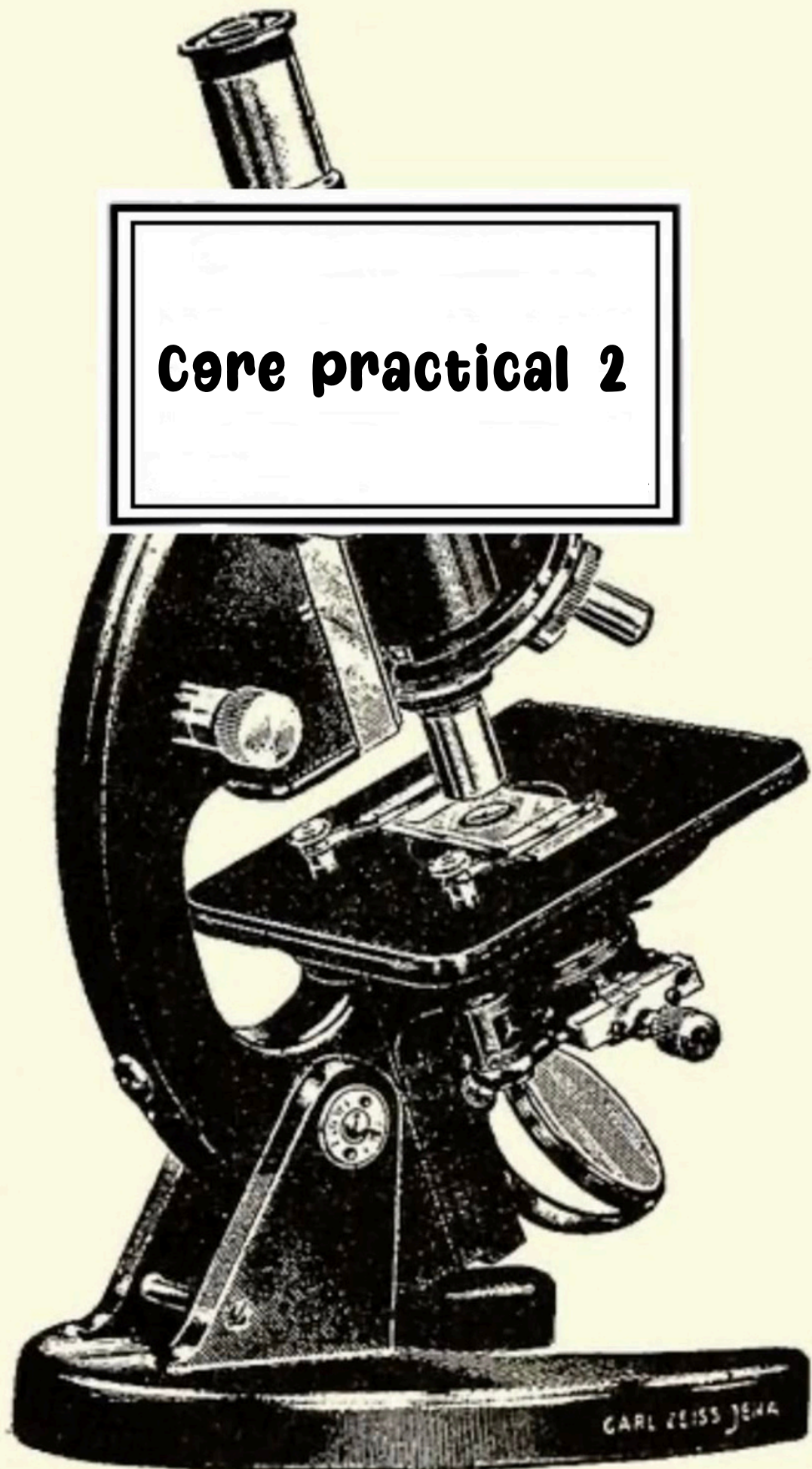
Complete the table below by writing the names of the foods in order from the highest concentration of reducing sugars to the lowest concentration.

(2)

highest —————> lowest

Apple	Sweet potato	Potato	Bread	Cashew nut
-------	--------------	--------	-------	------------

Core practical 2



core practical 02: Testing for vitamin C

independent variable: storage time, temperature or using different food samples

dependent variable: vitamin C concentration

DCPIP indicates the presence of vitamin C

procedure

1. Take 1cm^3 of 1% DCPIP in a conical flask
2. Take standard vitamin C solution in a burette and titrate against DCPIP.
3. Shake conical flask gently after each drop
4. Record the volume of Vitamin C needed to decolourise DCPIP.
5. Repeat the titration and calculate the mean volume of vitamin C solution needed using the formula: $c_1v_1=c_2v_2$
6. Titrate the juice against DCPIP until the colour changes from blue to colourless.
7. Record the volume of juice added
8. Repeat the procedure three times and find out the mean volume of juice added - increases reliability and helps calculate a mean and standard deviation and plot error bars



safety precautions

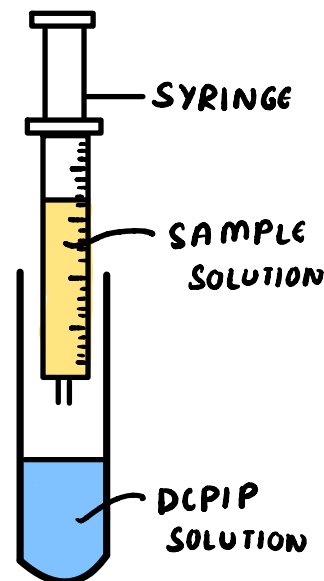
- wear eye protection
- avoid contact with the DCPIP and test tube solution hence wear a lab coat and gloves
- Do not taste the sample solutions

Uses of vitamin C

During the reaction between DCPIP and vitamin C, DCPIP gain electrons from vitamin C hence vitamin C is described as vitamin C loses electrons and becomes oxidized (turns DCPIP solution from blue to colorless indicating the end-point of the investigation).

Vitamin C helps to reduce risk of CVD

calculation example:- if the volume of 1% vitamin C (10 mg) solution needed to decolorise the DCPIP 1.4 cm^3 and the volume of fruit juice was 2.6 cm^3 , calculate the concentration of fruit juice



$$\begin{aligned}\text{Concentration of fruit juice} &= \frac{\text{volume of standard solution}}{\text{volume of fruit juice}} \times \text{concentration of standard solution} \\ &= \frac{1.4}{2.6} \times 10 \\ &= 0.54 \times 10 \\ &= \underline{\underline{5.4\text{ mg}}}\end{aligned}$$

why were syringes used in this investigation instead of burettes?

using very small volumes of solution requires more precise piece of apparatus rather than a burette.

Syringes are easier to control, allowing smaller volumes to be added.

Ease of use.

Relatively low cost

why we should not shake vigorously while titration?

To avoid any sort of spillage.

Shaking too vigorously introduce oxygen to DCPIP hence DCPIP will be reoxidised. So blue color will return making it difficult to determine the end point

1 Young fruits are unripe. As the fruits develop, they become ripe.

A student suggested that the vitamin C content of fruits increases as they become ripe.

To test this, the student carried out the following investigation:

- unripe apricots, peaches, apples and mangos were obtained
- a sample of each fruit was crushed
- each crushed sample was placed into a separate test tube
- distilled water was then added to each of the crushed samples
- each mixture was shaken and filtered to give four extracts, one of each fruit
- the vitamin C content of each extract was determined by adding it drop by drop to 1 cm³ 0.1% DCPIP solution until this solution changed colour
- this method was repeated using ripe apricots, peaches, apples and mangos.

(a) (i) State the independent variable and the dependent variable in this investigation.

(2)

Independent variable..... Ripeness of fruit.....

Dependent variable..... Vitamin C content of extract.....

(ii) State **two** variables, other than the volume and concentration of DCPIP, that should be controlled in this investigation.

