

# Determination of Refractive Index of Highly Concentrated Solutions of NaCl and Glycerin using Total Internal Reflection (TIR) (14 points)

Please read the general instructions in the separate envelope before you start this experiment.

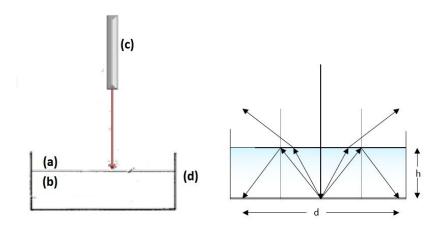
#### Dependence of Refractive Index of a Solution on Concentration

#### Introduction:

**Basic Principle:** When a beam of light is incident on a surface, reflection and refraction take place. When the surface is uneven (rough), these phenomena result in scattering light in all directions. If the surface is that of a transparent medium, then most of the light is transmitted (refracted) and a small portion of it is reflected. If the surface is opaque and polished (like a metallic surface), all the light is reflected.

In this experiment, the green laser light is shone normally into the water inside a container. The container used has vertical walls. The laser beam first meets the smooth top water surface then it gets scattered from the rough bottom surface of container while producing a bright green spot on the bottom surface. This scattered light travels back into the water in all directions. This light again meets the top smooth surface of water and undergoes reflection, refraction and another phenomenon called total internal reflection (see Figure 1).

The scattered rays that reach the top surface of the water at an angle greater than the critical angle, get totally internally reflected which results in a bright ring enclosing a dark region.



(a) Air (b) Liquid (c) Laser Pointer (d) Container with Liquid

**Figure1**: (*Left*) Arrangement to observe the phenomenon. (*Right*) The ray diagram.



Q1-2

English (Official)

From the definition of refractive index and critical angle we have:

$$\mu = \frac{1}{Sin\left(\theta_{C}\right)} = \frac{\sqrt{(\frac{d}{4})^{2} + (h)^{2}}}{\frac{d}{4}} = \frac{\sqrt{(d)^{2} + 16 \times (h)^{2}}}{d} - - - (1)$$

Where  $\mu$  is the refractive index (RI) of liquid, d is the diameter of the dark disc and h is the height/depth of liquid. This formula can be applied to any transparent liquid medium.

From equation-(1)

$$(d)^2 \times (\mu)^2 = (d)^2 + 16 \times \frac{(V)^2}{(A)^2}$$

Where, **A** is the effective area of the horizontal cross-section of the container and **V** is the volume.  $h = \frac{V}{A}$ The diameter (d) as a function of refractive index and area of the container (**A**) is

$$d = \frac{4}{A \times \sqrt{\mu^2 - 1}} \times V = S \times V - - - - - - - (2)$$

The diameter is proportional to volume and S is the proportionality constant given by

Using a liquid whose refractive index we know , we can calculate **A.** The effective area of the horizontal cross-section of the container is given by

$$A=\frac{4}{S\times\sqrt{\mu^2-1}}------(4)$$

The present experiment is to determine the refractive indices of salt and Glycerin solutions at certain high concentrations, using the refractive index of water (1.33).

#### **Percent Concentration of Solutions:**

Percent concentration **volume per volume** (V/V) is defined as the volume of solute in ml in 100 milliliters of solution. Hence 50% solution of any solute is 50 ml of solute in 100 ml of solution).



#### Aim:

- 1. Determination of refractive index of 30% NaCl solution and Glycerin.
- 2. Determine the dependence of RI on the concentration of Glycerin water solution.

**Equipments:** You are supplied with the following equipment for this experiment:

Sr. No.	Item	Specifications	Quantity
01	Green LASER Pointer	Wavelength-532 nm	1 no + 1 spare
02	Burette stand	As shown in final assembly	1 no
03	Beaker	500 ml	3 no
04	Syringe	50 ml	1 no
05	Digital thermometer	To measure Room Temp	1 no
06	Glass stirrer	For making solutions	1 no
07	Container	As shown	1 no
08	Sodium Chloride (NaCl) Solution	30 % from AR grade salt	500 ml
09	Glycerin	AR grade	500 ml
10	Distilled water	Solution + washing	5000 ml
11	Tissue paper		
12	Safety goggle	Polaroid	1 no
13	Divider	Screw adjustable	1 no
14	Steel scale (ruler – optional)	0.5 mm Least Count	1 no
15	Reading lens	High quality	1 no

#### Warning:



Avoid direct eye exposure and through reflections.

Avoid staring at the laser spot for too long, advise to turn off the laser when it's not used for performing measurements



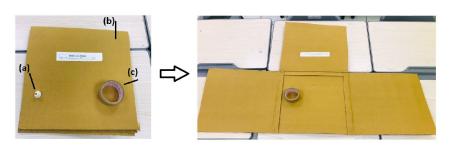
#### The Experiment

#### Part-0 Measurement of the room temperature (0.2 points)

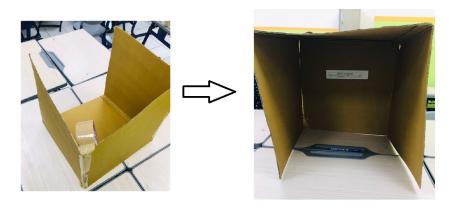
A.0 Measure the room temperature using the thermometer provided and record your reading in the answer sheet.(Get the supervisor's sign after taking this reading)

#### Steps for setting up the equipment .

#### Step-1: Making a cardboard box.



(a) Cardboard Holder (b) Cardboard Box (c) Tape





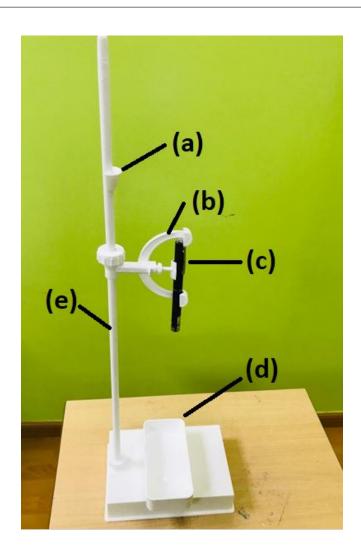
Wear your safety googles all the time. If you are already wearing spectacles, wear the safety googles above that. Do not look directly into the laser light.

Switch off the laser light when you are not taking the readings.

Glycerin should be kept covered when not in use.

Part 1. Calculation of effective area of cross section (A) of the container using distilled water (3.6 points)

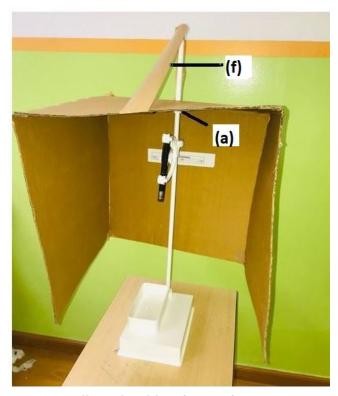
Step-2: Attaching the Green LASER Pointer on the burette stand. The laser pointer should be vertical .