(2)

1 Mass of the fruit.....

2 Volume of distilled water added.....

(iii) Vitamin C is water-soluble. The method used assumes that all the vitamin C from the fruit is in the extract.

Suggest how this assumption could be tested.

(3)

Take the residue of the fruit from the filter paper

Add distilled water to the residue

Test the residue for vitamin C with DCPIP.

No decolourisation of DCPIP indicates that the assumption was correct

(b) The table below shows the results of this investigation.

Fruit	Volume of extract added to decolourise 1 cm ³ of 0.1% DCPIP / cm ³		Vitamin C content of extract / mg cm ⁻³	
	Unripe	Ripe	Unripe	Ripe
Apricot	2.6	2.1	2.3	2.9
Peach	4.0	2.4	1.5	2.5
Apple	1.6	2.4	3.8	2.5
Mango	0.5	2.2	12.0	

(i) The student found that 6 mg of vitamin C decolourised 1 cm³ of 0.1% DCPIP solution.

Calculate the vitamin C content of the ripe mango extract.

Show your working.

(2)

$$C_2 V_2 = C_1 V_1$$

$$C_2 \times 2.2 = 6 \times 1$$

$$C_2 = \frac{6}{2.2} \times 1$$

$$= 2.73$$

$$\approx 2.7 \text{ mg cm}^{-3}$$

$$2.7 \text{ mg cm}^{-3}$$

1 Plant foods in the diet contain antioxidants, such as vitamin C. Storage time and conditions affect the concentration of vitamin C in these foods.

(a) Explain how increasing the quantity of plant foods that contain vitamin C in the diet may protect against heart disease.

(3)

vitamin C is an antioxidant and decreases free radicals

This prevents damage to the endothelium of blood vessels which reduces plaque formation

(b) The effect of storage time and temperature on the vitamin C content of four foods was investigated.

(i) Describe a method to allow a quantitative comparison of the vitamin C content of two of these foods.

(5)

Use equal masses of these two food types

Use the same method of food extraction for each food and add the same volume of distilled water.

Measure out the same volume of DCPIP solution into separate test tubes

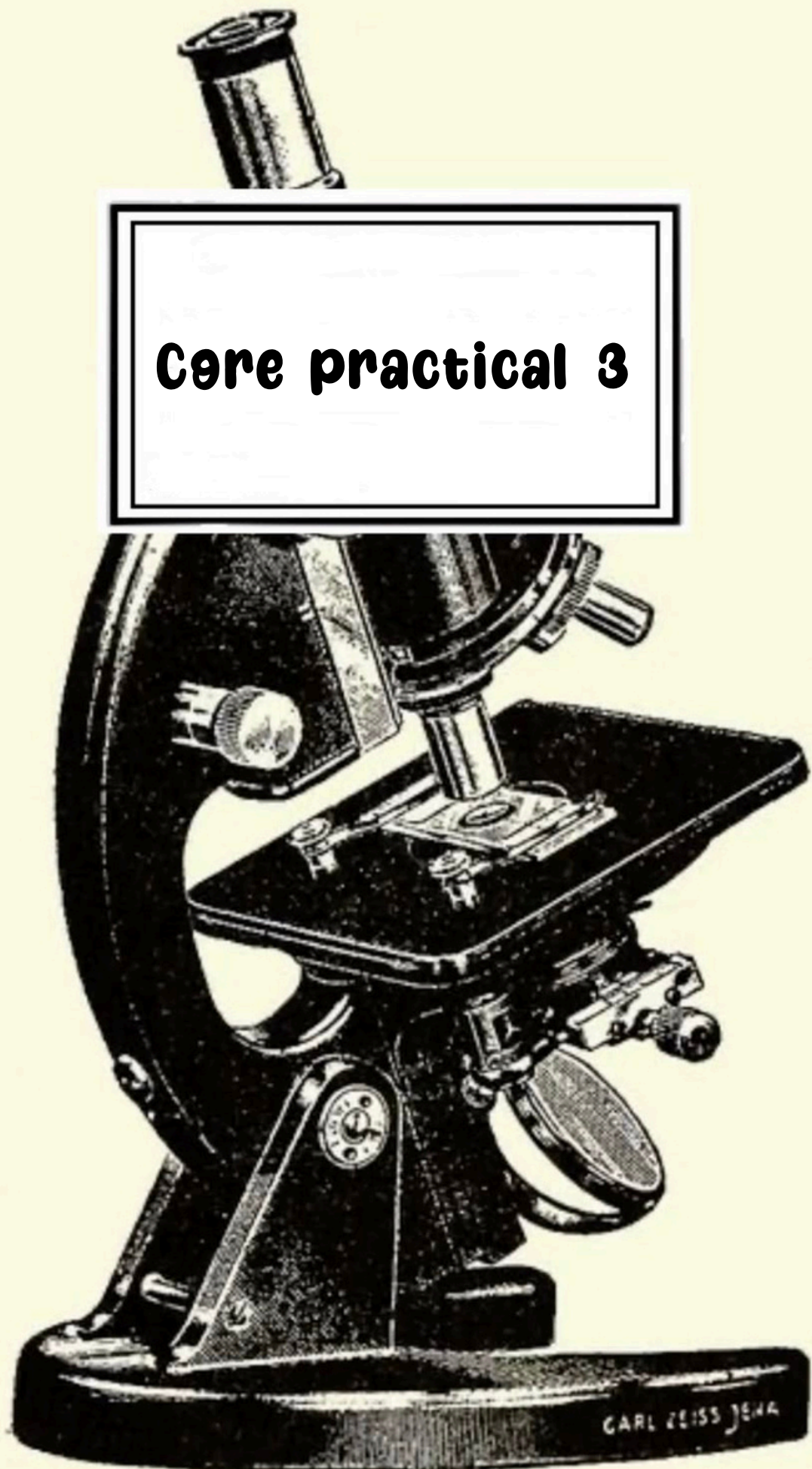
Add vitamin C standard solution of known concentration dropwise into one test tube and extract into the other test tube until DCPIP turns colorless.

Record the volume of each solution that decolourises DCPIP and calculate concentration of vitamin C in the food extract by using the equation

$$C_1 = \frac{C_2 V_2}{V_1}$$

Repeat the experiment for the other food sample and compare

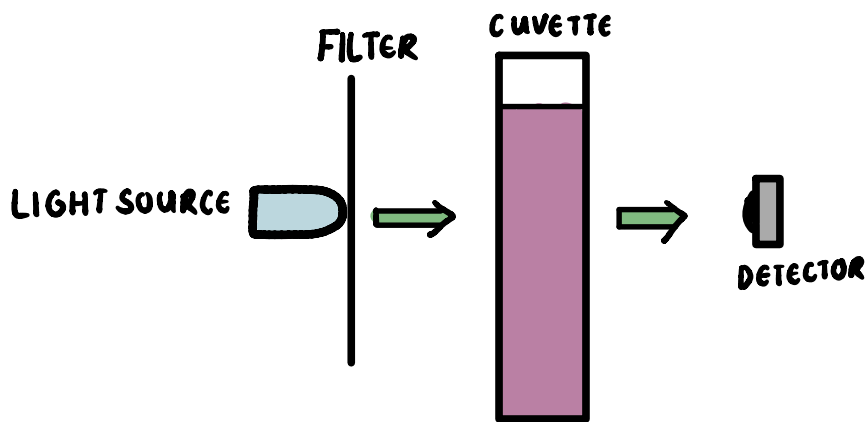
Core practical 3



core practical 3: Investigating effect of temperature and alcohol on cell membrane

independent variable: Temperature or concentration of alcohol

Dependent variable: absorbance or transmission of light in the colorimeter



How to use a colorimeter

1. Set the colorimeter to zero by placing a cuvette filled with distilled water or alcohol
2. Shake the cuvettes to distribute the pigment evenly
3. A green filter is used to adjust the wavelength of light to the one absorbed by the red pigment
4. Clean the cuvettes by wiping them
5. Measure the absorbance of light which increases as the amount of pigment increases



safety precautions

ethanol is highly flammable so keep away from naked flame and keep the stoppers on bottles
do not handle electric plugs socket or switches with wet hands wash hand with soap and water
when finished, Use of sharp items to cut the beetroot discs.
Always cut away from yourselves

Procedure for effect of temperature :

1. Carry out the experiment at 5 different temperatures (10°C TO 50°C) using a thermostatic water bath
2. Get 5 test tubes of same size and add equal volume of water
3. Cut 5 pieces of beet roots (containing Anthocyanin pigments) using a cork borer all of same size and from beetroots of same species and age
4. Rinse and dry to remove pigments from surface due to cutting
5. Allow equilibration of the 5 test tubes of water to reach to experimental temperature by leaving them in the water bath at required temperature for 5 mins.
6. Add the beet root pieces to the water
7. Use the colorimeter to measure the degree of absorbance
8. Zero the colorimeter using a blank cuvette filled with distilled water

At higher temperature:

- Kinetic energy of molecules increase
- Increasing movement and diffusion of pigment molecules
- Phospholipid of membrane becomes more fluid, bond/interaction between fatty acid tails begin to separate.
- Increasing permeability of the membrane.
- So pigment molecules can pass more freely through the cell membrane to the liquid outside
- So less light can pass through liquid.
-

At very high temperature:

- The protein molecules in the membrane become completely denatured (losing their tertiary structure by disrupting the bonds that hold their tertiary structure in place), (also phospholipid may melt) and gaps are formed in the membrane through which the pigment can flood out.
- The change in transmission levels out as the concentration of pigments is the same inside and outside the cells (equilibrium).

Procedure for effects of alcohol

1. Carry out the experiment at 5 different alcohol concentrations
2. Get 5 test tubes of same size and add equal volume of alcohol
3. Cut 5 pieces of beet roots using a cork borer all of same size and from beetroots of same species and age
4. Rinse and dry to remove pigments from surface due to cutting
5. Leave them in the different alcohol concentrations for 15 mins.
6. Use the forceps to remove the cylinders from each tube and discard the cylinders but keep the supernatant liquid.
7. Use the colorimeter to measure the degree of absorbance
8. Zero the colorimeter using a blank cuvette filled with alcohol

The change in alcohol concentration affects the integrity of the phospholipid bilayer.

Because:

- phospholipids are soluble in alcohol, where alcohol dissolves the fatty acids in cell membrane.
- so the membrane loses the ability to orientate towards and away from water (altering the hydrophobic interaction)
- The loss of hydrophobic and hydrophilic interaction disrupts the phospholipid bilayer,
- As well as at very high concentration, proteins in cell membrane denature resulting in holes forming in the membrane which allow the pigment out of the cells.
- The increase in pigment outside the cells reduces the ability of light to penetrate the solution therefore decreasing transmission.

suggest why the tubes were placed in the water baths for 5 mins before the cylinders were added?

the temperature must be equilibrated to ensure the tubes contain water at the correct temperature before the experiment begins. This allows confidence that the effect of the correct temperature is being assessed

Why were the beetroot cylinders washed with distilled water and dried before the experiment began?

The cylinders are washed and dried to remove excess surface pigment from the cut. This excess pigment would distort the transmission readings, giving inaccurate results

Explain why were the discs removed carefully

To avoid physical damage to the membranes of the cell. This would cause more pigment release hence results will not be valid

2: Some alcohols, such as methanol, increase the permeability of cell membranes.

Cholesterol can reverse this effect.

(a) Membrane permeability in the presence of these chemicals was investigated.

Beetroot cells contain a red pigment called betalain. Membrane permeability of the beetroot cells was estimated by measuring the loss of betalain from slices of beetroot.

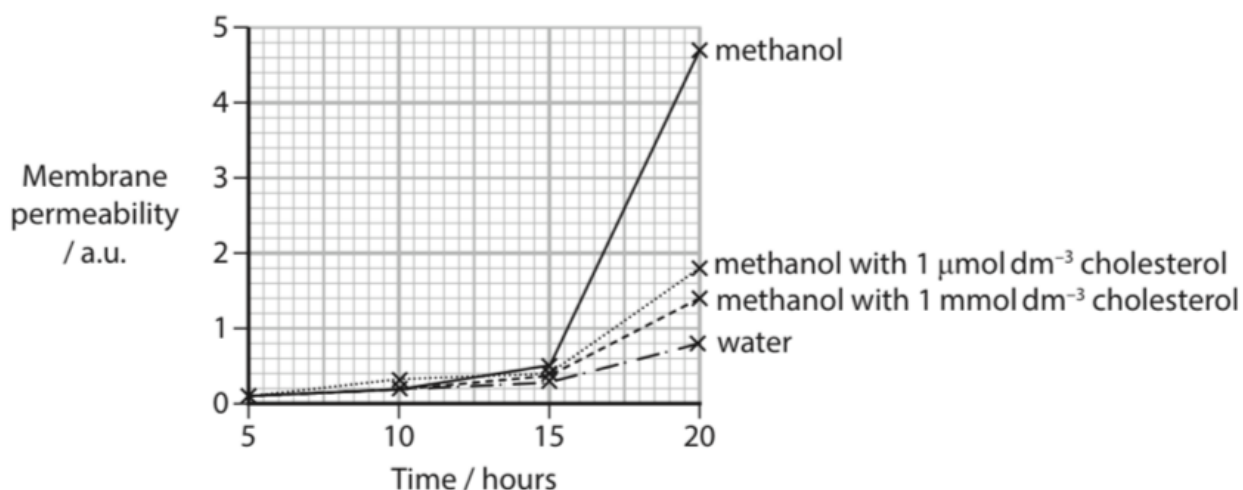
Samples of beetroot were immersed in four liquids:

- methanol only
- methanol with $1\mu\text{mol dm}^{-3}$ cholesterol
- methanol with 1mmol dm^{-3} cholesterol
- water.

Samples of each solution were taken every five hours.

Membrane permeability was estimated from these samples.

The graph shows the results of this investigation.



(i) Justify each step of a method that could be used to obtain the results shown in the graph.

(5)

Cut equal sized beetroot discs and place some number in search of the four liquids.

This keeps size and number the same so that each liquid receives equal quantity of pigment

Beetroot pieces are rinsed under running water.

This removes any excess pigment that leaked out during cutting

Keep temperature constant as it affects pigment leakage

Use the same species, age, type of beetroot - this ensures same quantity of pigment

Use same volume of liquid solvent - this ensures same level of pigment dilution when leaking and will ensure valid measurements

Solution is shaken to disperse the pigment. Use a colorimeter as it is a quantitative method