(a) Cardboard Holder (b) Fisher Clamp (c) Laser (d) Opaque Rectangular container(TIR Container) (e) Stand Rod



## Step-3: Attaching the cardboard box on the burette stand.



(a) Cardboard Holder (f) Tape for support



#### **Procedure for Observation:**

readings.

#### Apparatus set up:

Clamp the laser light in the burette stand in such a way that the switch is pressed by the middle grip of the stand. (When it is to be switched off, simply rotate the laser around the vertical axis so that the pressure on the switch is released. When it is to be switched on, rotate it back to the original position).

Make sure that the base of the stand on which the container is placed is horizontal.

#### Switch off the laser light when you are not taking reading.

Place the container below the light so that the laser beam falls on the bottom of the container.

- (i) **Add 50 ml of distilled water** into the container. Switch on the laser, a bright ring with a dark disc inside becomes immediately visible.
  - A.1 (ii) Using the divider and ruler provided, measure the diameter of the dark disc. Use the reading lens for better observation of the disc diameter. Record your readings in the Table 1 of the answer sheet.

    (iii) Repeat steps (i) and (ii) by adding water in steps of 50 ml to obtain six
  - **A.2** (iv) **Plot a graph on the given graph sheet (Graph 1 plot 1)** with diameter of the dark disc (d) on the vertical axis and the volume (V) of water on the horizontal axis using the given graph page in the answer sheet. (1.8pt)

Note: (0,0) will be an additional data point to be plotted on the graph (The total number of points to be plotted is 7) while plotting use symbol

( ) dot = water

(.) dot = water For marking the points on the graph

- **A.3** (v) Calculate the slope from the graph (S = d/V) (0.2pt)
- **A.4** (vi) **Calculate the effective area (A)** of cross-section of the container from the slope and equation (4)



#### Part 2: Determination of Refractive index of 30% NaCl Solution (3.4 points)

You are supplied 500ml of 30% NaCl Solution.

(i) Clean the container dry it by dabbing it with tissue paper.

- **B.1** (ii) Using the salt solution with fixed concentration, follow the steps (i) to (iii) of (1.2pt) **Part 1**. Enter your readings in **Table 2** of your answer sheet.
- (iii) Plot a graph on the same graph sheet in the answer sheet (Graph 1 plot
   2) overlapping graph plotted in Part 1) with diameter on the vertical axis and volume on the horizontal axis. Label the points for distinction.
   Note: (o,o) will be an additional data point to be plotted on the graph (The total number of points to be plotted is 7)

while plotting use symbol (+) plus = NaCl solution For marking the points on the graph

- B.3 (iv) Calculate the slope from the graph (0.2pt)
  - **B.4** (v) Calculate the refractive index of 30% NaCl solution from the slope and the value of A calculated in Part 1.



#### Part 3-A: Determination of refractive index of Glycerin (3.4 points)

You are supplied with 500 ml of Glycerin.

(i) Clean the container and dry it by dabbing it with tissue paper.

**C-1.1** (ii) Using the pure glycerin provided **follow the steps (i) to (iii) of Part 1**. Enter your readings in **Table 3a** of your answer sheet. (1.2pt)

(iii) Plot a graph on the same graph sheet in the answer sheet (Graph 1 - plot
 3) overlapping graph plotted in Part 1 and 2) with diameter on the vertical axis and volume on the horizontal axis. Label the points for distinction.

Note: (o,o) will be an additional data point to be plotted on the graph (The total number of points to be plotted is 7) while plotting use symbol

(\*) star = Glycerin

For marking the points on the graph

(iv) Do not disturb this solution at this point as it is required for part 3B of this experiment.

C-1.3 (v) Calculate the slope from the graph. (0.2pt)

C-1.4 (vi) Calculate the refractive index of glycerin from the slope calculated in this part of experiment and the value of A already calculated in Part 1 of this experiment. (0.4pt)



# Part 3B: Relation between Refractive index and concentration of Glycerin solution. (3.4 points)

Glycerin is miscible with water in all proportions; however, it takes thorough stirring to obtain a homogeneous mixture. In this part you will be measuring the refractive index of different concentrations of aqueous solutions of Glycerin.

(i) Using the syringe provided, remove 150 ml of Glycerin from the container, so the remaining amount of glycerin is 150 ml in the container.

- **C-2.1** (ii) Measure d and enter values of volume and diameter in Table 3b in the answer (1.6pt) sheet.
  - (iii) Now add 50 ml of water to the container, stir the mixture gently and thoroughly to make
  - a homogeneous solution.
  - (iv) Calculate the new concentration of the solution.
  - (v) Measure the diameter of the ring and record values of volume, diameter and concentration in Table 3b in the answer sheet.
  - (vi) Repeat steps (iii) to (v) for two more dilutions.

Calculate the values of S and Refractive Indices of the solutions and enter the values in the Table 3b in the answer sheet.

C-2.2 (vii) Plot the values refractive index on the vertical axis against the concentration on the horizontal axis (graph -2) in the answer sheet.

Note: (o,1.33) will be an additional data point to be plotted on the graph.

(The total number of points to be plotted is 5)

At this stage you have measured the refractive indices of 30 % NaCl solution and glycerin. You have also determined the relation between concentration and refractive index for glycerin solutions.

#### Answer the following questions in the answer sheet by choosing correct option:

- C-2.3 How does the refractive index change with the concentration of glycerin (0.2pt) solutions?
  - a. Increases with concentration
  - b. Decreases with concentration
  - c. Does not change with concentration
- C-2.4 How would you expect the refractive index of NaCl solution to change with (0.2pt) concentration?
  - a. Expected to increase with concentration
  - b. Expected to decreases with concentration
  - c. Expected not to change with concentration





# Determination of Refractive Index of Highly Concentrated Solutions of NaCl and Glycerin using Total Internal Reflection (TIR) (14 points)

Instruction - column on the right side is for office use only - do not write in this space

Part-0 Measurement of	the room temperature	e(Get the supervisor's	s signature after taking
the temperature) (0.1 բ	points)	•	

<b>A.0</b> $(0.2 \text{ pt})$	
The room temperature is	

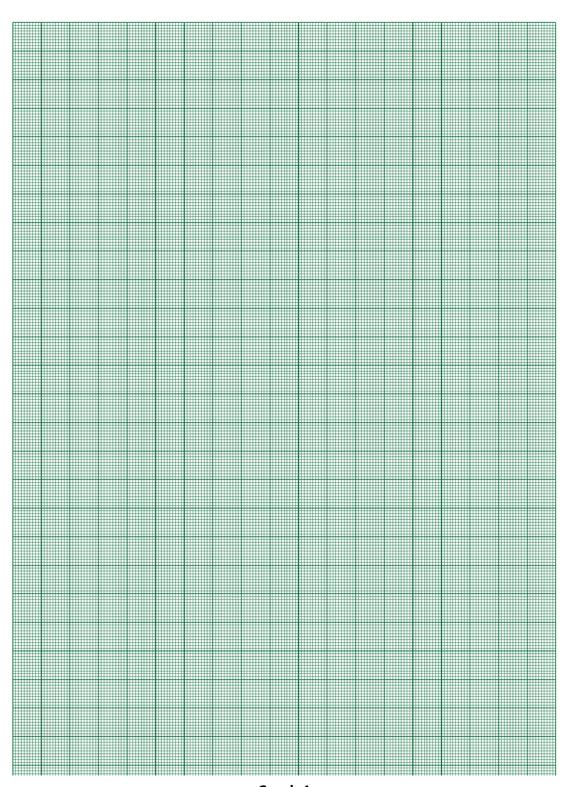
# Part 1. Calculation of effective area of cross section (A) of the container using distilled water (3.4 points)

le 1:						
S No.	1	2	3	4	5	6
Volume of water, V (ml)						
Diameter of ring, d (cm)						