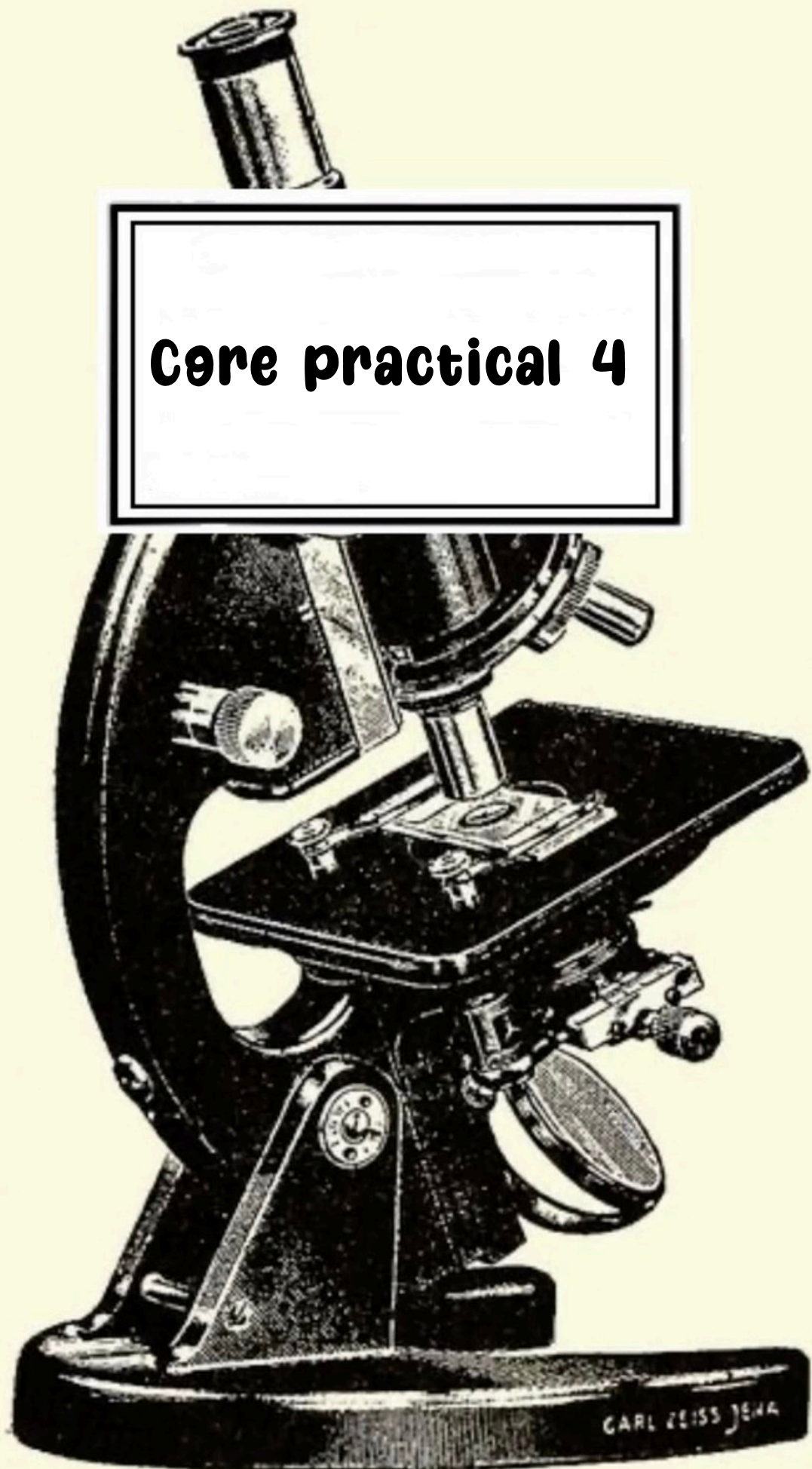
Zero/calibrate the colorimeter with a plain solvent to remove systematic error

Use water as a control for comparison

Repeat experiment to calculate a mean to look for anomalies and check for reliability

Calculate SD and error bars to check for variability and significance of data

Core practical 4



core practical 04: Effect of temperature, pH, Enzyme concentration and substrate concentration on initial rate of enzyme activity

independent variable:- temperature, pH, enzyme concentration, substrate concentration
dependent variable:- initial rate of enzyme activity

How to control?

- Temperature → thermostatically controlled water bath
- pH → Buffer solution
- Enzyme concentration → same volume and concentration of enzyme solution
- Substrate concentration → same mass or volume of substrate



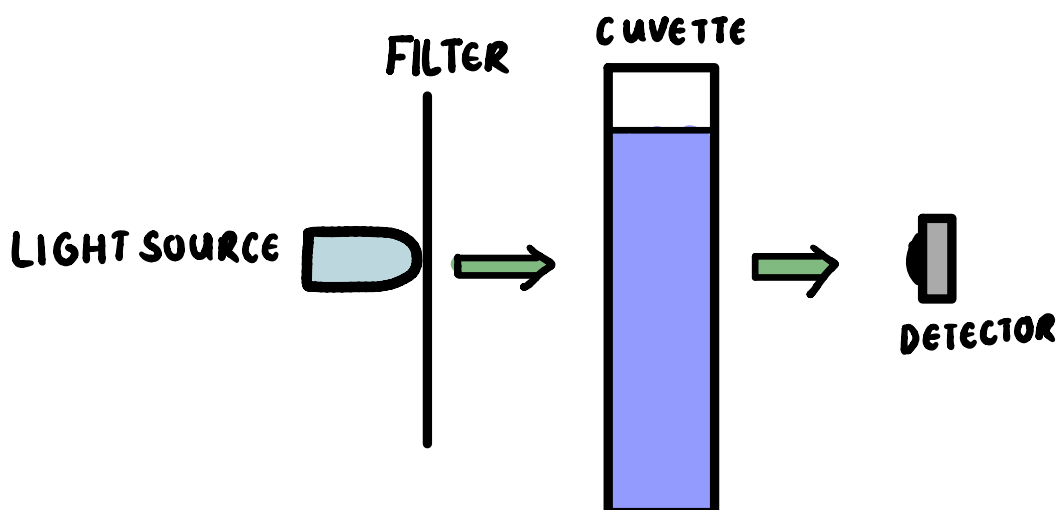
Safety precautions

1. Wear eye protection.
2. Avoid skin contact with the trypsin solution. If you get it on your skin, wash it off with cold running water.
3. Hot water from the water baths may be a hazard. If a water bath is above 50°C, take care not to scald yourself.

METHOD 1

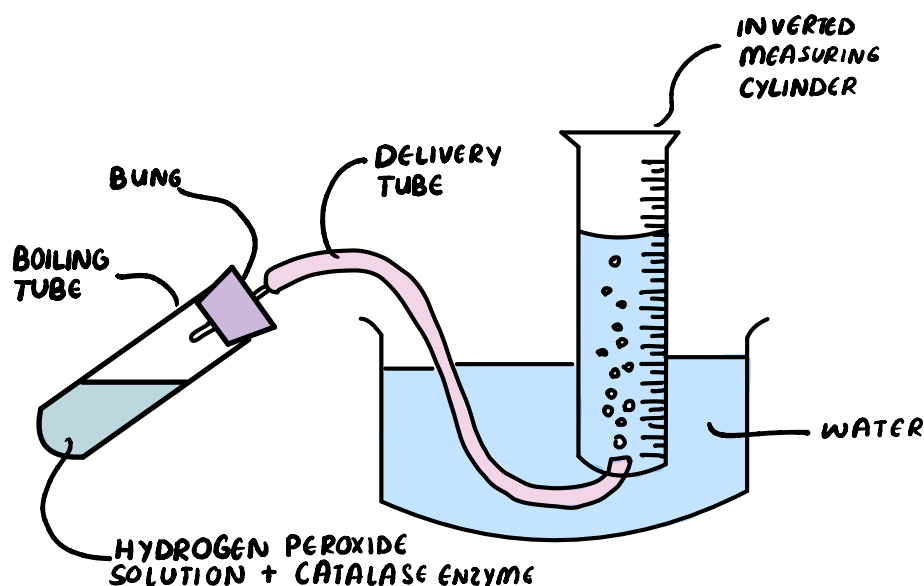
Procedure

- Make up 5 different concentrations of enzyme (0%, 1%, 2%, 3%, 4%) using distilled water.
- Zero/calibrate colorimeter using 2cm³ of 2% trypsin and 2cm³ of the distilled water in a cuvette.
- Take 2cm³ of the 0.2% milk suspension (casein) in a cuvette.
- Add 2cm³ of 2% trypsin solution to the milk in the cuvette and mix them quickly.
- Place the solution into the colorimeter and measure the absorbance immediately and then at 15 seconds intervals for 5 minutes.
- Rinse the cuvette with distilled water and repeat the procedure for other concentrations of trypsin (enzyme) as well.
- Record the data collected in a table.



METHOD 2

1. Set up the apparatus – conical flask/boiling tube connected to gas syringe through a delivery tube
2. Cut 10 discs of potato, each 0.2 mm thick. Place these in the boiling tube/conical flask with 5 cm³ of buffer solution.
3. Add 5 cm³ of hydrogen peroxide solution to the potato discs. Immediately place the bung and delivery tube firmly into the boiling tube.
4. Start a stopwatch. Collect any gas produced at suitable time intervals for 5 minutes or until there is little change in the volume. Shake the boiling tube gently throughout the reaction period to keep the contents well mixed.
5. Plot a graph of volume of gas produced against time. Use the graph to determine the initial rate of reaction. This is the initial gradient of the graph and should be the steepest part. Calculate the initial rate by dividing the change in the y-axis by the change in the x-axis values and use the units you have plotted on your x- and y-axes.
6. Repeat steps 1 to 5 of the experiment using a range of numbers of potato discs, ensuring that other conditions are kept unchanged
7. To keep temperature constant, place boiling tube in thermostatically controlled water bath



Why is the initial rate of reaction measured in enzyme experiments?

At the start of the reaction, substrate concentration is highest.

This means there are more successful collisions between enzyme and substrate molecules.

As the reaction continues, substrate is used up and product builds up, so the reaction rate decreases.

Therefore, measuring the initial rate gives the maximum and most reliable rate under the chosen conditions.

3 The enzyme urease catalyses the following reaction:



The effect of pH on this reaction was investigated using the following method.

- One test tube containing 5 cm³ of urease solution was placed in a water bath at 40 °C and left for 10 minutes.
- Another test tube containing 5 cm³ of urea solution in a buffer at pH 3.0 was placed in the same water bath and left for 10 minutes.
- After 10 minutes, the contents of both tubes were mixed together in one test tube.
- This test tube was replaced in the water bath.
- The concentration of ammonia was measured after 15 minutes and again after 60 minutes.
- The procedure was repeated for pH values of 4.0, 6.5, 6.8, 7.3, 8.0 and 9.0.

- (a) (i) Explain why the urease solution and the urea solution were kept in the water bath at 40 °C before and after being mixed.

(4)

to ensure both solutions were at 40 °C because mixing solutions of different solutions would make the temperature of the solution not equal to 40 °C.

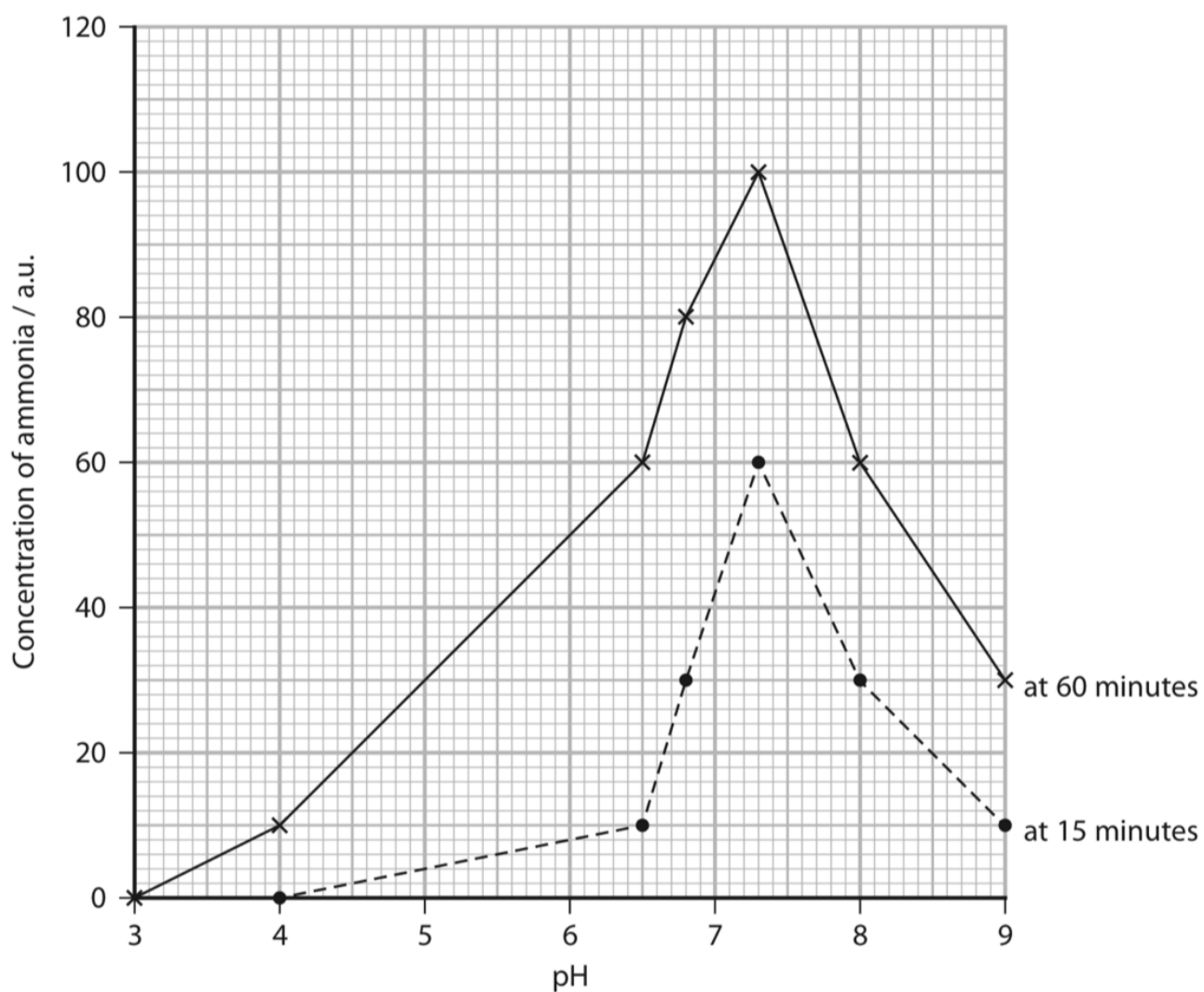
40 °C maybe the optimum temperature for urease. This also ensures that only the pH is caused

- (ii) Explain how you could determine suitable concentrations of urea and urease solutions to use in this investigation.

(2)

Carry out the above experiment at a range of different concentration of urease solution. Choose a combination where the rate of change is not too fast or too slow

(b) The graph shows the results of this investigation.



(i) Draw a suitable table to include the results for the experiment at 60 minutes.

(4)

pH	concentration of ammonia (a.u.)
3.0	0
4.0	10
6.5	60
6.8	80
7.3	100
8.0	60
9.0	30

- (ii) Calculate the rate of reaction at a pH of 7.3 during the first 15 minutes of this investigation.

Include appropriate units with your answer.

(2)

$$\text{rate} = \frac{60 \text{ a.u.}}{15 \text{ min}} = 4 \text{ a.u. min}^{-1}$$

Answer 4 a.u. min⁻¹

- (iii) Compare and contrast the results shown in the graph at 60 minutes with those at 15 minutes.