<b>A.2</b> (1.8 pt) <b>Graph 1.</b>		







**Graph-1** 





<b>A.3</b> $(0.2  ext{ pt})$	
The slope from the graph (S = d/V)=	
•	
<b>A.4</b> (0.4 pt)	
A.4 $(0.4~\mathrm{pt})$ Effective area of cross section of the container (A) =	





# Part 2: Determination of Refractive index of 30% NaCl Solution (3.4 points)

					ı	1
S No.	1	2	3	4	5	6
Volume of solution, V (ml)						
Diameter of ring, d (cm)						

B.2  $(1.6~\mathrm{pt})$ Graph 1 Plot 2 (overlapping with previous Part).

B.3 $(0.2~\mathrm{pt})$ Slope from the plot 2 =	
•	





B.4 $(0.4~\mathrm{pt})$ Refractive Index of 30% NaCl solution						



# Part 3-A: Determination of refractive index of Glycerin at different concentrations (3.4 points)

C-1.1  $(1.2 \mathrm{\ pt})$  Table 3a:

S No.	1	2	3	4	5	6
Volume of solution, V (ml)						
Diameter of ring, d (cm)						

C-1.2  $(1.6~\mathrm{pt})$  Plot the graph in Graph 1 plot 3 (overlapping with previous Parts).

C-1.3 $(0.2 \mathrm{\ pt})$ Slope from the plot 3 =	





C-1.4 $(0.4 \mathrm{\ pt})$ Refractive Index of glycerin=						
•						



# Part 3B: Relation between Refractive index and concentration of Glycerin solution. (3.4 points)

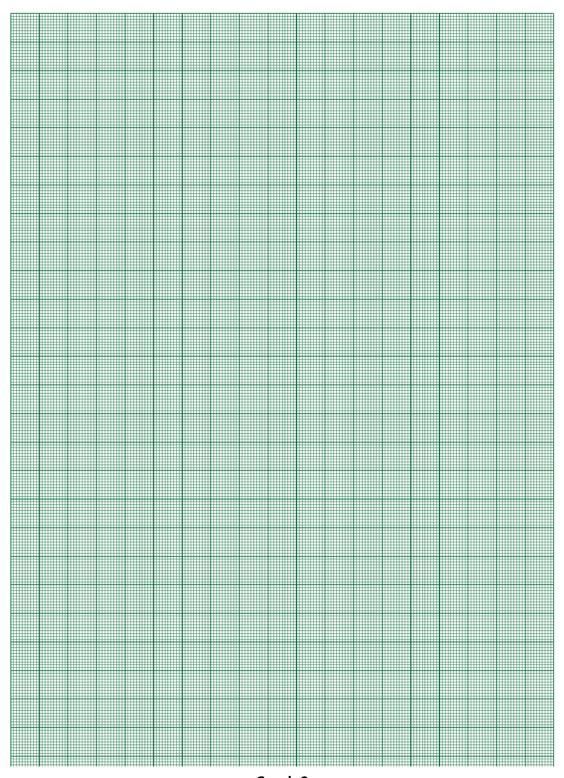
**C-2.1** (1.6 pt) **Table 3b:** 

S No.	1	2	3	4
Volume, V (cc)				
Diameter, d (cm)				
Concentration %				
S= d/V (cm <sup>-2</sup> )				
R.I.				

<b>C-2.2</b> (1.4 pt)
Graph 2.







**Graph-2** 



Calculations for concentrations
· 
Calculations for RI
•

At this stage you have measured the refractive indices of 30 % NaCl solution and glycerin. You have also determined the relation between concentration and refractive index for glycerin solutions.

From your measurements, answer the following:





**C-2.3** (0.2 pt)

#### Choose the correct answer from the options given and fill in the blank

Refractive index of Glycerin.....

- a. Increases with concentration
- b. Decreases with concentration
- c. Does not change with concentration

From the observations made above, give your prediction for NaCl solutions.

 $\mathbf{C-2.4} \ (0.2 \ \mathrm{pt})$ 

#### Choose the correct answer from the options given and fill in the blank

Refractive index of NaCl solution.....

- a. Expected to increase with concentration
- b. Expected to decreases with concentration
- c. Expected not to change with concentration



# **Determination of the Glucose Content of Date Syrup Sample** (8 Marks)

#### Please read the general instructions before you start this problem.

Date palms (*Phoenix dactlylifera*) are found in abundance in the Middle East and in desert areas. The latin name of the tree is believed to have been derived from Greek *Phoenix daktulos*, which means purple or red finger. In the UAE, the late Sheikh Zayed Bin Sultan Al Nahyan was the founder of the country's agricultural renaissance. He encouraged the people of UAE to cultivate dates beyond the borders of the oasis where they traditionally grew dates, which played a great role in the flourishing dates throughout the nation. This great achievement turned the desert into an abundant paradise for its dwellers; UAE today brings this ancient super fruit to a new global market, which made the UAE the leader of date's cultivation in the world. From dates, a variety of food products are made.



Dates are rich in sugar which consists of two isomeric carbohydrates-Glucose (Molecular Formula  $C_6H_{12}O_6$ ) and Fructose (Molecular Formula  $C_6H_{12}O_6$ ). Glucose is the most important source of energy in all organisms.

Glucose contains an aldehyde group (-CHO) and hence is an aldose. Fructose contains a keto group (-C=O) and hence is a ketose. Glucose can be quantitatively oxidized to Gluconic acid ( $C_6H_{12}O_7$ ) by iodine in alkaline medium.This enables the estimation of Glucose in presence by Fructose.