(3)

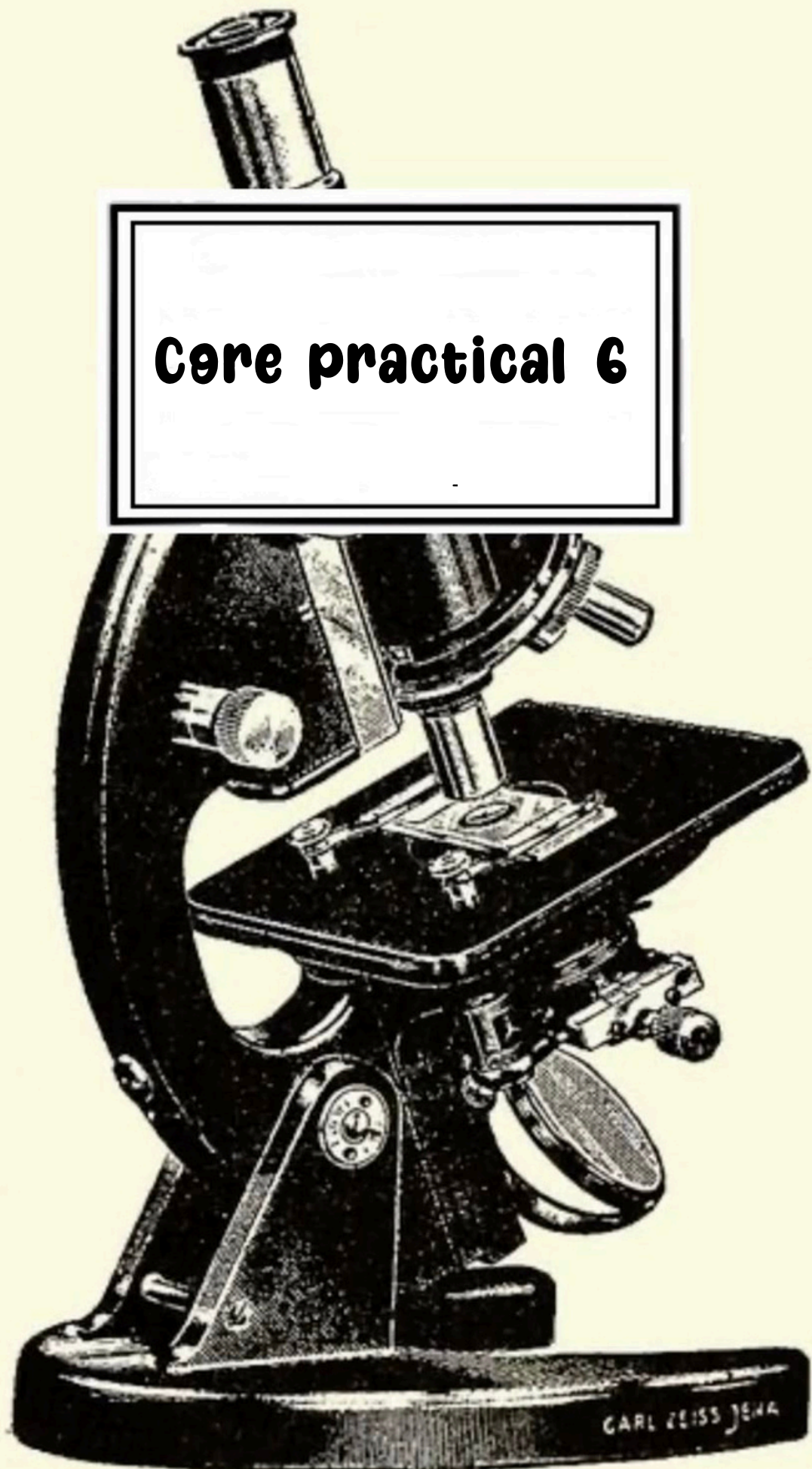
Both sets of results have a similar position

Both set of results have an optimum pH of 7.3.

More ammonia produced at every PH after 60 minutes than after 15 minutes.

No ammonia produced after 15 minutes at pH 4 but after 10 a.u. Of ammonia produced after 60 minutes

Core practical 6



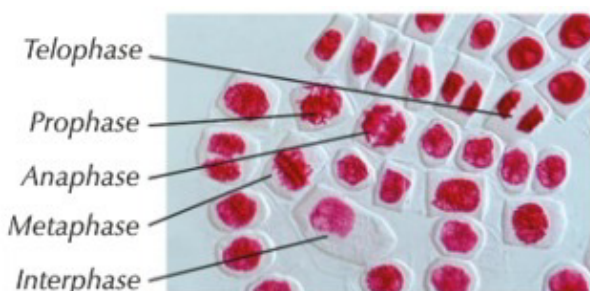
Core practical 06: Observing stages of mitosis

independent variable: distance from the tip or external factors (light, time of the day or certain chemicals that stimulate or inhibit mitosis)
Dependent variable: number of dividing cells per unit area

procedure

1. Removal of the last 5-10 mm of root tips (meristem).
2. Place root tip in hydrochloric acid to separate the cells/ macerate tissue/ soften tissue (making it easier to stain)
3. Addition of appropriate stain such as toluidine (blue), acetic orcein, methylene blue to high light chromosomes.
4. Place the root tip on microscope slide, teasing cells (using needle to spread cells), Squashing the cells underneath a cover slip to separate cells (for easier view), and heat the slide to intensify the stain.
5. Ensure the students view the preparation under the microscope on low power first to identify the area of dividing cells and to focus. Higher power will be required to examine the dividing cells for the stages of mitosis
6. Counting number of cells in mitosis, then counting total number of cells, then Calculate the mitotic index

$$\text{MITOTIC INDEX} = \frac{\text{NUMBER OF CELLS WITH VISIBLE CHROMOSOMES}}{\text{TOTAL NUMBER OF CELLS OBSERVED}}$$



safety precautions

1. Eye protection must be worn.
2. Acetic orcein stain is corrosive, causes burns, has an irritating vapour.
Wear eye protection and avoid contact with skin. If contact does occur, wash the area thoroughly with water for 10 minutes and tell your teacher.
3. Avoid skin contact with the hydrochloric acid, by using gloves (irritant/corrosive)

Q.Explain why the root tip is heated with acid?

To break up the tissues into individual cells. The cellulose cell walls of plant cells are held together by pectins such as calcium pectate. Treatment with hydrochloric acid breaks this down.

Q. State the effect of maceration and pressing the slide preparation on the dividing cells?

Separate cells in the meristem tissue into individual cells in a single layer. This makes it easier to see the chromosomes and to identify the stages of division

1: Trifluralin is a chemical used to stop the growth of weeds in fields of crops.

Mitosis is a process that is essential for the growth of weeds.

(a) State the **four** stages of mitosis in the order in which they occur.

(2)

1 Prophase

2 Metaphase

3 Anaphase

4 Telophase

(b) The effect of trifluralin on mitosis in wheat plants was investigated.

Wheat grains were germinated on moist filter paper.

The wheat seedlings were transferred to Petri dishes containing discs of filter paper, as shown in the photograph.



(Source: © Africa Studio / Shutterstock)

The filter paper discs were soaked in a solvent or the solvent containing 7 mg cm^{-3} of trifluralin.

The Petri dishes containing the seedlings were incubated at 25°C .

(i) Explain why a temperature of 25°C was used.

(2)

The optimum temperature for enzymes in mitosis hence prevents slower rate of

enzyme activity and denaturation of enzymes

(ii) State the independent variable in this investigation.

(1)

Presence of trifluralin

(iii) Give **two** reasons why the grains were germinated before being transferred to the Petri dishes.

(2)

1 To ensure the viability of seeds

2 All started treatment at the same time

(iv) Explain why some seedlings were placed in the solvent **without** trifluralin.