Date syrup is treated with iodine solution in the presence of  $Na_2CO_3$  solution.

$$2Na_{2}CO_{3\;(aq)}\;+I_{2\;(aq)}\;+H_{2}O_{\;(l)}\;\rightarrow\;NaI_{\;(aq)}\;+\;NaOI_{\;(aq)}\;+2NaHCO_{3\;(aq)}$$

 $Glucose + Sodium hypo Iodite \longrightarrow Sodium Gluconate + Sodium Iodide$ 

Glucose: Sodium hypoiodite = 1:1

The iodate (I) (hypoiodite) is converted back to iodine after adding acid.

$$NaI_(aq) \, + NaIO_(aq) \, + \, 2HCl_(aq) \, \longrightarrow \, I_2(_aq) \, + \, H_2O_(aq) \, + 2NaCl_(aq)$$

On the completion of the oxidation, the excess iodine is back titrated against  $Na_2S_2O_3$  solution using starch as an indicator.



$$I_{2~(aq)}~+2Na_2S_2O_{3~(aq)}~\longrightarrow~Na_2S_4O_{6~(aq)}~+2NaI_{~(aq)}$$
 ( In acidic medium)

#### You are supplied with the following:

Chemicals	Labeled as	Quantity Supplied
Date Syrup	Date Syrup Sample	Supplied in Plastic Container
Iodine solution in a closed bottle	Iodine	100 mL
0.10 M $Na_2S_2O_3$ in a beaker	$0.10\;M N a_2 S_2 O_3$	150 mL
15% $Na_2CO_3$ in a beaker	15% $Na_2CO_3$	50 mL
2M $HCl$ in a beaker	2M HCl	100 mL
Starch	Starch Indicator	15 mL in Falcon Tube
Distilled Water	Distilled Water	1000 mL in a bottle
Apparatus	Labeled as	Quantity supplied
25 mL burette on stand	$B_1$	1
10 mL volumetric pipettes	$P_1$ , $P_2$	2
Pipette fillers		2
250 mL Glass stoppered bottles		3
150 mL conical flasks	$C_1$ to $C_3$	3
Funnel		1
100 mL Volumetric flask	$V_1$	1
10 mL measuring cylinder		1
Wash bottle with distilled water		1
Dropper		1
Beaker 150mL		1

- 10 mL measuring cylinder is to be used for 15% Sodium Carbonate, 2M HCl and starch indicator. Make sure that you wash it before every use.
- Feel free to use beakers, conical flasks and funnels that are available in your laboratory that fit with the experimental requirements, if necessary.

#### **Procedure**

i)Standardization of Iodine solution



- 1. Rinse burette  $B_1$  with 3 to 5 mL of 0.1 M  $Na_2S_2O_3$
- 2. Fill burette  $B_1$  with 0.1 M  $Na_2S_2O_3$  using a funnel.

#### Note down the initial burette reading in Observation Table 1 in the answer sheet provided to you.

- 3. Using pipette  $P_1$ take exactly 10 mL iodine solution in a 150 mL conical flask  $C_1$ .
- 4. Using measuring cylinder add approximately 20mL of distilled water to the Iodine solution taken.
- 5. Titrate the iodine solution against 0.1M  $Na_2S_2O_3$  from burette  $\mathsf{B}_1$  till a yellowish or light brown colour appears.
- 6. Add 2 mL of starch indicator using 10 mL measuring cylinder , the solution turns blue, and continue to titrate. The endpoint is colour change from blue to colourless.
- 7. Repeat the titration twice using conical flasks  $C_2$ . and  $C_3$ .

2.1	Record your titration readings in Observation Table 1 and note down the	(3.0pt)
	burette reading.	

2.2 Calculate the molarity of Iodine solution.

(0.5pt)

#### ii) Estimation of Glucose in Date Syrup

- 1. Using a funnel fill burette  $B_1$  with 0.1M  $Na_2S_2O_3$ .
- 2. Note down the initial burette reading in Observation Table 2 in the answer sheet provided to you.
- 3. Dissolve the date syrup supplied in the plastic container with warm distilled water. Transfer the solution quantitatively in the 100 mL volumetric flask  $V_1$  and dilute with distilled water up to the mark using dropper. (You can use funnel to transfer the glucose solution.)
- 4. Insert the stopper and shake the solution to homogenise it. Transfer the homogenised solution to a correctly labelled 150 mL beaker.
- 5. Using pipette P<sub>2</sub>, take exactly 10 mL of the homogenised solution in a 250 mL glass stoppered bottle.
- 6. Using a 10 mL measuring cylinder, add approximately 10 mL of 15%  $Na_2CO_3$  solution and add exactly 10 mL Iodine solution using pipette  $P_1$ .
- 7. **Stopper the bottle** and **keep aside in the dark for about 30 minutes**. Repeat Steps 5 and 6 using two other 250 mL glass stopper bottles and keep them aside in the dark for about 30 mins each.
- 8. After 30 minutes, add 10 mL of 2M HCl using measuring cylinder, stopper the bottle and shake well (Iodine will liberate).
- 9. Add  $Na_2S_2O_3$  drop wise from the burette  $\mathsf{B}_1$  till a light yellowish / light brown colour appears and then add 2 mL of starch solution indicator (dark blue colour will be obtained) using a 10 mL measuring cylinder and continue to titrate.
- 10. The endpoint is colour change from dark blue to colourless.
- 11. Repeat the titration twice.



$$I_{2~(aq)}~+2Na_{2}S_{2}O_{3~(aq)}~\longrightarrow~Na_{2}S_{4}O_{6~(aq)}~+2NaI_{~(aq)}$$

2.3	Record your titration readings in Observation Table 2 and note down the burette reading.	(3.0pt)
	Molar mass of Glucose=180 g/ mol	

Ask for the mass of Date syrup given to you m(g)= g.
Observations
1.Calculate the number moles of Iodine solution added to 10 mL date syrup
=
2. Calculate the number of moles of Iodine which remain unused in the glucose oxidation reaction =
moles
3. Calculate the number of moles of Iodine used up for oxidation of 10 mL date syrup
=moles
4. Calculate the total number of moles Iodine used up for the oxidation of the given date syrup
the number of moles of Iodine =moles



2.4 Calculations (1.5pt)

Determine a) the number of moles b) mass c) percentage of Glucose present in the given date syrup sample.





# **Determination of the Glucose Content of Date Syrup Sample** (8 Marks)

Consider 1 Decimal place when recording your burette reading.

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Standardisation of Iodine Solution (Titration-1)

**Observation Table 1** 

- 5 % deviation = 2.5 mark
- 10 % dev. = 1.5 mark
- 10-15% dev = 0.5 mark
- Consider the burette reading to one decimal place.

Sr.		Titration	Titration	Titration
		I	II	III
1	Initial burette reading mL			
2	Final burette reading mL			
3	Difference in burette readings mL			

Burette reading =	mL
(0.5 mark)	

<b>2.2</b> (0.5 pt)	
Molarity of Iodine solution =M	
Wolarity of fourtie Solution	



A2-2
English (Official)

# 2.3 $(3.0\ \mathrm{pt})$ Estimation of glucose in date syrup (Titration-2) Observation Table 2

Sr.		Titration	Titration	Titration
		I	II	III
1.	Initial titration reading mL			
2	Final Titration reading mL			
3	Difference in burette readings mL			

Burette reading =.....mL





2.4 $(1.5~{\rm pt})$ Calculations 1. Determine the mass of Glucose in the given sample of Date syrup. (1 mark total) -Calculate the number if moles of glucose in the given sample (0.5 marks)
- Calculate the mass of glucose in the given sample (0.5 marks)
2.Determine the percentage of Glucose in the given sample of Date syrup. (0.5 marks)
Results:  1. Mass of Glucose in the given sample of Date syrup =g
2. Percentage of Glucose in the given sample of Date syrup =%



# pH Titration using Universal Indicator (6 marks)

Please read the general instructions before you start this problem.

An acid base indicator is a substance that changes colour depending on the pH of the solution to which it is added. Such substances are used in solution or powder form for the determination of pH of a solution or to detect changes in pH during acid-base titrations.

A Universal indicator is able to change colours in a wide range of pH, and is hence used to determine how acidic or basic a solution is. Universal indicator that can be used to indicate the pH of the solution. It is a mixture of indicators that shows different colours at different pH values, and can be used in solution form or as paper strips. A colour chart supplied with the indicator solution or strips enables the determination of pH value.

pH range	Description	Colour
< 3	Strongly acidic	red
3 -6	Weakly acidic	orange, yellow
7	Neutral	green
8 – 11	Weakly alkaline	blue
> 11	Strongly alkaline	violet

For the ease of the translations use the following color index to express the color as a letter, so it is easier for the markers to identify.

R	Red					
0	Orange					
Υ	Yellow					
G	Green					
В	Blue					
٧	Violet					

In case if you **ONLY** have Universal indicator as strips, use the following procedure. For every successive additions from the burette, stir the solution well, then touch the solution with a glass rod or dropper to take a very little of the solution and put it on the indicator strip and record the color. Repeat this until the end of the titration.

**Warning:** This procedure is written so that error in using pH strips is minimised. However, it will have an important error compared to the use of an indicator solution.

In this experiment, the equivalence point of an acid-base titration will be determined using Universal indicator to determine pH during the titration of weak acid with strong base.

You are supplied with the following:



	Apparatus	Labelled as	Quantity supplied
1	10 mL pipettes	$P_3,P_4,P_5$ and $P_6$	4
2	25 mL burette	$B_2$	1
3	100 mL volumetric flasks	$V_2,\!V_3,\!V_4$	3
4	250 mL beaker		1
5	150 mL conical flasks	C <sub>4</sub> , C <sub>5</sub>	2
6	Funnel		1
7	Indicator bottles		2
8	Dropper*		1

<sup>\*</sup> Use Dropper from Q-2

Chemicals	Labelled as	Quantity supplied		
0.1M Succinic acid	0.1M Succinic acid	100 mL in a beaker		
0.1M $NaOH$ (approx)	0.1M $NaOH$ (approx)	100 mL in a beaker		
Weak acid solution	Weak acid	In 100 mL volumetric flask $V_4$		
Universal indicator solution	Universal indicator with colour chart	In indicator bottle		
Phenolphthalein indicator	Phenolphthalein	In indicator bottle		
Distilled water	Distilled water	1000 mL in a bottle		

• Feel free to use beakers, conical flasks and funnels that are available in your laboratory that fit with the experimental requirements, if necessary.

#### **Procedure**

#### 1. Preparation of 100 mL of 0.01 M Succinic acid solution.

Using pipette  $P_3$  take 10 mL of the supplied 0.1 M succinic acid solution in volumetric flask  $V_2$  and dilute up to the mark using distilled water.

#### 2. Standardisation of the diluted ${\it NaOH}$ solution.

- 1. Using pipette  $P_4$  take 10 mL of the supplied 0.1 M(approx) NaOH solution in volumetric flask  $V_3$  and dilute up to the mark using distilled water.
- 2. Rinse burette  $B_2$  with 3-5 mL of diluted NaOH.
- 3. Using a funnel fill the burette with diluted NaOH.

#### Note down the initial reading in Observation Table 1 in the answer sheet provided to you.

- 4. Using pipette  $P_5$  take 10 mL of 0.01M Succinic acid solution in the conical flask  $C_4$  and add 2 drops phenolphthalein indicator.
- 5. Titrate the solution against the diluted NaOH till a faint pink colour persists.
- 6. Repeat the titration until you get three reasonable readings.



- 3.1 Record your titration readings in Observation Table 1. Note down the reading. (1.5pt)

#### 3. Titration of weak acid with strong base

- 1. Using a funnel fill burette  $B_2$  with diluted NaOH
- 2. Dilute the weak acid solution supplied in volumetric flask V<sub>4</sub>to 100 mL using distilled water.
- 3. Using pipette  $P_6$  take10 mL of the diluted weak acid solution in a conical flask  $C_5$  and add 4 drops Universal indicator.

In this titration, diluted NaOH is added in instalments to the weak acid solution. After the addition of each instalment of diluted NaOH, record the colour of the solution, and pH from the chart supplied to you.

Put down pH value only when exact colour match is seen. If the colour is intermediate between colours in the chart, write down the pH range.

- 4. Add the burette solution in instalments of 0.5 mL each till the colour of the solution changes to purple. Thereafter take four more readings adding instalments of 0.5 mL each.
  - 3.3 Record your observations in Observation Table 2 in the answer sheet provided to you. (2.5pt)
  - 3.4 Plot a graph of pH vs volume of diluted NaOH and determine the 5 mL (0.5pt) range of the equivalence point.
  - 3.5 Find  $\Delta pH$  for every successive change in volume (0.5mL) and then plot a graph of  $\Delta pH/\Delta V$  vs volume of diluted NaOH only in the range identified in 3.4 above
  - **3.6** Determine the equivalence point from the data above. (0.5pt)





# pH Titration using Universal indicator ( 6 Marks)

Consider 1 Decimal place when recording your burette reading.

**3.1** (1.5 pt)

#### **Observation Table 1**

- 5 % deviation = full marks
- 10 % dev. = 1 mark
- 10-15% dev = 0.5 mark

Sr.		Titration	Titration	Titration
		I	п	III
1	Initial burette reading, mL			
2	Final burette reading, mL			
3	Difference in burette readings, mL			

If only one reading has been taken, the mark will be 0.5

<b>3.2</b> (0.5 pt)	
Molarity of diluted $NaOH$ used for the titration =M	





3.3  $(2.5~\mathrm{pt})$ Observation Table 2

Volume of diluted	Colour	рН	ΔрН	ΔV	Δ pH /Δ V
NaOH added in mL	of solution				

Observation Table 2 continued on the next page.





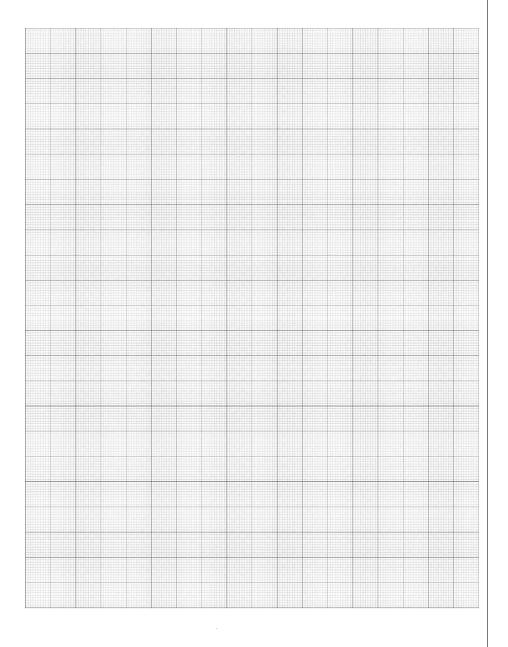
# 3.3 (cont.)