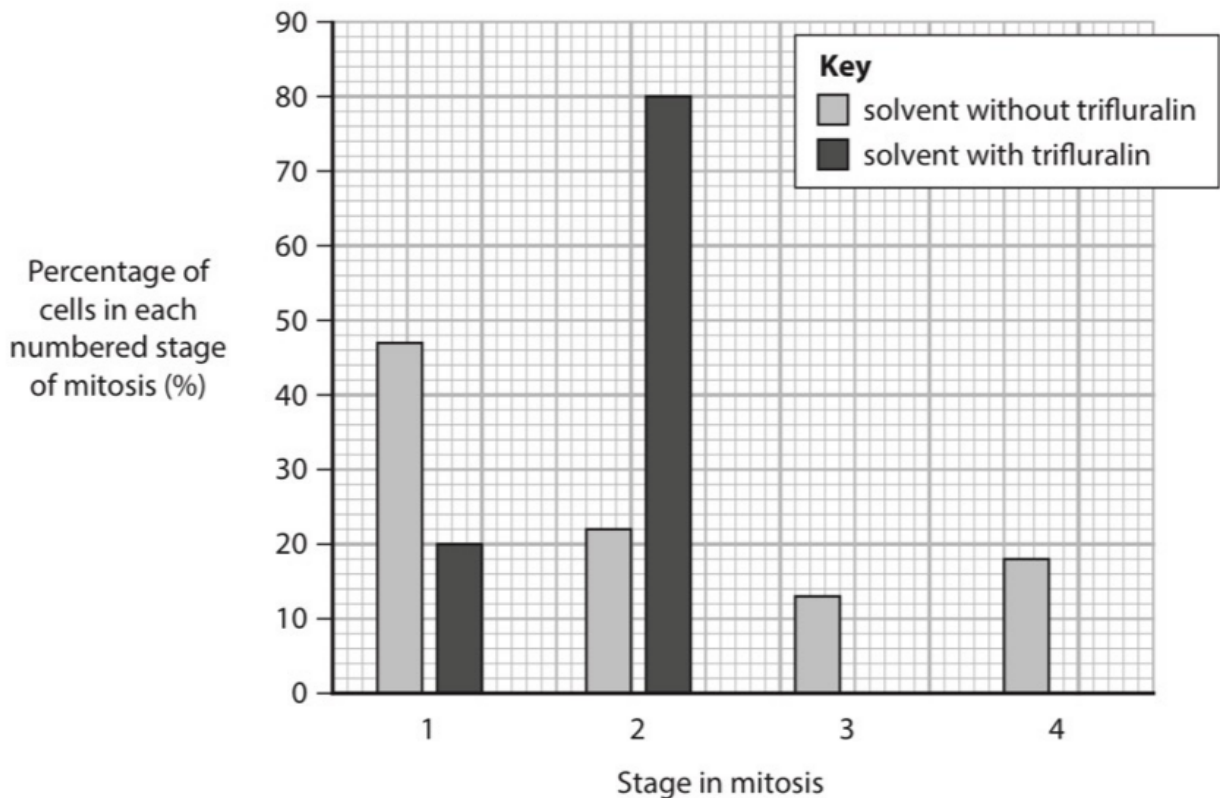
(2)

This acts as a control in order to prevent the effect of other factors than the presence of trifluralin. So that any changes using trifluralin could be attributed to trifluralin only

(c) After 12 hours of incubation, root samples were taken from seedlings in each Petri dish.

The root samples were examined to determine which stages of mitosis were present and how many cells were in each stage.

The bar chart shows the percentage of cells in each stage of mitosis, numbered 1 to 4.



(i) Describe how a root sample could have been prepared and examined to obtain these results.

(4)

cut the root to obtain the tips from the meristematic region.

Treat the root tip in warm dilute HCl for a set amount of time to soften the cell walls to squash easily

Macerate the root tip sample

Place the macerate root tips into a suitable staining agent like acetic orcein to stain the chromosomes

Transfer the stained sample to a microscope slide

Examine under high power, counting the cells in each mitotic stage across several fields of view and then calculate the percentage of cells in each stage

- (ii) There were 98 cells seen in total in the samples from the seedlings in the solvent with trifluralin.

Calculate how many cells were at stage 2 in the samples from the seedlings in the solvent with trifluralin.

(2)

$$\begin{aligned} \text{total \%} &= 20 + 80 \\ &= 100 \end{aligned}$$

$$\begin{aligned} \text{in stage 02} &= \frac{80}{100} \times 98 \\ &= 78.4 \\ &= \underline{\underline{78 \text{ cells}}} \end{aligned}$$

Answer 78 cells

- (iii) Explain the effect that trifluralin could have on the processes in mitosis.

(3)

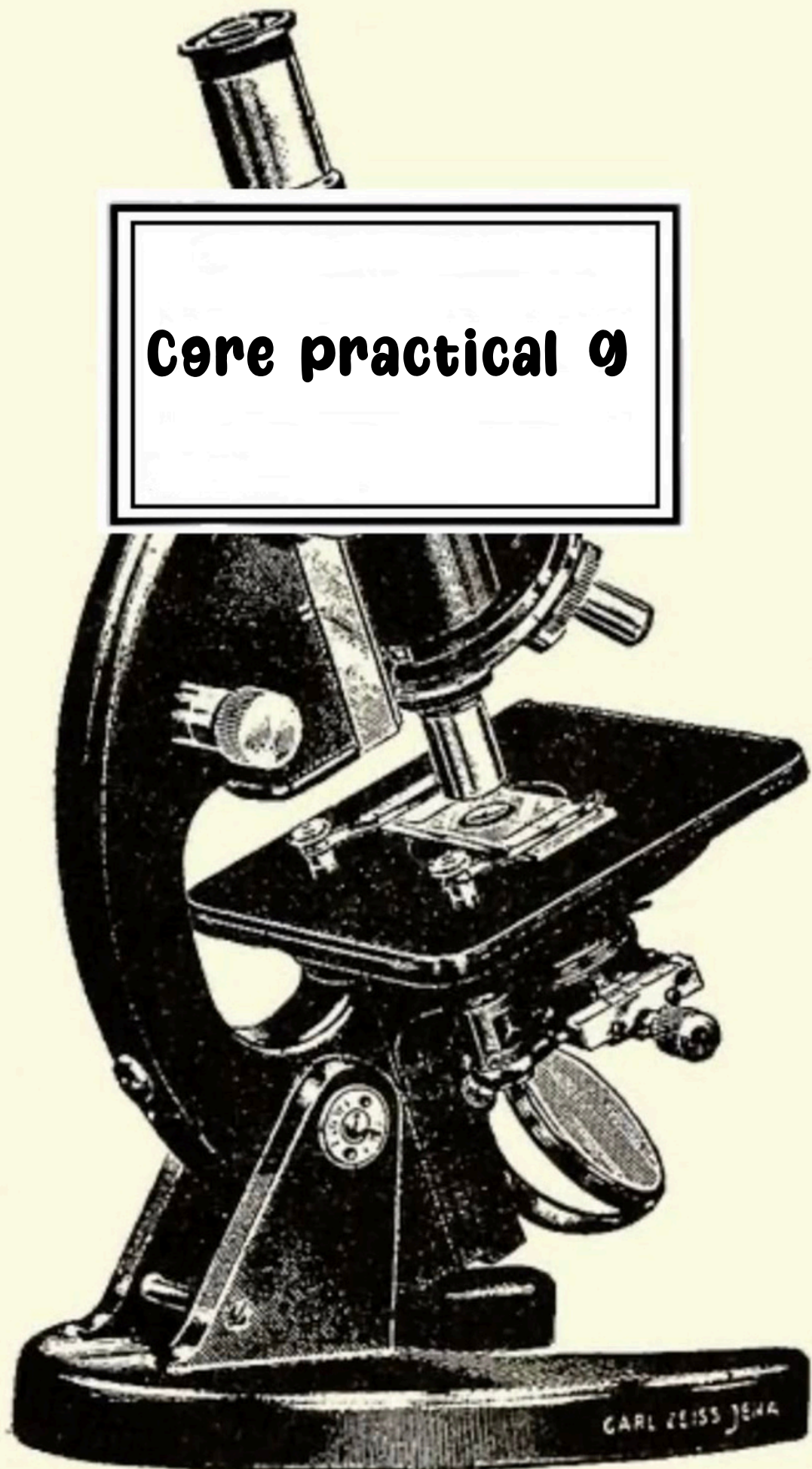
There is a lower or no mitotic index after stage 02

Hence, this indicates that the cell division stops after stage 02

This is because the chromosomes are not moving apart.