Volume of Diluted	Colour	рН	ΔрΗ	ΔV	Δ pH /ΔV
NaOH added in mL	of solution				



A3-4
English (Official)

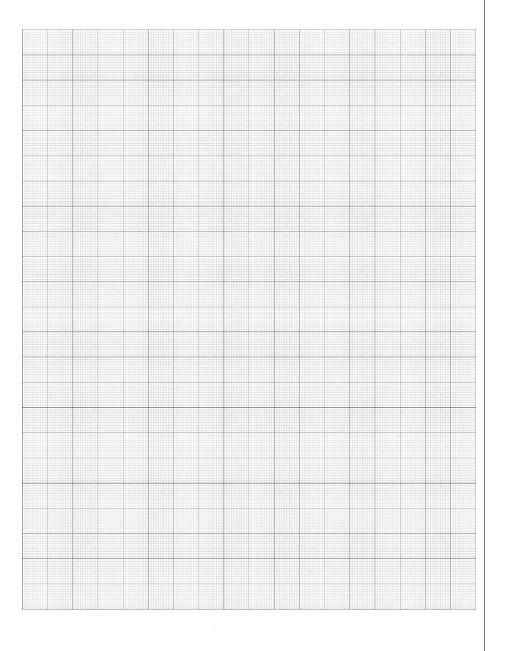
3.4  $(0.5~\mathrm{pt})$  Graph of pH vs. volume of NaOH added







3.5  $(0.5~\mathrm{pt})$  Graph of  $\Delta \mathrm{pH/\Delta V}$  vs volume of NaOH added





	<b>3.6</b> (0.5 pt)	
Equivalence point= mL	Equivalence point= mL	



### **General Instructions:**

1. Wherever asked to mark the cell with a cross (X), mark as follows.



2. Wherever asked to mark the cell with a dash (-), mark as follows.



#### Q.4. Investigating mixup of newborn babies in a hospital

This is an investigative experiment to identify parents of three newborn babies who got mixed in a hospital. The identification will be done with the help of blood groups. Untill the 1980s blood groups were used in forensics, but this has now been replaced by other techniques that are more reliable. ABO blood typing is still important for blood transfusions.

The presence or absence of A and B antigens on the Red Blood Cells (RBCs) determines the ABO blood group of an individual. A person with only antigen A will be of blood group A. A person with only antigen B will be of blood group B. A person with both antigens A and B will have blood group AB, while one who has neither of the antigens has a blood group of O type. These antigens in an individual are governed by the alleles of the gene responsible for their synthesis. Antigen A is determined by the allele  $I^A$ , while antigen B is determined by the allele  $I^B$ . No antigens are produced when an individual carries the  $I^A$  alleles. The alleles  $I^A$  and  $I^B$  are co-dominant. The allele  $I^A$  is recessive to both  $I^A$  and  $I^B$ . alleles.

The presence of a given antigen can be identified by the use of antibodies. For example, if an antibody against antigen A is added to blood from a person with blood group A, RBCs will clump together or agglutinate. In this experiment, you will not use blood samples but solutions that mimic the process of agglutination (clumping) of blood in the presence of a given antibody. The process has been mimicked using chemicals and precipitation represents the process of agglutination. The use of chemicals to demonstrate the concept of blood groups was developed by Magdalena Wajrak of Edith Cowan University in Perth, Australia (Harrison T, 2015, Science in school, 32: 33-36)

#### **Materials provided:**

#### Glassware and miscellaneous items

- 1. Mimicked blood samples (13 in all) in 1.5 ml plastic tubes (in stand) labelled as follows:
  - (i). Four samples W, X, Y and Z (to be used in Exercise 1).
  - (ii). Nine samples C, D, E, 1F, 1M, 2F, 2M, 3F, 3M (to be used in Exercise 2).
- 2. 3 X 15 ml plastic tubes labeled as Anti A, Anti B and NA which have mimicked antibodies against antigen A, antigen B and no antibody, respectively. These have been placed in a 250 ml plastic beaker.
- 3. Four Cavity slides with 3 wells in each.
- 4. 7 plastic droppers.
- 5. 250 ml beaker with distilled water



- 6. Permanent marker
- 7. Rectangular labels
- 8. A4 sheet of black paper for placing slides
- 9. Waste bin
- 10. Additional distilled water in a bottle

Note: Please raise your hand, if you require additional distilled water.

Tissue papers and waste beaker will be provided by the supervisor

#### Exercise 1: Identify the blood groups of the blood samples W, X, Y and Z

1. Arrange four cavity slides to make a grid similar to the one shown in Figure 1.1. The slides should be placed on the black sheet provided to you.

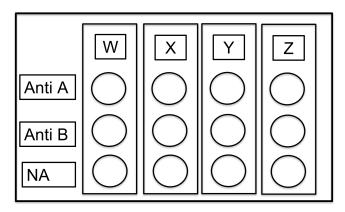


Figure 1.1

- 2. With the marker pen and labels provided, mark the wells of the grid as above.NA stands for no antibody.
- 3.1 Mark three droppers one as Anti-A (to be used only for taking Antibody A), one as Anti-B (to be used only for taking Antibody B) and one as NA (to be used for taking the solution marked as NA).
- 3.2 Use the remaining droppers for blood samples.
- 3.3 Before starting and in between taking two different samples, flush the dropper several times (15 to 20 times) with distilled water to ensure that they are clean.
- 3.4 Ensure that the droppers are clean so that samples do not become cross-contaminated. Please avoid touching the added solutions in any of the wells of the cavity slide.
- 4. Using a clean dropper, place a drop of blood sample W in each of the wells in column 1.
- 5. Continue the same with the other three blood samples (X, Y and Z).
- 6. In the first row, add 1 drop of Anti A (antibodies against A-antigen) in each of the wells.
- 7. In the second row, add 1 drop of Anti B (antibodies against B-antigen) in each of the wells.
- 8. In the third row, add 1 drop of NA solution.



A.1.1 Observe the wells and record the presence of white precipitate (mimicking ag- (0.75pt) glutination of blood) in the appropriate cells in Table 1.1 by marking a cross (X). Mark a dash (-) in cells representing wells where no precipitation was observed.

Table 1.1							
W	Х	Υ	Z				

**A.1.2** Request your supervisor to take a photograph of the plate. The supervisor will (0.25pt) submit the photograph on your behalf.

Based on your observation, identify the blood groups of the samples W, X, Y and Z in Table 1.2 by **marking** a cross (X) in the appropriate cell.

**A.1.3** (0.25pt)

Table 1.2						
	Blood Group					
Sample	Α	В	AB	0		
W						
X						
Υ						
Z						

Identify the row(s) (Anti A, Anti B and NA) in Figure 1.1 that act(s) as the control for the experiment with a **cross (X) in the correct cell(s)**. Mark dash (-) in the remaining one(s).



A.1.4			
	Anti-A	Anti-B	NA

# Exercise 2: Identify the blood groups of the parents and the babies in an attempt to restore the babies to their respective parents.

There are three newborn babies (C, D and E), whose tags indicating their parents have been mixed up. In order to identify the correct parents of the three babies, blood samples were taken from the babies and the possible parents (1 to 3). The experiment attempts to identify the parents of the three babies based on their blood groups.

For the identification you are provided with mimic of 9 blood samples labeled as follows:

Note: In the table, F stands for Father (not female) and M stands for Mother (not male).

Sample No.	Label	Blood sample from
1	1F	Parent 1 father
2	1M	Parent 1 mother
3	2F	Parent 2 father
4	2M	Parent 2 mother
5	3F	Parent 3 father
6	3M	Parent 3 mother
7	С	Baby C
8	D	Baby D
9	Е	Baby E

**2.1.** For each of the blood samples, identify the blood group following the procedure described in the first exercise.

#### Note:

- Before reusing the cavity slides, wash them carefully with distilled water and dry them well with tissue paper before putting samples in them.
- Ensure that the droppers are clean before taking the blood samples.

In Table 1.3, **mark a cross (X) in the appropriate cells** for the presence of precipitate. Mark a dash (-) in cells representing wells where no precipitation was observed.



**A.2.1.1** Take photographs of the labeled slides as done in 1.2. Request your supervisor to take photographs of the labeled slide. The supervisor will submit the photograph on your behalf. (4.5pt)

Table 1.3									
	1F	1M	2F	2M	3F	3M	С	D	Е
Anti-A									
Anti-B									
NA									

Based on the results of the experiment, identify the blood groups of each of the nine samples by **marking** a cross (X) in the appropriate cell in Table 1.4.

**A.2.1.2** (0.50pt)

	Table 1.4							
	Blood group of babies							
Baby	Blood group A	Blood group B	Blood group AB	Blood group O				
С								
D								
E								
	Blood group of parents							
1F								
1M								
2F								
2M								
3F								
3M								

Based on the different blood groups identified by you, indicate the possible parent pairs for the babies C, D and E by marking a cross (X) in the appropriate cell(s) in Table 1.5. There could be more than one possibility. Mark a dash (-) in the remaining cell(s).





A.2.2					(1.0pt)
		Ta	able 1.5		
		Parent pair 1	Parent pair 2	Parent pair 3	
	Baby C				
	Baby D				
	Baby E				

Based on your interpretations of the blood groups which baby(ies) (C, D, E) can be matched to their parent pairs (1, 2, 3) with certainty based on the evidence?

Write the number of the parent pair (1, 2 or 3) in the cell to the corresponding baby(ies).

Mark a dash (-) in cell(s) corresponding to a child with multiple possible parent pairs.

Predict the genotype of the child and the parent pair that can be matched with certainty based on your answer in 2.3.

Indicate **one possible genotype** of the child and the corresponding parent pair.

Mark a dash (-) in cell(s) corresponding to a child with multiple possible parent pair.

<b>A.2.4</b>							(0.25p
			Genotype		Genotype	e of the parents	
			Genotype of the child		Father	Mother	
	Child	С		Parent			
	Child	D		Parent			
	Child	Е		Parent			



# Q.4. Investigating mix up of newborn babies in a hospital

**A.1.1** (0.75 pt)

Table 1.1						
	W	Х	Y	Z		
Anti-A						
Anti-B						
NA						

**A.1.2** (0.25 pt)

Request your supervisor to take the photograph of the plate. The supervisor will submit the photograph on your behalf.



**A.1.3** (0.25 pt)

Table 1.2						
	Blood Group					
Sample	Α	В	АВ	O		
W						
X						
Y						
Z						

**A.1.4** (0.25 pt)

Anti-A	Anti-B	NA



**A.2.1.1** (4.5 pt)

Table 2.3									
	1F	1M	2F	2M	3F	3M	С	D	E
Anti-A									
Anti-B									
NA									

### A.2.1.1 (cont.)

Request your supervisor to take the photographs of the plate. The supervisor will submit the photograph on your behalf.





.2.1.2	(0.50  pt)			
		Table 1	.4	
		Blood group o	of babies	
Baby	Blood group A	Blood group B	Blood group AB	Blood group O
С				
D				
Е				
		Blood group o	f parents	
1F				
1M				
2F				
2M				
3F				
3M				

### **A.2.2** (1.0 pt)

Table 1.5					
	Parent 1	Parent 2	Parent 3		
Baby C					
Baby D					
Baby E					



**A.2.3** (0.25 pt)

Child	С	Parent	
Child	D	Parent	
Child	Е	Parent	

**A.2.4** (0.25 pt)

		Genotype		Genotype of the parents		
		of the child		Father	Mother	
Child	С		Parent			
Child	D		Parent			
Child	Е		Parent			



## Q.5. Analyzing human chromosomes

The karyotype of a species represents the chromosomes of a cell, usually displayed as a systematic arrangement of chromosome pairs in descending order of size. In order to make a human karyotype, metaphase chromosomes are prepared from blood cells

The blood cells are induced to divide in culture and then treated with colchicine to block cell division at metaphase. The cells are then broken by hypotonic treatment and chromosomes are spread on glass slides. The chromosomes are stained and observed under a microscope. The photograph of the metaphase (as shown in Figure 5.1) is then used to make a karyotype. Karyotypes can be used to identify chromosomal abnormalities and aberrations. In the manual method, individual chromosomes are cut from the photograph and then lined up by size and the position of the centromere with their respective partners. In humans chromosomes can be of three types, which are determined by the position of the centromere (point of attachment of the mitotic spindle): (i) **acrocentric chromosomes** have the centromere located very close to the end resulting into one of the arms being very short ( sometimes not even discernable), (ii) **submetacentric chromosomes** have arms of unequal lengths and (iii) **metacentric chromosomes** have equal or almost equal arms. The karyotype prepared from the metaphase spread shown in Figure 5.1 is presented in Figure 5.2. A description of the chromosomes belonging to different groups (Figure 5.3) is given in Table 5.1.



Figure 5.1. A metaphase spread of human chromosomes





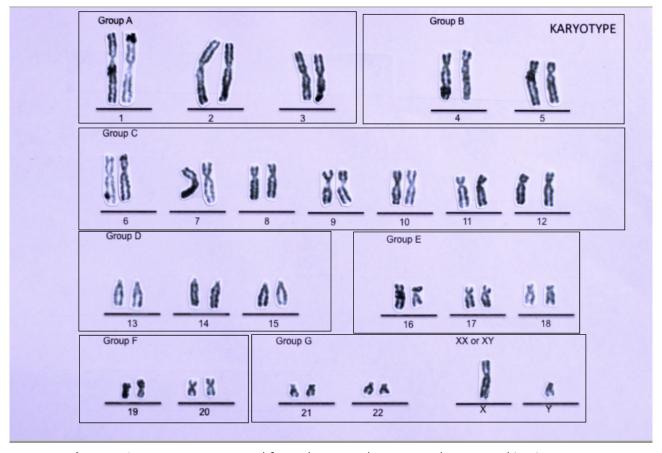


Figure 5.2. Karyotype prepared from the metaphase spread presented in Figure 5.1.