This is because spindle fibers are not formed

Core practical 9



core practical 09: Investigating antimicrobial properties of plants

Independent variable: different types of plant extracts/ different concentrations of a single plant extract/different types of solvents

Dependent variable: the size of the clear zone (also known as the inhibition zone)

Measuring the dependent variable: this can be measured in two different ways:

1. Measuring the diameter (several times) of the clear zone and then calculating the area using $A = \pi r^2$
2. Tracing the clear zone on graph paper and counting the boxes if the clear zone is not a circle
3. Either method requires the subtraction of the area of the disc in the middle of the clear zone



safety precautions

1. Wear eye protection.
2. Wash your hands with soap and water before and after the activity.
3. Do not open your inoculated plate. Instead, use the alternative plate provided by your teacher.
4. Do not decant ethanol near a naked flame and stopper the bottle after use.
5. Use aseptic techniques when transferring the bacteria to the Petri dishes.
6. Only open the Petri dish slightly when adding the discs with forceps (not fingers) and, once it is closed with some strips of tape, do not open it again.
7. Disinfect the bench before and after working. Leave the disinfectant on the bench for about 10 minutes.
8. If the agar plate shows signs of contaminating growth, do not open the plate. A technician will destroy it by autoclaving.

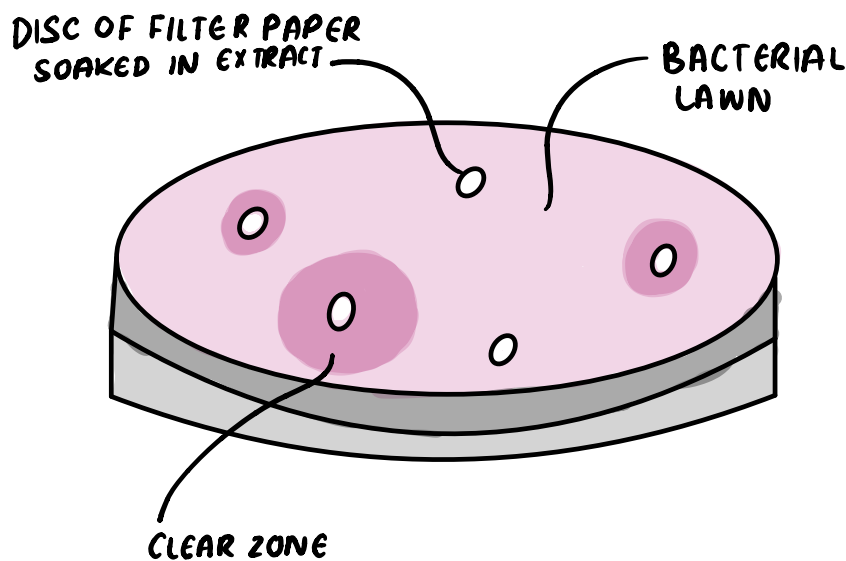
Aseptic techniques

Aseptic techniques: procedures to avoid contamination from bacteria which include all of the following:

1. Using sterilised Petri dishes, growth medium, and equipment
2. Heating metal equipment and glassware in an oven before using them
3. Sealing the containers
4. Using filtered air
5. Have a Bunsen burner lit to move microorganisms in the air away
6. Flaming, using a Bunsen burner, metal equipment before and after using them
7. Disinfecting surfaces by wiping them out with alcohol to kill bacteria

Procedure

1. Wash hands with soap and water and disinfect the bench area/use gloves/goggles
2. Take same mass of plant material, garlic, and mint
3. Place them in a mortar and grind into a paste with a pestle separately
4. Add 10cm³ of alcohol/ distilled water into the paste.
5. Take same sized sterile paper discs for all the extracts and soak them in respective plant extract.
6. One paper disc dipped in alcohol/ distilled water is used as a control.
7. Take sterile agar medium in a petri dish and allow it to cool.
8. Spread the microorganism evenly on the agar plate using sterile spreader.
9. Place the paper discs dipped in plant extract and the control disc in the agar plate using
10. sterile forceps.
11. Lid should be taped cross way.
12. Incubate it upside down at 25°C for 24 hours - 48 hours
13. Measure the diameter/ area of zone of inhibition using a ruler
14. Compare the result. (the larger the zone of inhibition the greater the antimicrobial effect)



Preparation of a bacterial lawn

- Sterilise the Inoculating loop by holding it in the Bunsen burner until it glows red hot and then leave it to cool.
- Dip the sterilised loop in the suspension of the bacteria. Streak the loop across the surface of the agar, avoiding digging into the agar.
- Replace the petri dish lid, tape closed (don't tape it all way around) and label. Turn dish upside down.

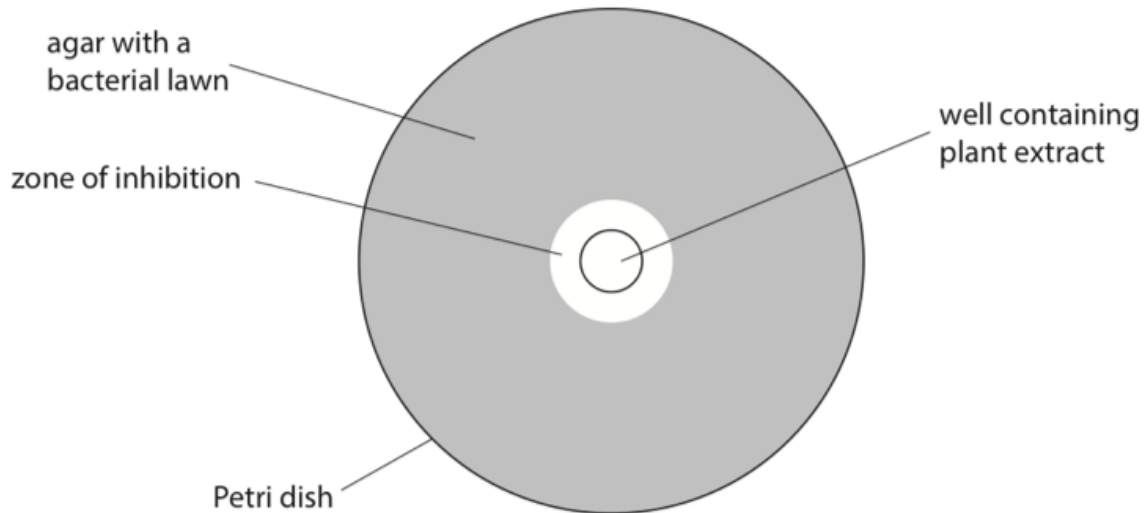
- 3 Petri dishes with nutrient agar were used in an investigation of the antimicrobial properties of plant extracts.

Bacteria were spread on the surface of the agar to form a lawn.

Plant extracts were placed in wells cut in the agar.

After incubation, the size of the zone of inhibition is a measure of the antimicrobial effect of the plant extract.

The diagram shows a Petri dish after the incubation period.



- (a) (i) Explain why the dishes were incubated for 48 hours at 30°C.

(2)

48 hours

Allows time for bacteria to become visible

30°C

Will not allow growth of human pathogens but still high enough for other bacteria to grow

(ii) Explain **three** aseptic techniques that should be used when transferring bacteria from one culture to another.

(3)

1 Flame the loop and neck of the culture bottle to kill the bacteria

2 Have a Bunsen burner lit to remove microorganisms in the air away

3 Lift the Petri dish or culture bottle partially to minimize entry of bacteria

(b) The technique described was used to investigate the antimicrobial effects of oil A and oil B, extracted from two species of plant.

Different bacterial lawns were made using *E. coli* and *B. subtilis*.

(i) Explain how the area of the zone of inhibition could be measured accurately.

(2)

Use squared paper measure the size of an individual square

Count the number of squares that either fully or more than half covered then add the area of all squares

(ii) State **one** independent variable in this investigation.

(1)

Type of species of extract

(c) The size of the zones of inhibition may also be affected by the rate of diffusion of the antimicrobial molecules through the agar.

(i) Suggest **two** factors that may affect the rate of diffusion of the antimicrobial molecules.

(2)

1 The size of the antimicrobial molecules affect its function

Solubility of antimicrobial molecule

Properties of agar

2 The concentration of antimicrobial reactions

Incubation temperature