Table 2	Table 2.1: Characteristics of the chromosomes in the human karyotype			
Group	Chromosomal Pairs	Description		
Α	1-3	Large almost metacentric chromosomes		
В	4-5	Large submetacentric chromosomes		
С	6-12 + X	Medium-sized submetacentric chromosomes		
D	13-15	Large acrocentric chromosomes		
E	16-18	Small submetacentric chromosomes		
F	19-20	Small metacentric chromosomes		
G	21-22 + Y	Small acrocentric chromosomes		
XY	X	Medium-sized sub metacentric chromosome		
	Υ	Small acrocentric chromosome		

In the following exercise you are required to prepare a karyotype from the photograph of the metaphase spread given to you. This chromosomal preparation is from an individual with an anomaly in their sex chromosomes.



### Materials given for karyotype preparation:

- 1. Photograph of a metaphase spread for karyotype preparation
- 2. Plastic petri-dish
- 3. Fine scissors
- 4. Forceps
- 5. Transparent tape
- 6. Sheet marked 'Karyotype' to paste the chromosome cutouts

#### **Procedure:**

Use the photograph of the metaphase spread for the following exercise

#### **Exercise 1: Count the number of chromosomes**

**A.5.1** Count the number of chromosomes and record in the answer book.

(0.25pt)

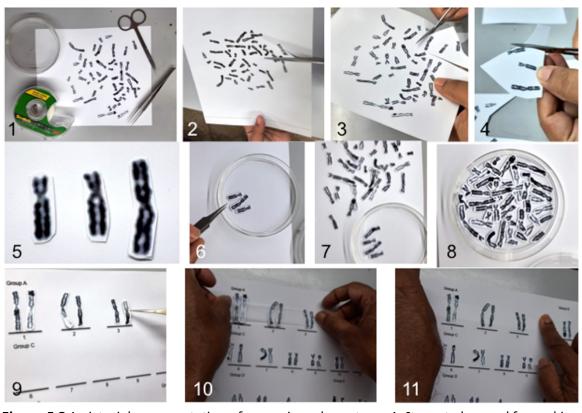


#### **Exercise 2: Make a karyotype**

- 1. With the help of fine scissors cut out each chromosome and place it in the given petri- dish. Make sure that you do not loose any of them.
- 2. Arrange the chromosomes (cut outs) on the sheet labeled **karyotype** according to the size of the chromosomes and position of their centromere. Use Figure 5.3 and Table 5.1 as your reference. After arranging the chromosomes stick them in place with transparent tape. Take a photograph and attach to the answer sheet.
- 3. The sheet labeled karyotype is part of your answer book and is placed at the end of the answer book.

In each group, arrange the chromosomes by their approximate length. There will be no penalty for errors in identification of specific chromosomes within a group.

Figure 5.3 is a pictorial guide on how to prepare a karyotype.



**Figure 5.3** A pictorial representation of preparing a karyotype. 1: Items to be used for making a karyotype, 2-5: Cutting the individual chromosomes, 6-8: Picking the chromosomes with the fine forceps and collecting them, 9: Arranging the chromosomes, 10-11: Sticking the chromosomes with the piece of tape.

**A.5.2** Please ask your supervisor to either scan or take a photograph of the karyotype on the answer sheet and submit. (3pt)



#### **Exercise 3: Answer the following.**

Can the following cells in human blood be used for preparation of metaphase chromosomes?

**A.5.3.1** Mark a cross (X) in the appropriate column (**Yes / No**).

(0.25pt)

S.No.	Cells	Yes	No
1.	Erythrocyte (Red Blood Cells)		
2.	Lymphocyte (White Blood Cells)		

You are asked to prepare chromosomal spread from mitotic plant cells. Can you use the following plant parts to successfully prepare the chromosomal spread?

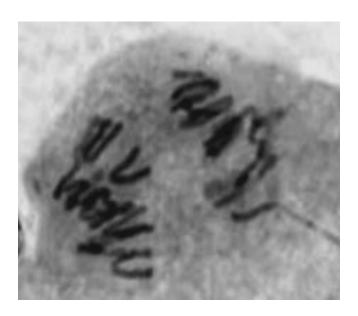
**A.5.3.2** Mark a cross (X) in the appropriate cells (Yes/No).

(0.25pt)

S.No.	Plant parts	Yes	No
1.	Leaf blade		
2.	Anther		
3.	Root tip		

The photograph represents chromosomes in a rodent cell undergoing division. All chromosomes in this rodent are **acrocentric**.





Does the following photograph represent the following stages of division?

**A.5.3.3** Mark a cross (X) in the appropriate column (Yes/No).

(0.25pt)

Stage of division	Yes	No
Mitotic Metaphase		
Mitotic Anaphase		
Meiotic Metaphase I		
Meiotic Anaphase I		
Meiotic Metaphase II		
Meiotic Anaphase II		



# Q.5. Analyzing human chromosomes

**A.5.1** (0.25 pt)

Number of chromosomes =

**A.5.2** (3.0 pt)

Please ask your supervisor, to either scan or take the photograph of the kary-otype ad submit.

**A.5.3.1** (0.25 pt)

S.No.	Cells	Yes	No
1.	Erythrocyte		
2.	Lymphocyte		

**A.5.3.2** (0.25 pt)

S.No.	Plant parts	Yes	No
1.	Leaf blade		
2.	Anther		
3.	Root tip		

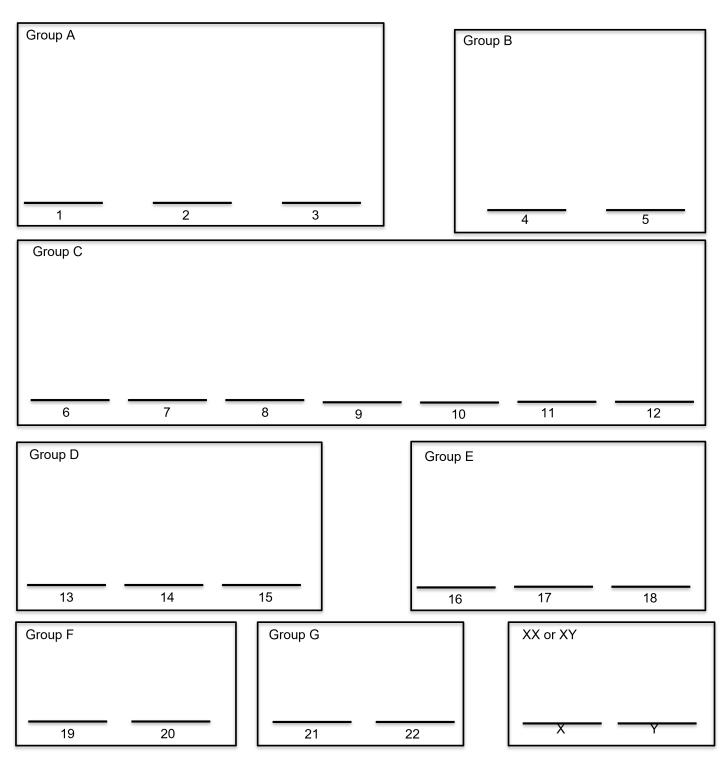


A5-2
English (Official)

**A.5.3.3** (0.25 pt)

Stage of division	Yes	No
Mitotic Metaphase		
Mitotic Anaphase		
Meiotic Metaphase I		
Meiotic Anaphase I		
Meiotic Metaphase II		
Meiotic Anaphase II		





Karyotype